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Implications for the use of *Osmia cornifrons* (Hymenoptera: Megachilidae) as pollinators

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Implications for the Use of *Osmia cornifrons* (Hymenoptera: Megachilidae) as Pollinators

Joseph Brent White

Thesis submitted to the College of Agriculture, Forestry and Consumer
Sciences at West Virginia University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Plant and Soil Sciences – Entomology

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Morgantown, West Virginia
2009

Keywords: Pollination, degree day, bioassay, hornfaced bee, hairy-footed mite

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ABSTRACT

Implications for the Use of *Osmia cornifrons* (Hymenoptera: Megachilidae) as Pollinators

Joseph Brent White

The Japanese hornfaced bees, *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae), is a key pollinator of spring blooming fruit crops such as apple, pear and blueberry. To maximize pollination efficiency, two implications must be considered. First, diapause termination of overwintering *O. cornifrons* adults needs to be synchronized with the bloom of the target trees because adult bees are active as pollinators for only a few weeks. Patterns of temperature-dependent diapause termination of *O. cornifrons* were modeled and simulated with linear and non-linear regression analyses. Results of the study indicated the upper and lower developmental thresholds, as well as the optimum temperature for diapause termination. This study provides a framework for manipulating diapause termination of *O. cornifrons* adults to synchronize their emergence with pollination periods of target crops. Second, *O. cornifrons* is associated with the hairy-footed mite, *Chaetodactylus krombeini* Baker (Acari: Chaetodactylidae), which can impede the propagation of *O. cornifrons*. This study was conducted to evaluate formic acid and wintergreen and determine if these compounds would effectively control *C. krombeini*. This study was conducted in two bioassays: to investigate *O. cornifrons* tolerance of formic acid and wintergreen and to find the lethal concentration of formic acid and wintergreen needed for mite control. Results of this study showed that the time and dosage required to induce *C. krombeini* mortality with either formic acid or wintergreen was significantly less than the time required to produce *O. cornifrons* mortality. Estimates of the LC₅₀ and LC₉₀ for *C. krombeini* hypopi treated with wintergreen were ca. five times lower than those with formic acid, indicating that wintergreen was a more effective chemical that can control the mites. This study provides evidence that *C. krombeini* can be managed effectively with formic acid and wintergreen without inducing mortality in *O. cornifrons*.

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TABLE OF CONTENTS

Chapter 1	Introduction	
	Thesis Organization	1
	General Introduction	1
	Objective of Study	2
	Literature Review	2
	References Cited	5
Chapter 2	Temperature-Dependent Diapause Termination of <i>Osmia cornifrons</i> (Hymenoptera: Megachilidae) Adults	
	Abstract	11
	Materials and Methods	13
	Results	16
	Discussion	19
	Acknowledgements	21
	References Cited	21
Chapter 3	Bioassay of Potential Fumigants to Control <i>Chaetodactylus krombeini</i> (Acari: Chaetodactylidae) Associated with <i>Osmia cornifrons</i> (Hymenoptera: Megachilidae)	
	Abstract	32
	Materials and Methods	35
	Results	37
	Discussion	39
	Acknowledgements	41
	References Cited	41
Chapter 4	General Conclusion	
	Conclusion	49
	References Cited	50

LIST OF TABLES

Chapter 2	Temperature-Dependent Diapause Termination of <i>Osmia cornifrons</i> (Hymenoptera: Megachilidae) Adults	
Table 1.	Parameter estimate ($\pm$ SE) of the Weibull model used to estimate median time (d) for diapause termination of overwintering <i>O. cornifrons</i> adults at constant temperatures	24
Table 2.	Parameter estimate ($\pm$ SE) of the Weibull model to describe variability of diapause termination rate for the overwintering <i>O. cornifrons</i> adults against normalized time (d/median)	25
Table 3.	Parameter values ($\pm$ SE) and associated statistics for non-linear model (Rueda et al. 1990) for temperature-dependant diapause termination of <i>O. cornifrons</i> adults	26
Chapter 3	Bioassay of Potential Fumigants to Control <i>Chaetodactylus krombeini</i> (Acari: Chaetodactylidae) Associated with <i>Osmia cornifrons</i> (Hymenoptera: Megachilidae)	
Table 1.	Required hours to death of <i>O. cornifrons</i> adults with formic acid and wintergreen Treatments	44
Table 2.	Estimates of lethal concentration (LC ₅₀) of wintergreen (WG) and formic acid (FA) in <i>O. cornifrons</i>	45

LIST OF FIGURES

Chapter 1	Introduction	
Figure 1.	<i>Osmia cornifrons</i> nesting sites	8
Figure 2.	Generalized diagram of <i>O. cornifrons</i> life cycle	9
Figure 3.	Generalized diagram of <i>C. krombeini</i> development	10
Chapter 2	Temperature-Dependent Diapause Termination of <i>Osmia cornifrons</i> (Hymenoptera: Megachilidae) Adults	
Figure 1.	Device to capture <i>O. cornifrons</i> adults after emergence to prevent them from escaping or returning back to the nests.	27
Figure 2.	Temperature-dependant diapause termination of <i>O. cornifrons</i> adults fitting with non-linear model.	28
Figure 3.	Cumulative frequency (%) of development completion for <i>O. cornifrons</i> adults fit with Weibull function to the cumulative frequency of normalized age (day/mean development duration)	29
Figure 4.	Simulated temperature-dependent patterns of diapause termination of <i>O. cornifrons</i>	30
Figure 5.	Median time (d) required for diapause termination of overwintering <i>O. cornifrons</i> males and females at constant temperatures.	31
Chapter 3	Bioassay of Potential Fumigants to Control <i>Chaetodactylus krombeini</i> (Acari: Chaetodactylidae) Associated with <i>Osmia cornifrons</i> (Hymenoptera: Megachilidae)	
Figure 1.	Procedures of image analysis to count numbers of <i>C. krombeini</i> hypopi using Adobe Photoshop CS3 Extended	46
Figure 2.	Mortality of <i>O. cornifrons</i> adults when wintergreen was applied directly to the bee.	47
Figure 3.	Survivor analysis of <i>C. krombeini</i> hypopi when fumigated with (A) wintergreen and (B) formic acid at three different amount of application: 0 (control), 5, 10, and 20 μl per 56.6 cm^3	48

CHAPTER 1: INTRODUCTION

Thesis Organization

This thesis is organized into four chapters; Chapter 1 is a general introduction to the project and a literature review. Chapter 2 provides a framework for temperature dependent diapause termination of Japanese hornfaced bee, *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae) adults. Chapter 3 addresses possible control methods for the hairy-footed mite, *Chaetodactylus krombeini* Baker (Acari: Chaetodactylidae) associated with *O. cornifrons*. Chapter 4 provides a general conclusion for this study. This thesis was prepared according to the publication guidelines established by the Entomological Society of America.

General Introduction

Crop and fruit pollination is a \$15 billion industry each year in the United States (Morse and Calderone 2000). With recent events such as parasitic mites (Finley 1995), Africanized honeybees (Sammataro and Avitable 1998) and Colony Collapse Disorder (Stokstad 2007) contributing to the decline of honey bees *Apis mellifera* (L.