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Nesting Biology of Osmia cornifrons: Implications for Population Management

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Nesting Biology of *Osmia cornifrons*: Implications for Population Management

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ABSTRACT

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The Japanese hornfaced bee, *Osmia cornifrons* (Hymenoptera: Megachilidae) is a palearctic mason bee managed for the pollination of early season fruit crops such as apple and blueberry. Since its adoption as a managed pollinator in Japan during the 1940s, a large body of literature has amassed with the goal of enhancing *O. cornifrons* management practices. This research makes important contributions to that literature in two ways. First, the research describes the in-nest relationship of *O. cornifrons* and the cleptoparasitic mite pest *Chaetodactylus krombeini*. Distribution of male and female *O. cornifrons* and of *C. krombeini* was determined using linear and non-linear regression analysis. Results indicated that *C. krombeini* is more frequently found in the cells of female *O. cornifrons* and that female *O. cornifrons* suffer greater mortality than male *O. cornifrons* due to *C. krombeini.* Second, the research describes trends in *O. cornifrons* activity resulting from abiotic factors by utilizing videography techniques. *Osmia cornifrons* daily activity was measured as the number of trips initiated from the nest every hour. The effects of time of day, temperature, and precipitation on *O. cornifrons* activity were determined using non-linear regression analysis and correlation analysis. Results of this study showed that *O. cornifrons* activity is limited days above 13.9 °C without rain, between the hours of 8:00am and 8:00pm. In addition, the videography techniques described in this research provide a new methodology for the study of solitary nesting bees.

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Chapter 1: Introduction

Thesis Organization

This thesis is organized into four chapters. Chapter 1 is an introduction to *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae) and its cleptoparasitic mite pest *Chaetodactylus krombeini* (Baker) (Acari: Chaetodactylidae). Chapter 2 describes how *C. krombeini* is distributed throughout *O. cornifrons* nests, and how it affects the bee's pollination potential and population growth. Chapter 3 discusses how time of day, temperature, and precipitation affect *O. cornifrons* nesting activities, and describes a new method for studying the nesting biology of solitary bees and wasps. Chapter 4 synthesizes the information in chapters 1-3 to form general conclusions on the management of *O. cornifrons*. This thesis was prepared according to the publication guidelines established by the Entomological Society of America.

General Introduction

It is estimated that 88.7% of the 250,000 species of plants are angiosperms, and that 90% of the angiosperms are pollinated by insects (Hoshiba and Sasaki 2008). Also, one-third of the food we eat comes from crops which require pollination (Mader et al. 2011). In addition to bees, pollination services are rendered by several species of bats (e.g., suborder Megachiroptera), birds (e.g., Apodiformes: Trochilidae), and terrestrial mammals (Allen-Wardell et al. 1998). Of all the pollinators, the most important are the bees (Hymenoptera: Apoidea) as they are the only animals known to deliberately gather pollen (Mader et al. 2011). The most economically important bee is the European honey bee *Apis mellifera* L. (Hymenoptera: Megachilidae) (Johnson 2010), whose value from pollination services has been estimated at $14.6 billion annually (Morse and Calderone 2000). Currently, colony collapse disorder (CCD), a mysterious disappearance of honeybees that is not well understood, threatens the pollination services gained from *A. mellifera* as colony declines occur (Stankus 2008). Mussen (2007) suggests that CCD may be caused by a combination of several factors that when present individually do not cause CCD symptoms. Factors which threaten *A. mellifera* and which may be partially responsible for CCD include severe winters, stress from moving and managing bee hives, hive damage from large mammals, insect pests (e.g., small hive beetles *Aethina tumida* [Coleoptera: Nitidulidae] and wax moths *Galleria mellonella* [Lepidoptera: Pyralidae]), mites (e.g., *Varroa destructor* [Parasitiformes: Varroidae]), fungi (e.g., *Nosema apis* and the invasive *Nosema ceranae* [Microsporidia, Dissociodihaplophasida, Nosematidae]), other fungi (e.g., chalkbrood *Ascophaera apis* [Onygenales: Ascosphaeraceae]), stone brood (*Aspergillus* spp.), bacteria (e.g., American foulbrood *Paenibacillus larvae* and European foulbrood *Melissococcus pluton*), and many viruses (Stankus 2008).

Because of the threats to *A. mellifera* and the vital importance of pollination, there is a need for alternate pollinators that can be easily managed and that are as effective as *A. mellifera* at pollinating. Currently managed alternate pollinators in the United States include three species of the family Megachilidae (blue orchard bees *Osmia lignaria*, Japanese hornfaced bees *Osmia cornifrons*, and alfalfa leafcutting bees *Megachile rotundata*), two species of the family Halictidae (alkali bees *Nomia melanderi* and grey-haired alfalfa bees *Rhophitoides canus*), and several species of the family Apidae (bumblebees *Bombus* spp.) (Bosch and Kemp 2002). Each of these alternate pollinators requires different management practices and several of them are specialist pollinators (i.e., oligolectic). The research presented in this thesis focuses on one of these alternate pollinators, *O. cornifrons*.

Objectives of Study

The goal of this study was to describe the nesting biology of *O. cornifrons*. The results of this study will be used to improve the management practices currently utilized for *O. cornifrons* populations so that it can become a more effectively implemented alternate pollinator. The objectives (i.e., chapters) of this research were:

Objective 1. To determine how the cleptoparasitic mite *C. krombeini* impacts developing male and female *O. cornifrons* larvae (Chapter 2).

Objective 2. To elucidate the role that certain abiotic factors play in determining the frequency of *O. cornifrons* nesting activities (Chapter 3).

Literature Review

The history of the hornfaced bee. The Japanese Hornfaced Bee *Osmia cornifrons* Radoszkowski (Hymenoptera: Megachilidae) was first described in 1887 as *Chalicodoma cornifrons* (Radoszkowski 1887). It was not until 1922 that it was placed in the genus *Osmia* by Cockerell (cited by Yasumatsu and Hirashima 1950). *Osmia cornifrons* became the first non-*Apis* bee to be managed for pollination (Batra 1998) in the 1940s when Eikyu Matsuyama, a Japanese farmer in the Aomori Prefecture of northern Japan, discovered its effectiveness for pollinating apples (Batra 1982, Maeta 1990). After Eikyu Matsuyama devised a technique for managing *O. cornifrons*, other growers in the Aomori Prefecture began to take notice. Two of these growers, Gisuke Takeshima and Masao Fukui, made efforts to spread the use of *O. cornifrons* to other growers. The use of *O. cornifrons* for pollinating apples came to be noticed by Dr. Masateru Yamada, Takashi Shôji, Taizo Kitamura, and Dr. Yasuo Maeta who were all agricultural researchers. Together, they developed the first highly effective management program for *O. cornifrons* (Maeta 1990).

The first attempt to release *O. cornifrons* for pollination in the United States was in 1965 in Utah, but the bees were unable to establish a population there. In 1976 two separate shipments of *O. cornifrons* were sent from Japan by Yasuo Maeta to the Beltsville Agricultural Research Center (BARC) in Maryland. The two shipments in 1976 were shipped as diapausing adults in cocoons that had been removed from their original nests, and the bees from both of those shipments were found to be either dead or dying upon arrival. Finally in 1977, diapausing adult *O. cornifrons* were sent to BARC in their original nests, which allowed them to survive the shipment process. After reaching BARC, the bees were placed into quarantine, and were then successfully introduced into the United States for orchard pollination (Batra 1978). North American Fruit Explorers were the first group to test *O. cornifrons* after it was introduced at BARC (Batra 1998).

Cultural significance of the hornfaced bee. Before *O. cornifrons* was discovered as a pollinator, apple pollination in Japan was done by hand. During the apple bloom period military personnel would be recruited to perform this task. Hand pollination required 30 workers/ha causing increased expense for apple growers (Batra 1982, Maeta and Kitamura 1981). In addition, hand pollinating practices were not as effective as *O. cornifrons* for pollinating apple orchards (Xu et al. 1995). The use of hand pollination and pollination by *A. mellifera* steadily declined between the 1950s and 1990s (Sekita and Yamada 1993). By 1996, 80% of 25,000 ha of apple orchards in northern Japan were being pollinated by *O. cornifrons* (Sekita 2001). *Osmia cornifrons* became such an important pollinator that Japanese growers modified traditional agricultural practices and worked with neighbors to support the proliferation of the bee over large areas. This in turn increased the profitability of Japanese apple orchards and allowed the orchards to support an increased number of employees (Batra 1982).

The biology of the hornfaced bee. *O. cornifrons* is a Palearctic species whose natural range is limited to northern Asia. Presence of *O. cornifrons* has been reported in Japan, Korea, China, and Russia. In addition, *O. cornifrons* may be found in the Nearctic as an introduced species primarily in the eastern United States (The Polistes Corporation 2011).

Osmia cornifrons has one generation per year (i.e., univoltine) (Batra 1978). It emerges in April close to the time of apple bloom (Fig. 1). Males emerge 2-3 days before females and wait outside the nest for females to emerge. When females emerge the males quickly approach the females to mate. Females are easily distinguished from males by the presence of facial horns and generally larger body size. During courtship a male mounts a female, and then attempts to entice the female by touching her face with his antennae and rubbing her abdominal terga with the end of his abdomen. This process can last for over an hour before the female permits the male to mate (Batra 1982). Once mating is finished the males go into the field where they may live and serve as pollinators for several weeks before they die (Matsumoto and Maejima 2010, Bosch and Kemp 2002). Mated females leave their nests for several days (Maeta 1978) presumably to complete ovarian development before nesting. Average number of oocytes of *O. cornifrons* is 56 ± 0.7 (SD) in three pairs of ovarioles (Maeta 1978).

Once ovarian development has ended the female searches for a suitable nesting site, often returning to the location of the nest from which she emerged. Female *O. cornifrons* can return back to their nests by memorizing the location during an orientation flight (Maeta 1978). Natural nesting sites for *O. cornifrons* are broken reeds, bamboo, or beetle created holes in trees (Maeta 1978). Nesting activities are broken up into several steps as outlined by Maeta (1978): First, *O.*

cornifrons cleans out the nest if there is material from a previous nest inside. Second, they create a preliminary plug of mud at the back of the nest. Third, after that they begin to provision the cell (single nest unit) with pollen, they lay an egg by inserting it partially into the pollen provision. Fourth, *O. cornifrons* seals off the cell with a mud wall and repeats the process. Fifth, after most of the nest is filled with provisioned cells, *O. cornifrons* creates empty cells known as vestibular cells which are used to fill the remaining space in the nest to prevent other bees from utilizing it (O'Toole and Raw 2004). To finalize the nest, *O. cornifrons* creates a thick mud wall called the closing plug (Maeta 1978) (Fig. 2).

Once nesting has begun, *O. cornifrons* forages within ~130 m of its nest, though they are capable of locating their nests from up to 700 m away (Maeta 1978, Kitamura and Maeta 1969). During this time *O. cornifrons* can visit as many as 2,450 flowers per day, which is 80 times more flower visits than *A. mellifera* visited on apples (Maeta and Kitamura 1981). Pollen gathering takes *O. cornifrons* 699.9 ± 81.87 (SD) seconds according to Lee et al. (2010). After gathering enough pollen to fully provision a cell, *O. cornifrons* lays an egg by inserting her abdomen partially into the slanted surface of the pollen ball (Yamada et al. 1971) and then inserting the egg 1/10 of its length into the pollen ball before pulling away her abdomen (Maeta 1978). A single *O. cornifrons* can oviposit 22.7 ± 1.1 (SD) eggs in her lifetime (Maeta 1978). *Osmia cornifrons* is believed be able to voluntarily control the sex ratio of its progeny through haplodiploid sex-determination; female progeny are diploid and male progeny are haploid (Maeta 1978, Maeta and Sugiura 1990). Their sex ratio is reported as 3 to 2 (male to female) (Maeda 1974), though this ratio is variable based on the period of nesting activity, abundance of forage available, and size (length and diameter) of the nest (Maeta 1978). *Osmia cornifrons* generally provides female larvae with more pollen than male larvae (Maeta and Sugiura 1990), and female bees tend to be larger than males (Maeta 1978, Yoshida and Maeta 1988). In addition to gathering pollen and laying eggs, *O. cornifrons* must gather mud to construct the cell partitions inside the nest. Lee et al. (2010) reported that mud gathering required 464.9 ± 151.17 seconds (see chapter 3 for further discussion of *O. cornifrons* activity time requirements). *Osmia cornifrons* may maintain up to six nests at one time (Maeta and Sugiura 1990), and may attempt to take the nest of another bee (i.e., supersedure) (Maeta 1978). *Osmia cornifrons* will continue nest building activity until the end of May after which female dies.

The larvae develop through four instars and the rate of development is temperature dependent. *Osmia cornifrons* larvae cannot develop at temperatures ≤ 12.4 °C, and 18 °C may be the optimum temperature for larval survival (Maeta 1978). By early summer the adults pupate, and by mid-summer adult eclosion occurs (Maeta 1978). At this time the bees go into summer diapause. To successfully break diapause and emerge the next spring, *O. cornifrons* must undergo a cold period lasting at approximately three months (Maeta 1978).

Management practices of the hornfaced bee. There are several advantages to using *O. cornifrons* for fruit tree pollination. First, the short foraging range (Batra 1989) of *O. cornifrons* combined with high flower constancy (i.e., a behavior in which a pollinator continuously returns to the same species of flower) (Matsumoto et al. 2009) keeps *O. cornifrons* pollination activities within the boundaries of the orchard. Second, *O. cornifrons* preference for Rosaceous flowers (Maeta 1978) is advantageous because *O. cornifrons* spends more time pollinating target crops than non-target flowering plants when compared to *A. mellifera*. Third, *O. cornifrons* is a very docile bee and rarely stings (Batra 1998), making it an easy pollinator for growers to manage. Finally, *O. cornifrons* is known to visit flowers in rapid succession and to have high anther and stigma contact (Batra 1998). Cho et al. (1995) reported that the percentage of malformed fruit increased with distance from *O. cornifrons* nests, and that trees in the presence of *O. cornifrons* had double fruit set and half as much malformed fruit as trees not in the presence of *O. cornifrons.* Matsumoto and Maejima (2010) reported that *O. cornifrons* was a more effective apple pollinator than *A. mellifera*, especially on 'Delicious' variety apples in which *A. mellifera* was able to work around the anther to get to the nectar.

Choosing suitable locations and crops for *O. cornifrons* is important for determining how the bee can be utilized and managed. Because *O. cornifrons* development is dependent upon temperature (Maeta 1978), it is not suitable for pollination in some regions. Plant hardiness zones 5-8 are similar to *O. cornifrons'* natural climate in Japan, and are considered the best places in the United States to use *O. cornifrons* for pollination (Batra 1998). *O. cornifrons* shows a preference for flowers in the family Rosaceae (Maeta 1978, Batra 1998), but is considered polylectic (i.e., foraging from a wide variety of plants) (Maeta 1978). There have been numerous reports on the variety of crops which *O. cornifrons* may pollinate (Maeda 1974; Kristjansson and Rasmussen 1991; Batra 1978, 1982, 1998; Maeta et al. 1990; Abel and Wilson 1998; Wilson et al. 1999, 2000; Goubara and Takasaki 2003) (Table 1).

To utilize *O. cornifrons* as an alternate pollinator, management techniques have been developed and refined over the last 60 years. One of the most important considerations that had to be made for managing *O. cornifrons* was the type and size of the nest to be used. The length and diameter of the nest can impact the sex ratio of *O. cornifrons* (Maeta 1978), and can affect the female bees body size (Bosch and Kemp 2002). Smaller female *O. cornifrons* tend to have reduced fecundity (Sugiura and Maeta 1989). Popular nest materials in Japan are bamboo or reeds placed in a sheltered area. In addition, cardboard straws are a commonly used nesting material (Maeta 1978).There are also more permanent nests constructed from wood such as Binderboards (Mills 1994, Mills 1997).

Previous studies (Maeta 1990; Batra 1989, 1998; Sekita and Yamada 1993) described general management practices for *O. cornifrons*. Management practices have been developed to increase pollination by *O. cornifrons* and to avoid *O. cornifrons* pests (Table 2). In apple orchards, pesticides need to be sprayed ten days after bud break (Sekita and Yamada 1993). Three days later *O. cornifrons* nests can be placed in a nest release site which receives adequate sunlight and is sheltered from wind and rain (Maeta 1978). Several sites like this should be located throughout the orchard. Nests release sites should be placed at 2.4 m off the ground (Wilson et al. 1999) and they should be placed about 60 m apart (Yamada et al. 1984) so that optimum nest establishment and pollination is achieved because *O. cornifrons* rarely visits trees great than 75 m from its nest site. Currently, it is recommended that 500-600 bees/ha be used for adequate pollination (Maeta and Kitamura 1981, Batra 1989), though release density can vary depending on the crops being pollinated and geographic location of the orchard (Yoshida and Maeta 1988). The nests may be placed in a garbage can or oil drum lying vertically with a board inside to act as support for the nests (Batra 1989). The opening of the garbage can is covered with a 3.8 cm mesh to keep out bird predators. Alternatively, a nest box can be made which is propped up on four legs. By applying grease to the legs, some pests, such as ants, cannot get to the nests (Maeta 1978). The nests are placed in an exclusion device which permits the bees to exit but not re-enter, and abundant new nesting material is supplied nearby. This is done to reduce pest buildup (e.g., *Chaetodactylus* spp.), which occurs when nests are reused (Maeta 1990, Sekita et al. 1996). By early June the nesting period is over and the nests are brought in from the field to avoid the parasitic wasp *Monodontomerus* spp. Nests are stored in a ventilated container in an area safe from rodent pests. By August, *O. cornifrons* is in summer diapause and can be heat treated (30-32 °C for 30-40 days) to kill *Chaetodactylus* spp. mites if necessary (Sekita and Yamada 1993). The bees are placed back into storage until early November when they can be put into a cold chamber at 5 °C until the following spring. Depending on the target crop for pollination, release of *O. cornifrons* may be delayed, though this can increase *O. cornifrons* mortality (Wilson and Abel 1996, White et al. 2009). *Osmia cornifrons* released after mid-June do not survive long (Abel and Wilson 1998). When *O. cornifrons* are well managed, populations may double or triple each year (Batra 1978, Maeta 1990)

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Table 1. List of plants reported to be pollinated by *O. cornifrons*

Scientific Name	Common Name	Scientific Name	Common Name
Acer rubrum	red maple	*Medicago sativa*	alfalfa
Acer spp.	maple	*Nepeta* spp.	catnip
Amelanchier	serviceberry	*Paeonia* spp.	peony
Astragalus spp.	milk-vetch	*Prunus armeniaca*	apricot
Berberis spp.	barberry	*Prunus domestica*	plum
Brassica napus	rapeseed	*Prunus persica*	peach
Brassica rapa	turnip mustard	*Prunus* spp.	cherry
Brassica spp.	coleseed	*Pyrus pyrifolia*	apple pear
Capsicum annuum	sweet pepper	*Pyrus* spp.	pear
Cercis spp.	redbud	*Rosa* spp.	rose
Chaenomeles spp.	flowering quince	*Rubus* spp.	raspberries
Cornus spp.	dogwood	*Rubus* spp.	blackberries
Fragaria spp.	strawberries	*Salix* spp.	willow
Helianthus petiolaris	sunflower	*Sinapis alba*	white mustard
Lactuca sativa	lettuce	*Spiraea* spp.	spirea
Linum spp.	flax	*Taraxacum* spp.	dandelion
Lonicera fragrantissima	winter honeysuckle	*Trifolium repens*	ladino clover
Lonicera tatarica	tartarian honeysuckle	*Vaccinium* spp.	blueberry
Malus domestica	apple	*Viburnum* spp.	viburnum
Malus spp.	crabapple		

Table 2. Currently known pests, predators, competitors, and commensals of *O. cornifrons*

Scientific Name	Common Name	Scientific Name	Common Name
Anthax jezoensis	bombyliid flies	*Mimus polyglottos*	mockingbird
Anthrax spp.	bombyliid flies	*Monodontomerus osmiae*	parasitic wasp
Anthrenus verbasci	carpet beetle	*Monodontomerus* spp.	parasitic wasp
Ascophaera spp.	chalk brood	*Nematopogon granellus*	moth
Attegenus japonica	carpet beetle	*Orius* spp.	flower bug
Chaetodactylus spp.	cleptoparasitic mite	*Pimpla hokkaidonis*	ichneumonid
Chrysis spp.	cuckoo wasp	*Pimpla* spp.	ichneumonid
Chrysura spp.	wasp	*Ptinus* spp.	beetle
Crematogaster spp.	ant	*Ptinus japonicus*	beetle
Ectopsocopsis cryptomeris	psocid	*Rattus* spp.	rat
Lasius niger	black garden ant	*Stylops* spp.	strepsipteran
Leucospis japonica	wasp	*Tortonia* spp.	cleptoparasitic mite
Liposcelis bostrychophilus	psocid	*Trichodes* spp.	beetle
Luecospis spp.	wasp	*Trogoderma longisetosum*	dermestid beetle
Mellitobia spp.	wasp	*Trogoderma* spp.	dermestid beetle
Mellitobia acasta	wasp	*Trogoderma varium*	dermestid beetle
Meloe corvinus	oil beetle		

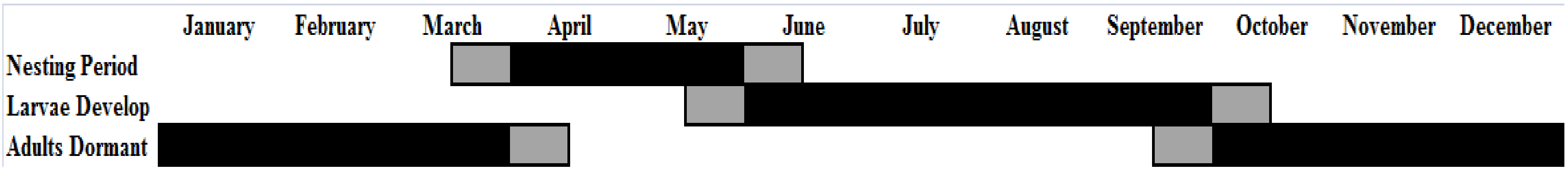

Fig. 1. Life cycle calendar of *Osmia cornifrons*. Black bars indicate time ranges for West Virginia. Gray bars indicate potential variation in activities for other areas where *O. cornifrons* is used.

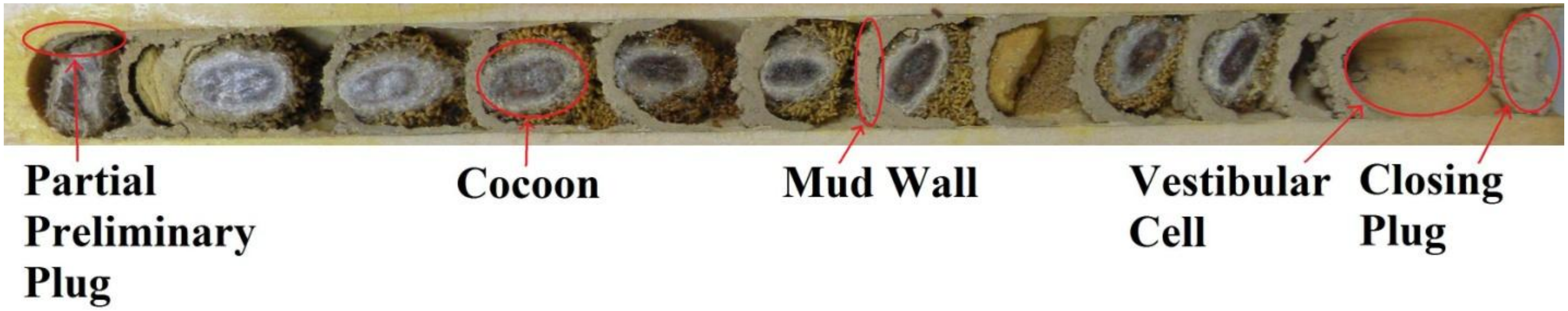

Fig. 2. Photo-diagram of an *O. cornifrons* nest.

Chapter 2: Distribution of *Chaetodactylus krombeini* (Acari: Chaetodactylidae) infestation in *Osmia cornifrons* (Hymenoptera: Megachilidae) nests

Abstract *Chaetodactylus krombeini* (Baker) (Acari: Chaetodactylidae) is a cleptoparasitic mite of *Osmia cornifrons* (Radozskowski) (Hymenoptera: Megachilidae), the Japanese hornfaced bee. *Chaetodactylus krombeini* may attack *O. cornifrons* larvae directly or may eat pollen reserves intended for *O. cornifrons* larvae. Direct attack by *C. krombeini* may cause *O. cornifrons* mortality, and if pollen reserves are eaten the size of adult *O. cornifrons* larvae may be reduced. The objective of this study was to determine the effect of *C. krombeini* on male and female *O. cornifrons.* A total of 30 *O. cornifrons* nests were observed and the distribution trend of male and female *O. cornifrons* within the nest was determined with regression analysis. Mite distributions based on presence/absence and categorical density values were analyzed for 89 additional nests and regression analysis was used to determine mite distribution within the nests. Cocoons from 20 infested *O. cornifrons* cells were examined to determine if mites could be found inside cocoons. Trends in *O. cornifrons* gender distribution showed that female bees were located in the rear of the nest and that males were located in the center of the nest. Regression analysis of *C. krombeini* showed a preference for the inner cells of the nest. No mites were found inside *O. cornifrons* cocoons. Given these trends, *C. krombeini* may have a greater effect on mortality in the egg and larval stages of female *O. cornifrons* than in male *O. cornifrons*.

Key words: Japanese hornfaced bee, Krombein's hairy-footed mite, solitary bee, mason bee, cleptoparasite

The value of pollination services from honeybees alone has been estimated at $14.6 billion annually (Morse and Calderone 2000). Cook et al. (2007) estimated that *Varroa destructor* (Acari: Varroidae) could cause $16.4-38.8 million in economic damages every year if introduced into Australia. These estimates highlight the importance of pollination services and illustrate the importance of pollinator pest control for economic gain. As numerous viruses, parasitic mites, and other factors related to colony collapse disorder reduce honeybee populations, the necessity for alternative pollinators grows.

Osmia cornifrons Radoszkowski (Hymenoptera: Megachilidae) was first managed for pollination in Japan during the 1940s. Over several decades, management practices were perfected and *O. cornifrons* became widely utilized for Japanese apple production, being found in over half of all apple orchards in Japan (Maeta 1990). Several decades later, in 1977, *O. cornifrons* was introduced to the northeastern United States for pollination of early season fruit crops (Batra 1978).

Osmia cornifrons has numerous pests, but the most important of these pests are the members of the mite genus *Chaetodactylus.* In Japan these include *C. nipponicus* and *C. hirashimai* (Qu et al. 2002, Qu et al. 2003). Krombein's hairy footed mite, *Chaetodactylus krombeini* Baker (Acari: Chaetodactylidae) is a cleptoparasitic mite pest of some *Osmia* spp. *Chaetodactylus krombeini* is native to North America and is not currently known to affect *O. cornifrons* in Japan. *Chaetodactylus krombeini* is one of two mite species associated with *Osmia* in the northeastern United States (Klimov and OConnor 2008).

Chaetodactylus krombeini is considered an important pest of *Osmia* spp. (Bosch and Kemp 2001, Kuhn and Ambrose 1984). *Osmia cornifrons* populations which are heavily infested with *C. krombeini* can be reduced by one-half (Batra 1998). *Chaetodactylus krombeini* consumes the pollen provision that female *O. cornifrons* supply to their larvae (Park et al. 2008). This may cause increased mortality in *O. cornifrons* larvae or reduce the size of the developing larvae. Smaller females have reduced fecundity (Sugiura and Maeta 1989). In addition, *C. krombeini* may feed directly upon *Osmia* spp. eggs (Krombien 1962).

Dispersal and within-nest distribution of *C. krombeini* was first described by Krombein (1962) who observed nests of *Osmia lignaria*. Krombein (1962) found that that the existence of both encysted and migratory hypopi stages allowed *C. krombeini* to maintain its normal host relationship and to gain new hosts as other cavity nesting bees come into contact with encysted hypopi. *Chaetodactylus krombeini* may also disperse on cleptoparasitic bees such as *Stelis montana* and *S. murina* (Klimov et al. 2007). Krombein (1962) also reported a low level of mite infestation in the nests he observed; only nine of 121 nests from 1958-1961 were infested and of those nine nests (95 cells) only 14 cells were infested. Previous studies noted that more mite-infested cells were found in the middle of the nests of *O. lignaria* (Krombein 1962) and *O. cornifrons* (Park et al. 2008). However, both studies were not designed to investigate the distribution of *C. krombeini* within *Osmia* spp. nests.

This study was assessed the impact of *C. krombeini* on male and female *O. cornifrons*. Objectives of this study were (1) to determine within-nest distribution of *O. cornifrons* gender, (2) to investigate within-nest distribution of *C. krombeini,* and (3) to determine if *C. krombeini* occurs in cocoons of *O. cornifrons.* By describing the distribution trends of *C. krombeini* in *O. cornifrons* nests, better management practices can be developed to reduce *O. cornifrons* mortality. In addition, the distribution patterns may elucidate the role that *O. cornifrons* gender plays in mite dispersal.

Materials and Methods

Developing observation bee nests. To observe activity of *C. krombeini* inside bee nests, observation nest blocks were developed. A total of six nests were made from white pine timber blocks (14 cm × 9 cm × 2 cm) by carving the nests with a router (Fig. 1). Transparency film (PP2500 plain transparency film, 3M, St. Paul, MN) was attached to the top of the block using tacky glue (Aleene's Original Tacky Glue, Duncan Enterprises, Fresno, CA). The transparency film allowed us to examine inside the nests and prevented mites from dispersing between nests in a block. When blocks were brought in from the field any blocks which had warped allowing mites to move between nests were discarded.

Within-nest distribution of O. cornifrons gender. Observation nest blocks were placed in an apple orchard in Lone Pine, PA (N 40.098838, W -80.183051), and at the West Virginia University (WVU) Organic Farm in Morgantown, WV (39.644261, -79.938852) in early April, 2010. In September 2010, three nest blocks from Lone Pine and two nest blocks from the WVU Organic Farm were randomly selected and brought to the entomology laboratory at WVU. The position of all *O. cornifrons* cocoons inside each nest was recorded and each cocoon was dissected with two perpendicular incisions to determine the sex of individual *O. cornifrons*. The relationship between *O. cornifrons* gender distribution and their location inside each nest was determined using regression analysis (PROC REG, SAS Institute 2008).

Within-nest distribution of C. krombeini. A total of 50 observation nest blocks were placed in a blueberry orchard in Independence, WV (N 39.469902, W -79.934651) in early April, 2009. The blocks were brought back to the laboratory in early August, 2009. Every nest cell was thoroughly checked through the transparency film for the presence of *C. krombeini*. The presence or absence of pollen and *O. cornifrons* cocoons was also noted for each nest cell.

Blocks contained 107 *O. cornifrons* nests of which 89 contained some number of mites (> 83% nest infestation). Relationship between distribution of *C. krombeini* and their location inside each nest was determined using regression analysis (PROC REG, SAS Institute 2008).

Determining if C. krombeini occurs in cocoons of O. cornifrons. To determine if *C. krombeini* can inhabit the inside of *O. cornifrons* cocoons, 100 *O. cornifrons* nests were observed for *C. krombeini* infestation. These nests were made in cardboard tubes (7.9 mm in diameter, 0.8 mm in thickness, and 152 mm in length; Jonesville Paper Tube Co., Jonesville, MI) in Independence, WV. The paper tubes were opened by making two cuts running from the nest entrance to the rear of the nest with a rotary tool (Dremel, Racine, WI). Cocoons were removed from *C. krombeini*-infested cells and cut open. Gender was determined for *O. cornifrons* and presence or absence of mites inside the cocoon was recorded. A total of 20 cocoons containing ten male and ten female *O. cornifrons* were examined.

Results

Within-nest distribution of O. cornifrons gender. We found that females were more frequently located within the inner part of the nest and males were found toward the center of the nest. Distribution of female *O. cornifrons* was fitted with a logarithmic model, $y = -30.47 \ln(x) + 79.87$ (d.f. = 1,9; $F = 116.9$; $P < 0.001$; $r^2 = 0.91$), where y is the number of female *O. cornifrons* and x is the cell position within the nest (Fig. 2). Females were found in the innermost cell (cell 1), and after cell 10 they were no longer found. Distribution of male *O. cornifrons* was fitted with a second order polynomial model, $y = -0.4531 x^2 + 7.573 x - 10.132$ (d.f. = 2,13; $F = 48.6$; $P < 0.001$; $r^2 = 0.88$), where y is the number of male *O. cornifrons* and x is the cell position within the nest (Fig. 2). No male *O. cornifrons* were found in the innermost nest cell. Also, no bees were found in the outermost (i.e., vestibular) cell. Vestibular cells are thought to prevent other cavity nesting species from utilizing the left over space in the nest (O'Toole and Raw, 1999).

Within-nest distribution of C. krombeini. The average number of cells per infested nest was 9.5 ± 4.28, and the maximum number of non-vestibular cells in a nest was 15. Of the 847 cells found in the nests 429 (50.6%) contained mites, 705 (83.2%) contained pollen, and 367 (43.3%) contained *O. cornifrons* pupae. An average of 4.0 ± 3.77 infested cells were found in each nest. Because only four nests had 15 cells, data on cell 15 was omitted from the regression analysis. Mite distributions followed negative linear trends indicating *C. krombeini* was more frequently present in the inner part of *O. cornifrons* nests (Fig. 3): $y = -4.070\,x + 75.585$ (d.f. = 1, 12; $F = 110.6$; $P < 0.001$; $r^2 = 0.90$), where y is the percentage of mite occurrence and x is the cell position within the nest. Of the four omitted cells one had mites present. Vestibular cells were included in the analysis unless they were open, and these cells rarely contained mites. Vestibular cells were considered open if the nest's final mud cap was missing or disturbed.

To determine how many *O. cornifrons* died, cells which contained mites and pollen but did not contain a pupa were assumed to be indicative of mite-induced *O. cornifrons* mortality. Cells that did not contain any traces of pollen were not included in the analysis because they would not have contained a pupa under natural conditions. This data was fit with a linear regression $y = -3.5484\,x + 55.164$ (d.f. = 1, 12; $F = 148.7$; $P < 0.001$; $r^2 = 0.93$) (Fig. 4), where y is the percentage of cells containing only mites and pollen and x is the cell position within the nest. The negative linear trend indicates that mites kill more *O. cornifrons* in the inner portions of the nest.

Determining if C. krombeini occurs in cocoons of O. cornifrons. In the ten female and ten male *O. cornifrons* cocoons observed from infested cells, no cocoons contained mites. This indicates that cocoons cannot be penetrated by the mites, and while eggs and larvae of *O. cornifrons* are susceptible to attack from *C. krombeini*, *O. cornifrons* pupae are not exposed to mite attacks.

Discussion

Trends in nest distribution of *O. cornifrons* and *C. krombeini* were similar, and mite induced mortality was found to occur more frequently in the rear of the nest. These results contradict previous observational data on mite distribution in *O. cornifrons* nests (Krombein 1962, Park et al. 2008). These trends are important for two reasons: 1) they indicate a hindrance on managed *O. cornifrons* population growth and 2) they indicate the potential for *C. krombeini* to negatively impact pollination. Both of these problems are caused by the loss of female *O. cornifrons.*

Because a single male *O. cornifrons* may inseminate multiple females, the loss of males should have less impact on annual population growth. Managers of *O. cornifrons* populations which are intended for commercial sale to growers should see reason to be concerned by the impact *C. krombeini* has on *O. cornifrons* females. Because *C. krombeini* more frequently impacts female *O. cornifrons*, and because female *O. cornifrons* are a limiting factor in offspring production, *C. krombeini* may have strong impacts on *O. cornifrons* population growth.

While it is known that male *O. cornifrons* may contribute to pollination, the contribution they make is insignificant compared to females (Bosch and Kemp 2002). When a female *O. cornifrons* is killed by *C. krombeini,* the capacity of an *O. cornifrons* population to pollinate is reduced. Furthermore, those female *O. cornifrons* not killed by *C. krombeini* may be reduced in size by the pollen provision consumption of *C. krombeini*. The reduction in size may increase brood mortality in *O. cornifrons* populations (Bosch and Kemp 2001).

Chaetodactylus krombeini distribution within *O. cornifrons* nests is most likely not a function of the mite's behavior, but of the bee's nesting biology. When *O. cornifrons* females emerge in the spring they carry loads of *C. krombeini* migratory hypopi (attached primarily to the bee's thorax via suctatorial plates). Once the female scouts and establishes a nest she starts nest construction from the back of the nest. At this time mites may drop off the female's body. As mud partitions are created the *C. krombeini* in the rear of the nest become imprisoned in these cells.

The implications for population growth and pollination hindrance found in this study make *C. krombeini* a more serious pest of *O. cornifrons* than previously understood. One method of controlling *C. krombeini* may be submerging cocoons in 0.007% endosulfan, as this method was used successfully for the control of *C. osmiae* (Krunic et al 2001). One problem with this method is that it requires the cocoons to be removed from the nest, which is time consuming. Other chemicals known to be effective for controlling *C. krombeini* include formic acid and wintergreen oil applied as fumigants (White et al 2009). A problem with using wintergreen oil is that direct contact can cause mortality in *O. cornifrons* (White et al 2009). In addition, delivering formic acid or wintergreen oil to the inner parts of *O. cornifrons* nests is difficult to accomplish with current management methods. Another *C. krombeini* control method, which requires no chemicals, is to heat *O. cornifrons* nests for 40 days at 32 °C when the bees are in summer diapause. This method takes advantage of the fact that *O. cornifrons* becomes very heat tolerant during its summer diapause, while the mites are still susceptible to high temperatures (Yamada

1990). This method may be the most promising for controlling *C. krombeini* populations, but it does not kill the mites in the early spring which may impact developing *O. cornifrons.*

Future work should focus on more efficient ways of controlling *C. krombeini*, especially in regard to a pesticide delivery method. Additional research investigating the difference in population growth between mite free and mite infested populations would be valuable for *O. cornifrons* population management practices.

Acknowledgements

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Fig. 1. Observation nest block design for observing *O. cornifrons* and *C. krombeini*. The transparency film window was removed from this observation nest before the picture was taken.

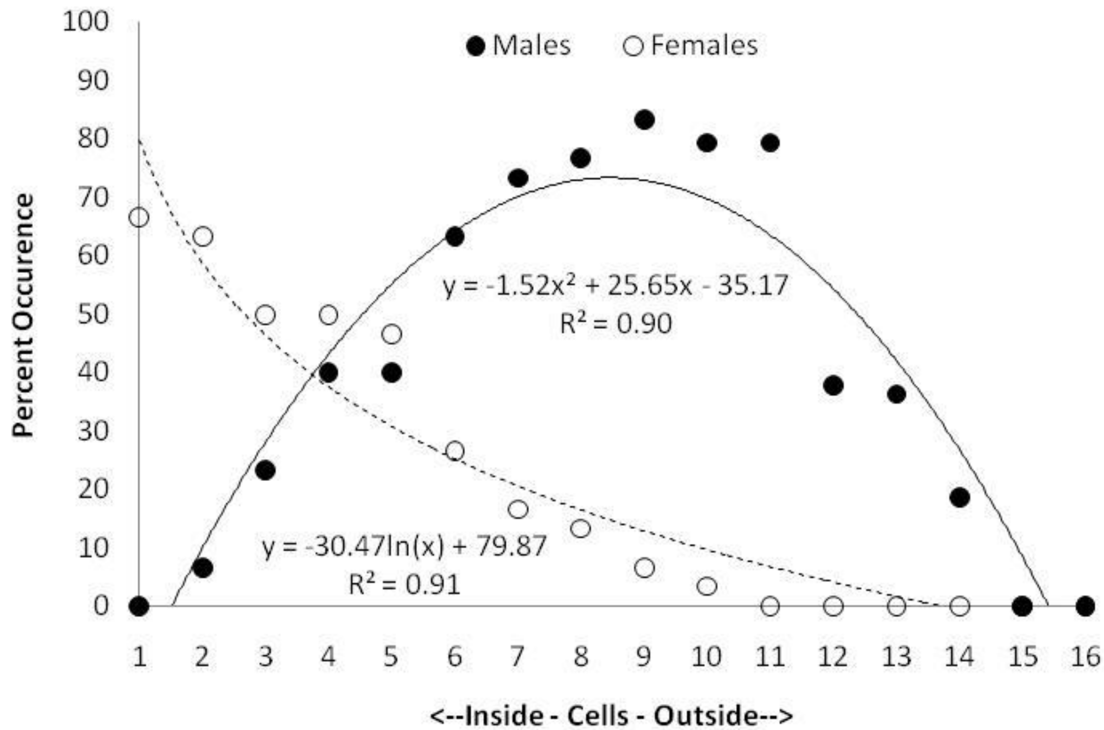

Fig. 2. Distribution of developing males and females in *O. cornifrons* nests.

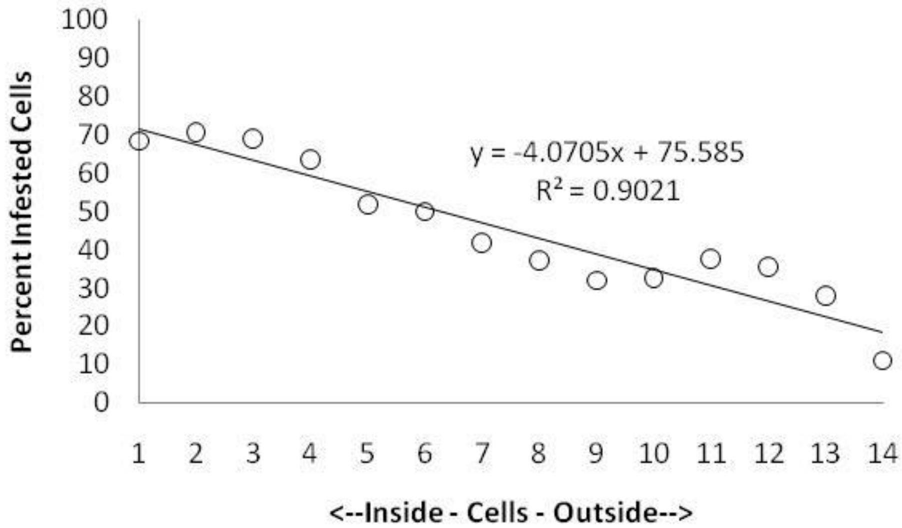

Fig. 3. Distribution of *C. krombeini* in *O. cornifrons* nests.

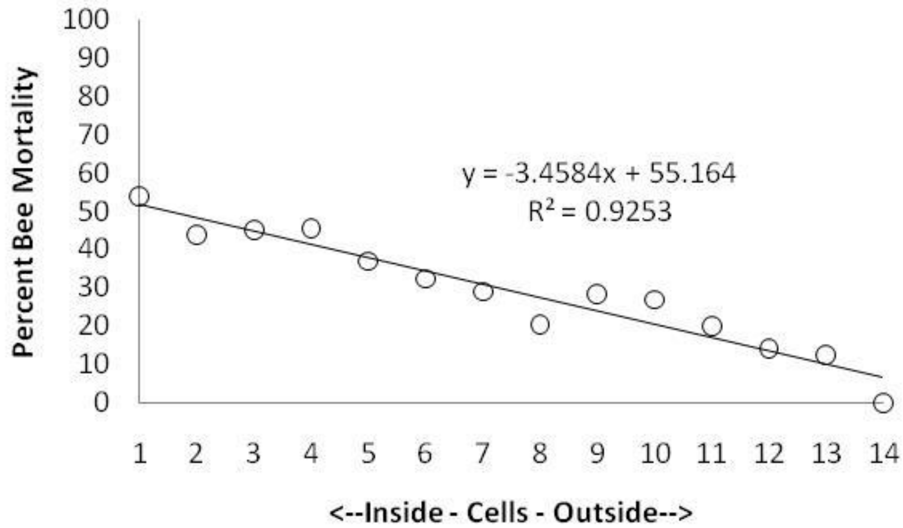

Fig. 4. Distribution of mite induced mortality in *O. cornifrons* nests. Cells which contained mite and pollen in the absence of bees were considered indicative of mite induced bee mortality.

Chapter 3: Nesting Activity and Behavior of *Osmia cornifrons* (Hymenoptera: Megachilidae) Elucidated Using Videography

Abstract *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae) is utilized as an alternate pollinator to *Apis mellifera* L. (Hymenoptera: Apidae) in early season fruit crops, most notably in apple production. While previous studies have described the nesting biology of *O. cornifrons*, those studies employed methods which may have had behavior altering effects on *O. cornifrons.* This study investigated nesting activities of *O. cornifrons* and described associated behaviors. *Osmia cornifrons* nesting activity was recorded by using a digital video recorder with infrared cameras. Behavior of ten female *O. cornifrons* was observed and the number of nesting trips per hour was recorded. Trends in daily activity were determined with regression analysis and chi square analysis was used to determine if *O. cornifrons* spent a greater amount of time performing certain activities. The percentage of time required to gather nesting resources and complete nest construction activities was recorded from the video footage. Pollen gathering was the most time consuming gathering activity, requiring 221.6 ± 28.69 min and cell provisioning was the most time consuming within-nest activity, requiring 28.9 min ± 3.97 min. Behavioral observations are described for scouting behavior, preliminary plug construction, cell provisioning, cell partitioning, oviposition, grooming behavior, resting behavior, sleeping behavior, fighting behavior, nest-searching behavior, nest repair, and nest supersedure. *Osmia cornifrons* activity was found to be related to time of day, temperature, and precipitation. This information is valuable for enhancing current management practices of *O. cornifrons.*

Key words: Japanese hornfaced bee, solitary bee, mason bee, nesting biology, videography

Pollination services are both economically valuable (Morse and Calderone 2000) and essential to many crop production systems (Klein et al. 2007). With colony collapse disorder and various pests threatening the honeybee *Apis mellifera* (Johnson 2010), and issues such as habitat fragmentation and pesticides threatening wild pollinators (Kearns et al. 1998), considerations for alternate pollination strategies by effectively managing solitary bees have become more relevant to agricultural production (Torchio 1990).

Osmia cornifrons Radoszkowski (Hymenoptera: Megachilidae) is an important pollinator of rosaceous fruit crops such as apple and pears. *Osmia cornifrons* has been managed in Japan for apple pollination since the 1940s and was introduced into the U.S. for pollination in 1977 (Batra 1978). Additionally, *O. cornifrons* is being used for orchard pollination in Korea and China (Lee et al 2008, Da-Yong and Long-Shi 2007). *Osmia cornifrons* has been shown to be up to 80 times more effective at pollinating apples than *A. mellifera* (Maeta and Kitamura 1981) and has several benefits over *A. mellifera* such as flower constancy and consistent anther contact (Batra 1982). Despite these benefits *O. cornifrons* remains an underutilized pollinator in the United States.

Understanding the nesting biology of *O. cornifrons* is important for management of the bees for growers, population managers (i.e., those who sell the bees to growers), and researchers. For example, by understanding *O. cornifrons* nesting biology, one can select release sites where *O. cornifrons* has access to adequate resources. Understanding the limiting factors of *O. cornifrons* activity, such as temperature thresholds, allows one to predict if *O. cornifrons* will be pollinating on a given day of the blooming season. In addition, knowing the nesting biology of *O. cornifrons* provides growers and researches with insights into the biology of other *Osmia* bees such as *O. lignaria,* a managed solitary bee pollinator in the United States.

Observing nesting behavior of solitary bees such as *O. cornifrons* can be challenging because their nests do not easily permit observation of bee activities inside the nest. Despite this challenge, several aspects of *O. cornifrons* nesting biology have been described previously (Yamada et al. 1971, Lee et al. 2010). Yamada et al. (1971) described nesting behaviors of *O. cornifrons* including cell provisioning, mud wall partitioning, and the time required to gather pollen and mud by utilizing glass tubes wrapped in paper as artificial nests. The paper could be removed from these glass tubes after the bee entered, which permitted *O. cornifrons* nesting activities to be observed. A major disadvantage of using glass tubes is that *O. cornifrons* could be disturbed by a sudden and unnatural increase in light levels in the innermost portion of the tube when the paper is removed from the glass tube; Lee et al. (2010) noted that luminance is an important factor affecting *O. cornifrons* activity. In addition, the presence of visible observers has been found to alter the frequency of activities in some insects, such as damselfly nymphs (Baker and McGuffin 2007).

This study investigated the nesting biology of *O. cornifrons* and described in detail the behaviors associated with nesting activities. There were four objectives in this study: (1) developing an unobtrusive and novel method to observe the nesting behavior of solitary bees, (2) investigating the factors that affect *O. cornifrons* activity levels, (3) determining how much time is allocated to gathering nesting resources and constructing the nest, and (4) describing the behaviors that occur during nest construction.

Materials and Methods

Experimental insects. *Osmia cornifrons* used in this experiment were acquired from a population that had been successfully established and managed for several years prior to the experiment on a blueberry farm in Independence, WV (N 39.46992, W -79.934651). In early November 2009, the bees were brought into the laboratory at West Virginia University (Monongalia County, WV) and placed into cold storage at 5 °C for overwintering. On May 9th, 2010, the bees were released in a residential area in Morgantown, WV (N 39.666871, W -79.965523) where a power source for prolonged video recording was readily available. Video data was recorded from 9 May 2010 - 1 June 2010. *Osmia cornifrons* has been successfully released and propagated on landscape plants in a city previously (Wilson et al 1999a).

Developing a protocol for observing O. cornifrons in-nest activity. To effectively record in-nest activities of *O. cornifrons,* three camera housings were constructed from white pine and masonite boards (Fig. 1). An opening was cut into the front of the box to allow six observation nest blocks (see Fig. 1 in chapter 2) to sit below the camera. A masonite board roof with a 3.5 cm × 4 cm × 1.5 cm block of white pine attached to the center held an infrared camera (The Hawk Eye Nature Cam, West Linn, OR) with the lens 44.3 cm from the bottom of the release box. The camera emits infrared light allowing for continuous observation without disturbing *O. cornifons.* Cameras were connected to a 4-channel digital video recorder (DVR) (Falco Model LX-4PRO, Falco Pro Series, Taiwan) to record continuously for the entire duration of *O. cornifrons* nesting activity. The three camera housings were placed next to a building facing south and were covered with plastic to help shelter the nests from rain. A fourth camera was set outside the three camera housings facing the nest entrances. This camera was used to observe *O. cornifrons* searching behaviors and to record the weather.

Determining factors affecting patterns of O. cornifrons activity. To determine the daily activity pattern of *O. cornifrons,* the number of trips initiated per hour was recorded for ten bees from 15 May 2010 – 21 May 2010. Data were taken from the start of nesting to six days later.

Only those trips where *O. cornifrons* gathered nesting materials (i.e. pollen or mud) were used to determine daily activity levels. The relationship between the number of trips *O. cornifrons* initiated and the time of day was determined using non-linear regression analysis (SigmaPlot 11, Systat Software, Inc., San Jose, CA).

To determine the effect of temperature on *O. cornifrons* activity levels, hourly climate data were obtained from a National Climate Data Center (NCDC) weather station located ca. 3.3 km from the study site. The weather station (i.e. MGTN RGNL-W L B HART FD AP located at N 39.642867, W -79.919947) is an automated surface observing system (ASOS) weather station which reports NCDC version 3 climate data; the weather station logged data every hour at the 53-min mark. Activity data (i.e. number of trips initiated per hour) from 18 May 2010 (sunny day) were used to correlate temperature with activity. Correlation between precipitation and bee activity from 16-17 May 2010 (rainy days) was analyzed to determine the effect of precipitation on *O. cornifrons* activity. Because the weather station reported trace precipitation (< 0.25 mm rain) without a numerical value, hours of trace precipitation are considered to be 0.025 mm of rain. Pearson product moment correlation was used for correlation analysis by using SigmaPlot 11.

Within-nest activity of O. cornifrons. To determine the amount of time spent by *O. cornifrons* on different in-nest activities, video data were logged for ten bees from 15 May 2010 – 21 May 2010: three bees from camera 1, three bees from camera 2, and four bees from camera 3. Within-nest activities included nest scouting, construction of preliminary plugs, cell provisioning, oviposition, cell partitioning, resting, grooming, sleeping, fighting, and other activities. For each activity, duration was measured as follows: (1) the start time was taken from the point at which the bee reached the area of the nest being constructed, (2) the stop time was taken from the point work activity ceased, (3) if the bee stayed in the nest for >20 seconds after building activity ceased, this extra time was recorded along with noting post work activities. A chi-square test determined if the time requirements of within-nest activity differed significantly.

Gathering activity of O. cornifrons. To determine the amount of time *O. cornifrons* requires for gathering nesting materials, time away from the nest was recorded for ten bees during every trip made. Trip times were recorded from the start of nesting (15 May 2010) until six days later (21 May 2010). Only trips in which nesting materials were brought back to the nest were used in data analysis. A threshold of 1 h was set for pollen gathering trips and 30 minutes for mud gathering trips. Any trips exceeding these thresholds were excluded from the data used in calculating the time requirements of gathering activities. The thresholds were not used to calculate the number of trips that *O. cornifrons* took to complete one part of a cell. This was done to account for trips in which *O. cornifrons* engaged in both gathering and non-gathering activity (e.g., resting), while still being able to report an accurate number of trips required to complete the provisioning and partitioning of a cell. A chi-square test determined if time requirements of gathering activity differed significantly.

O. cornifrons behaviors. To describe behaviors associated with *O. cornifrons*, nesting behavior of 30 *O. cornifrons* was observed from the time nesting was initiated (15 May 2010) until nesting ceased or six days later (21 May 2010), whichever came first. Behaviors were divided into nesting behaviors and non-nesting behaviors. Any behaviors performed during nest constructing activities were considered nesting behaviors and all other behaviors were considered non-nesting behaviors. Nesting behaviors included scouting behavior, preliminary plug behavior, cell provisioning behavior, oviposition behavior, and cell partitioning behavior. Non-nesting behaviors included grooming behavior, resting behavior, sleeping behavior, fighting behavior, nest-searching behavior, nest repair, and nest supersedure. Additionally, abnormal behaviors that did not fall under any of the listed categories were also recorded.

Results

Activity patterns of O. cornifrons. Data of daily nesting activity was fitted with a second order polynomial trend (Fig. 2): $y = -0.0728x^2 + 1.9543x - 10.442$ (d.f. = 2,13 ; $F = 33.30$; $P < 0.0001$; $r^2 = 0.86$), where y is the number of trips initiated per hour and x is time of day. Daily activity was tested for normality using the Shapiro-Wilk normality test and was found to be normally distributed (W = 0.9053 ; $P = 0.1346$; $\alpha = 0.05$). Variance of daily activity data was constant when disregarding the time of day based on the constant variance test ($P = 0.3642$). All activity occurred between 7:00 am and 8:00 pm, and the most trips initiated per hour occurred between 10:00 am and 6:00 pm. *O. cornifrons* was not active on days when it rained. Individuals responded to rain by staying in their nests and occasionally walking to the nest entrance and looking out, but not exiting the nest.

Results of the Pearson product moment correlation test showed that activity and temperature were significantly correlated (n = 24; $\rho = 0.856$; $P < 0.0001$). The positive correlation coefficient indicates that *O. cornifrons* activity increased with temperature (Fig. 3). *O. cornifrons* were not active below temperatures below 13.9 °C, and were not active on rainy days (Fig. 4).

Within-nest and gathering activities of O. cornifrons. The average total duration of labor required for cell completion (i.e. pollen provisioning, oviposition, and mud wall partitioning) was 50.6 min ± 6.45 min, and average time to complete the preliminary plug was 26.7 min ± 2.50 min. Provisioning the cell took the most total time, requiring 28.9 ± 3.97 min (i.e. 57% of the total time to complete a cell). Building the mud-wall partition required 19.9 ± 1.80 min (i.e. 40% of the total time to complete a cell). Oviposition required only 3% of the total time to complete a cell, requiring 1.7 ± 0.68 min. Time allocated to provisioning, partitioning, and oviposition were found to be significantly ($P < 0.0001$) different (Table 1).

The total time required for *O. cornifrons* to gather pollen and mud for one cell was 254.8 ± 36.75 min, and gathering mud for a preliminary plug required 44.9 ± 13.73 min. Gathering pollen took 221.6 ± 28.69 min with an average of 19.8 trips. Gathering mud for the cell partition took 33.25 ± 8.05 min with an average of 11.5 trips. Time allocated by *O. cornifrons* to gather pollen was significantly greater ($P < 0.0001$) than that to gather mud (Table 1).

Nesting behavior of O. cornifrons. Most nesting behaviors were distinct and consistent throughout the recorded video. Scouting behavior was the most variable behavior observed. When *O. cornifrons* searched for nests, they entered empty nests and moved to the back of the nest. Then they performed a series of forward and backward movements accompanied by turning upside down and left to right, inspecting the nest thoroughly. Finally, they turned and left the nests, occasionally coming back and performing these behaviors again.

During preliminary plug activity *O. cornifrons* focused on plugging the upper edges of the nest, where the transparency film was attached to the observation block. During many of these trips *O. cornifrons* moved back and forth repeatedly. This is likely a method used by the bees to measure distance (Yamada et al. 1971). They used their middle legs for support by holding them out perpendicular to their bodies and grasping the sides of the nest. Then, while holding mud with their mandibles, they bent their abdomen up until the apex of the abdomen was nearly in contact with the mandibles. Then, moving backwards, the mud ball was spread like a paste onto the nest surface. Use of the abdomen for nest building only occurred during the preliminary plug activity, and only when the corner formed by the nest and the transparency film was being plugged.

Cell provisioning started with mandibulating. *Osmia cornifrons* females approached the rear of the nest where the pollen ball was being made. Then they manipulated the pollen ball with their mandibles by either "pecking" at the pollen ball or pushing the pollen ball with the mandibles, using them like a shovel. During this time nectar from the crop was added to the pollen ball. After mandibulating the pollen ball, they turned in the nest and backed up so that their abdomen was over the pollen ball. Then they scraped the pollen from their scopa with their hind legs.

Oviposition behavior looked deceptively similar to cell provisioning behavior. Before oviposition *O. cornifrons* mandibulated the pollen ball, and then turned to oviposit. The primary difference in behavior between oviposition and provisioning was that the abdomen moved vigorously during nest provisioning, but it was very still during oviposition.

Osmia cornifrons females started building a mud wall partition by creating a mud ring around the inner circumference of the nest. Building the ring usually took several trips. Then they spread mud around the ring in concentric circles. Once there was just a small opening left in the ring they placed mud in the hole and then rotated their entire body several times with their face seemingly directly in contact with the mud wall.

Non-nesting behavior of O. cornifrons. Grooming behavior and resting behavior were the most commonly observed non-nesting behaviors. An *Osmia cornifrons* female often groomed itself right after provisioning a cell. Grooming entailed using the front legs to clean off the antennae as well as shaking the abdomen back and forth and rubbing it with the hind legs, seemingly to clean the scopa before the next pollen load was gathered. Frequently *O. cornifrons* would groom itself as it made a hasty exit from the nest. Usually the process did not take more than 20 sec to complete and did not slow down nest building activity. When grooming took more than 20 sec it was often followed by resting activity. *Osmia cornifrons* was considered resting when it was in the nest but was not performing any noticeable activity.

Sleeping behavior was defined as all activity that occurred between the final trip of one day and the first trip made the following day. Most frequently, after the final activity of the day, *O. cornifrons* would move about the nest, seemingly giving the nest a thorough inspection. After inspection, activity would cease for several minutes at a time, and if *O*. *cornifrons* moved it was only a few centimeters (ca. 4 cm). Finally activity would cease for several hours at a time, and if the bee moved it would most often simply turn sideways. The bees often slept sideways or upside down inside the nest. Many bees did not sleep in the nests at all, but returned the next day, presumably having slept in the field overnight. If it rained on the morning after a bee had been sleeping outside its nest, the bee did not return to its nest during the rainy day, but did return the following day. Some *O. cornifrons* also slept in empty nests. In the morning, as light entered the nest entrances, *O. cornifrons* would begin to move again. Most often, *O. cornifrons* moved a few centimeters (ca. 4 cm) then ceased movement again for some time. Eventually *O. cornifrons* would go to the nest entrance and look outside. Sometimes they left immediately, but more frequently they moved back into the nest and waited. On a few occasions a bee took flight only to return a few minutes later and resume a resting state.

There was only one observation of an attempt to repair a damaged nest. The nest became damaged when one corner of the transparency film cover became detached from the nest, and when that occurred one individual attempted to repair the uncovered area. First it spent a great deal of time inspecting the damaged area, then it began gathering mud and trying to patch the open area at the back of the nest. It made 13 trips and patched a large area of the opening, but was unable to successfully close it. After the bee's unsuccessful attempt to repair the nest, it seemed to abandon the nest.

Discussion

Solitary bee activity levels could be affected by time of day, temperature, or precipitation. *O. cornifrons* has previously been observed foraging as early as 6:10 am and as late as 6:00 pm (Matsumoto and Maejima 2010). The earliest time *O. cornifrons* became active in our study was 8:00 am and activity continued until as late as 8:00 pm. The actual difference between the starting activity times in this study and in Matsumoto and Maejima (2010) may be only one hour due to daylight savings time. Lee et al. (2010) reported that temperatures above 20 °C caused an increase in *O. cornifrons* activity. Our study showed that the minimum temperature for *O. cornifrons* to be active was 13.9 °C and bee activity increased with temperature. Matsumoto and Maejima (2010) observed *O. cornifrons* activity at temperatures as low as 10.7 °C. The difference in the observed minimum temperature for *O. cornifrons* activity in our study and Matsumoto and Maejima's (2010) observations may be due to temperature tolerance differences within the populations of *O. cornifrons* observed or caused by differences in light levels or other abiotic factors between the two study sites. In this study, *O. cornifrons* did not fly on rainy days, though this might be attributed to low temperatures on those days; the maximum temperature on the rainy day analyzed in this study was 14.4 °C which is 0.5 °C above the minimum temperature threshold for activity. *O. cornifrons* was most active on warm, sunny days. Therefore, *O. cornifrons* can be expected to be most active from 10:00 am to 6:00 pm on warm days (>13.9 °C) without precipitation.

The majority of time that *O. cornifrons* spent performing nesting activities was used for gathering pollen and provisioning the nest, which agrees with information reported by Lee et al (2010). The average number of cells in *O. cornifrons* nests is 9.5 (see chapter 2), and results of this study showed that the average number of trips to complete a cell was 31.3. This means that it takes an average of 297 trips for *O. cornifrons* to complete a nest, though this could vary as the nesting season progresses. While the amount of time required to gather pollen was found to be significantly different from the time required for *O. cornifrons* to gather mud, the number of trips required for the two activities were not found to be significantly different. Because the number of trips required was not significantly different, it can be assumed that a single pollen gathering trip requires more time to complete (i.e., the higher time requirement of pollen gathering is not an artifact of a higher trip requirement for nest provisioning).

In addition to observing *O. cornifrons* behaviors described previously, two unusual behaviors were observed that have not been described in detail previously. First, one case of nest supersedure was observed in this study. An *O. cornifrons* female had oviposited in the back of its nest and began building a mud wall. For an unknown reason the bee seemed to abandon the nest, but may have been a victim of predation. Two days later, another bee entered the nest, destroyed the original egg, laid a new egg, and finished the mud wall. Second, individual *O. cornifrons* females showed some inadequacy in locating their own nest. Many times a female would enter a nest and immediately turn around and leave, then enter an adjacent nest. Sometimes a bee would enter two or three nearby nests before finally entering its own nest. When *O. cornifrons* entered a nest occupied by another female *O. cornifrons,* an aggressive fighting behavior took place. During fighting *O. cornifrons* utilized its mandibles to fight off intruding *O. cornifrons*. Also during fighting, *O. cornifrons* would bend its abdomen forward putting its body in a C-shape. It was difficult to observe from the video footage if the abdominal behavior was being used for offensive or defensive purposes. The duration of fighting was usually several minutes, and most often the original bee displaced the intruder. On some occasions nest constructing activity was interrupted by intruding bees. When an interruption like this occurred, the bee failed to complete the activity it had been working on prior to the interruption, and instead inspected the back of the nest and began the behavior all over again.

Osmia cornifrons behaviors described by Yamada et al. (1971) were found to be similar to those observed with the video. This indicates that *O. cornifrons* is likely not disturbed by using glass tubes to view their nesting behaviors. Still, the video method has advantages over using glass tubes which make it a valuable tool for studying solitary bees: (1) it does not require the physical presence of the researcher, (2) it can gather data on activities non-stop for weeks at a time which is nearly impossible to do otherwise, (3) video footage can be rewound, sped up, or slowed down as needed to analyze the data, (4) video footage can be archived and used in other studies. The biggest disadvantages of using the video are the power requirements to run the equipment, the time consuming nature of watching video footage, and that the technology can sometimes fail.

This study showed that *O. cornifrons* was most active between 10:00 am and 6:00 pm, and they spent most of their active time gathering pollen and provisioning their nests. It also showed that temperature and precipitation have strong effects on the activity of *O. cornifrons.* This information is important as it can be used to avoid pesticide application during *O. cornifrons* peak activity. Results indicate that pesticide application should be avoided between the hours of 11:00 am–4:00 pm to reduce *O. cornifrons* exposure to pesticides. Ideally, pesticide application should not occur between 7:00am and 8:00pm, but this is an impractical recommendation for most growers. Furthermore, observations of *O. cornifrons* sleep habits indicate that they frequently sleep in outside the nest, which means that it may be impossible to completely avoid affecting *O. cornifrons* with pesticide sprays. Previous management practice has been to place *O. cornifrons* in the field seven to ten days before crop bloom (Batra 1982). From the data gathered it is recommended that growers wait for several days of temperatures above 13.9° C so that the bees can maintain activity after emergence. Releasing *O. cornifrons* in colder weather than this will hinder their ability to perform pollination duties, and may cause the bees harm as they cannot forage in the cold temperatures.

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Table 1. Comparison of time spent and trips made for different *O. cornifrons* activities. Maximum effort is the activity which required the greatest amount of time or number of trips

Activities Compared (n = 10)	Maximum Effort	Observed Value	χ^2	d.f.	*P*
Time provisioning vs. partitioning vs. oviposition*	Provisioning	1,736 sec	1,368.18	2	< 0.0001
Time gathering pollen vs. mud*	Pollen	13,296 sec	8,382.55	1	< 0.0001
Trips made to gather pollen vs. mud	Pollen	20 trips	2.00	1	0.1573

*Significant ($P < 0.05$) differences were found in the time allotted to these activities.

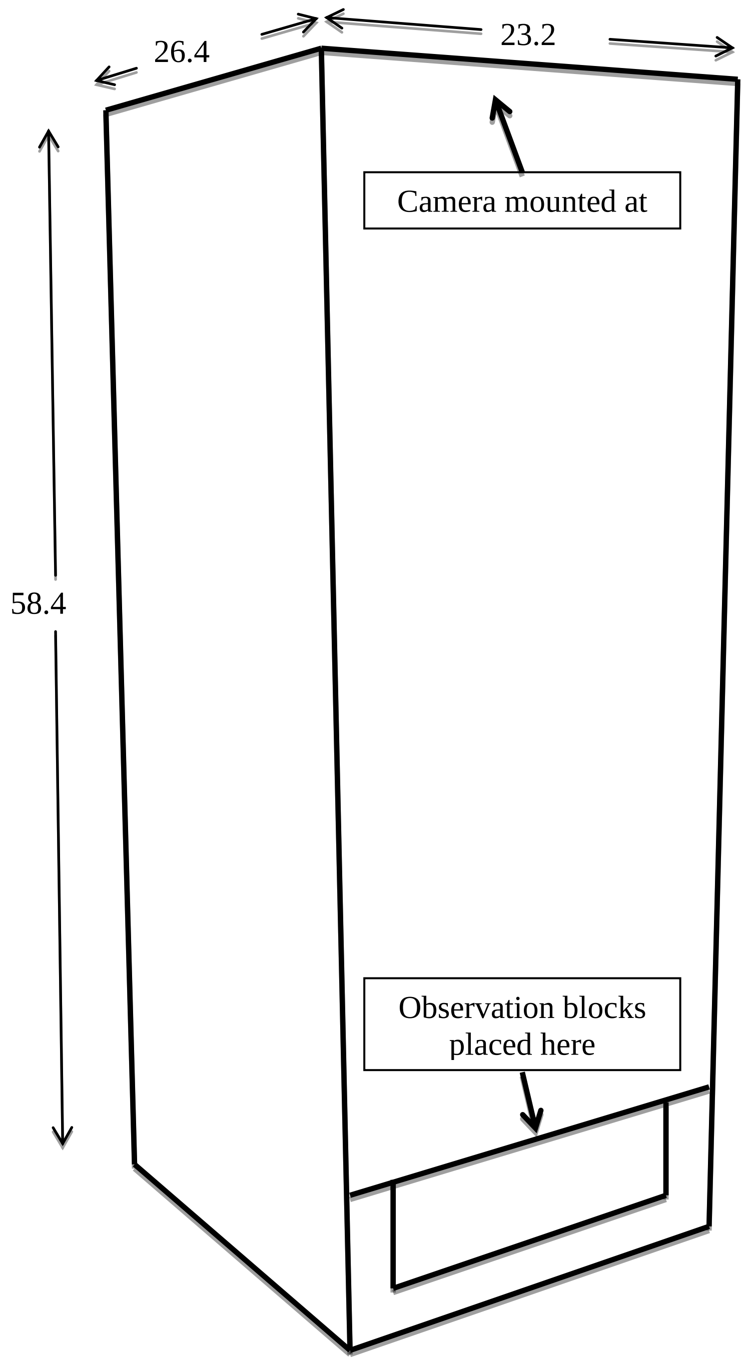


Fig. 1. Diagram of camera housings for recording within-nest behavior of *O. cornifrons*. The frame was made of white pine timber and the walls were made from masonite boards. The camera was mounted to the lid and faced down toward the observation blocks. A small groove was cut into the back of the frame to allow space for the camera cord.

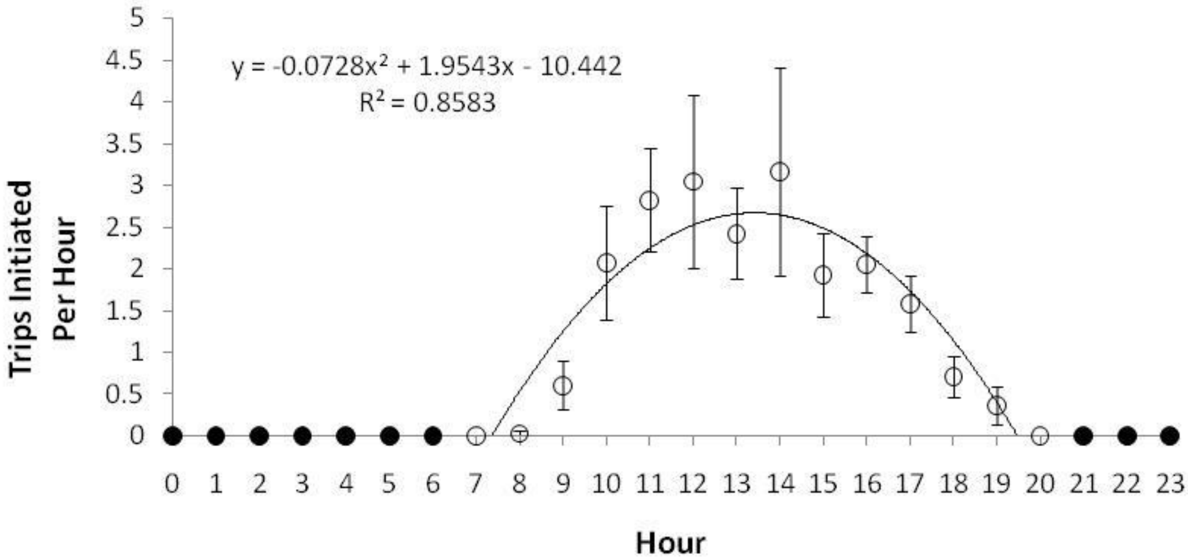


Fig. 2. The average number of trips taken per hour by *O. cornifrons*. Hour 0 is 12:00am and hour 23 is 11:00pm. Error bars indicate standard error. Only the open circles were used to determine the regression equation.

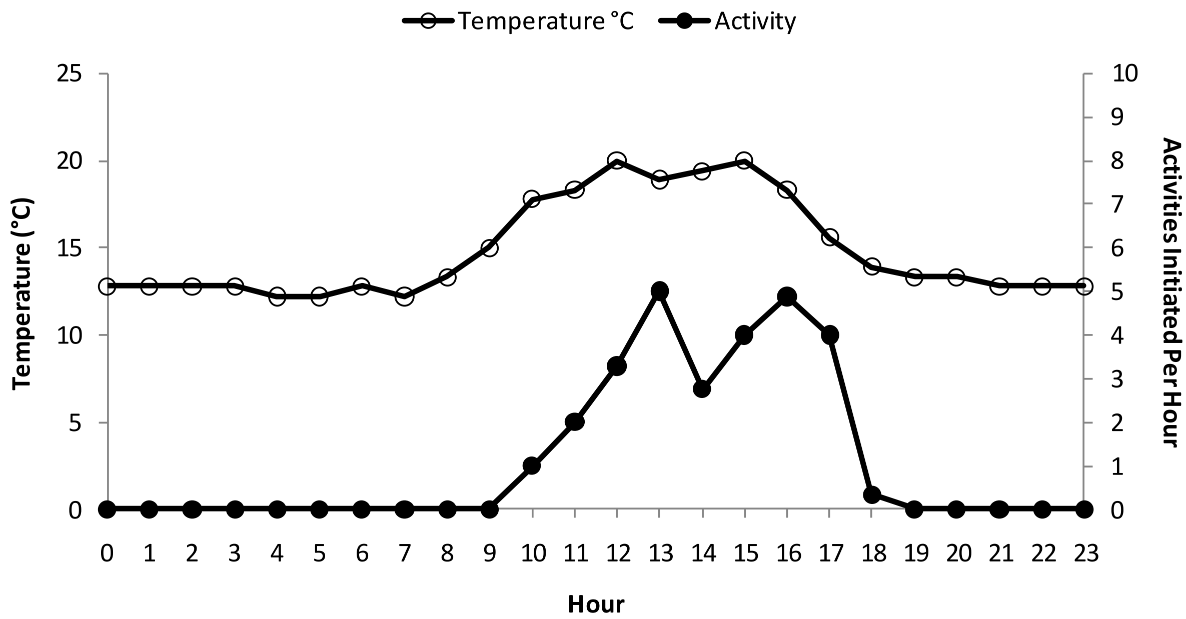
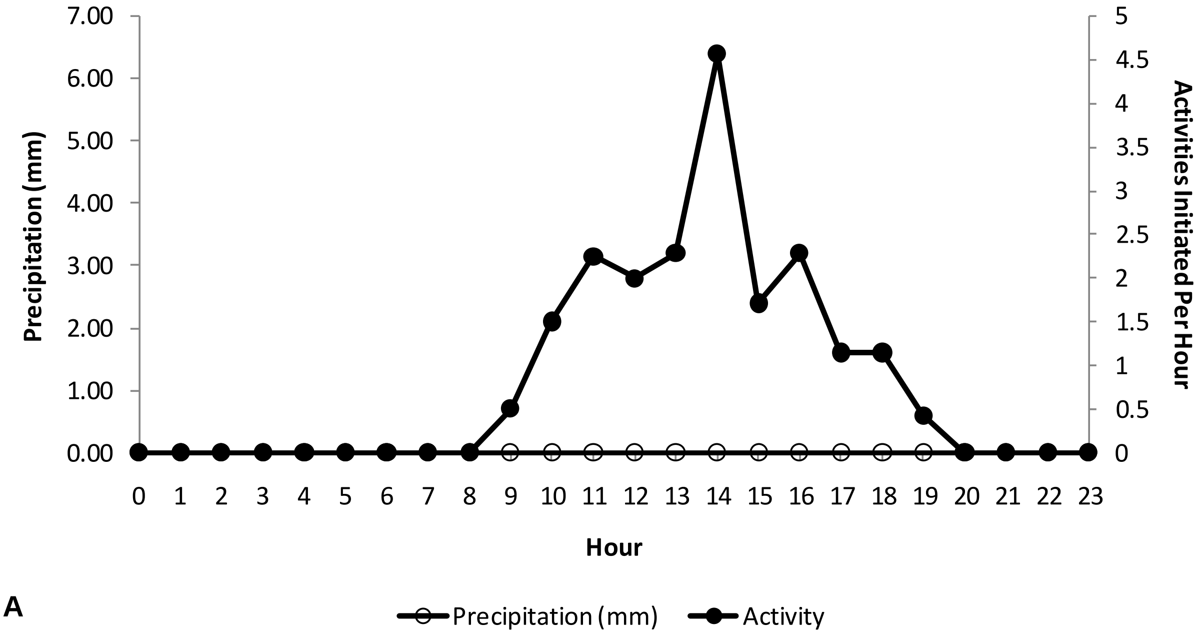
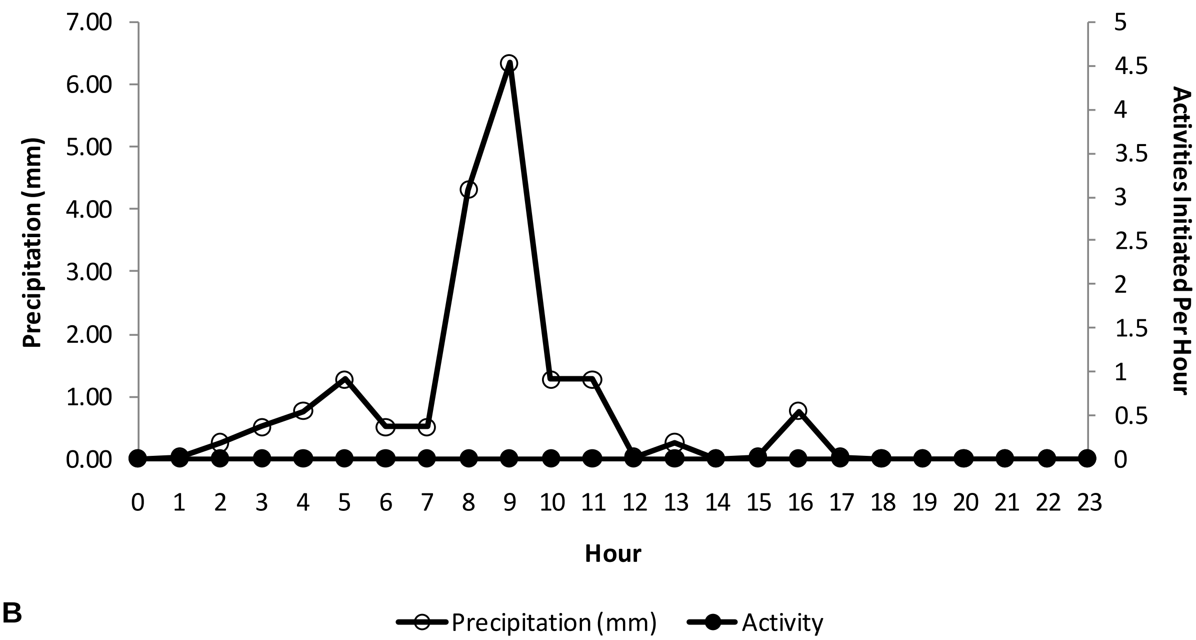


Fig. 3. Relationship between *O. cornifrons* activity and temperature. Hour 0 is 12:00 am and hour 23 is 11:00 pm.



A



B

Fig. 4. Relationship between *O. cornifrons* activity and precipitation on a day without rain (16 May 2010) (A) and a day with rain (17 May 2010) (B). Hour 0 is 12:00 am and hour 23 is 11:00 pm.

Chapter 4: Conclusion

Pollination, a vital ecosystem service, is important to both humans and wildlife. Currently there are several major threats to *A. mellifera*, the most important and economically productive pollinator. *Osmia cornifrons* is an alternative pollinator with great potential for use in orchard pollination. Understanding *O. cornifrons* nesting biology should be a valuable part of designing effective and efficient management strategies for its implementation as an alternative pollinator.

This study (Chapter 2) showed that female *O. cornifrons* suffer higher mortality than male *O. cornifrons* as a result of exposure to *C. krombeini.* The implications of this are two-fold: (1) *O. cornifrons* pollination potential is greatly reduced by high *C. krombeini* mite infestation, and (2) *O. cornifrons* annual population increase is greatly reduced by high *C. krombeini* infestation. Though there are management techniques (disposing of old nest material) and chemical treatment options (endosulfan and formic acid) to help control *C. krombeini*, there is currently no method to control mites in the nest as it is being constructed in the early spring. Since this is the time that developing *O. cornifrons* larvae are affected by *C. krombeini*, future work should be directed to develop a control method for *C. krombeini* during *O. cornifrons* nest building time in the early spring.

The results of this study (Chapter 3) indicated that *O. cornifrons* ability to build a nest is limited by time, temperature, and precipitation. Temperature seemed to have the greatest impact on *O. cornifrons* activity. This is important for two reasons: (1) growers can predict when *O. cornifrons* will be actively pollinating allowing them to determine if they will receive adequate pollination from *O. cornifrons* alone, and (2) the predictability of *O. cornifrons* activity allows growers to plan pesticide applications to avoid negatively affecting *O. cornifrons* during foraging activity in the field. This study also showed that videography and simple tools could easily become an effective and unobtrusive method for studying nesting biology in solitary bees and wasps. The methods used in this study can be applied to other potential alternative pollinators to gain similar insights into their nesting biology, possibly turning a potential alternative pollinator into a fully managed alternative pollinator.

In conclusion, pollination services are vital for forage crop production, wildlife, and human survival. Without these services our food supply would be reduced by 33% (Mader 2011). *O. cornifrons* has been shown to be an effective pollinator of several early season fruit crops, such as apple and blueberry, but is currently not utilized to its maximum potential. By describing the nesting biology of *O. cornifrons* and revealing the role of one of its major pests, *C. krombeini,* this research has taken one step forward toward better management practices for alternative pollinators.

References Cited

Mader, E., M. Shepherd, M.Vaughan, S. H. Black, and G. LeBuhn. 2011. Attracting native pollinators: protecting North America's bees and butterflies. Storey Publishing, North Adams, MA.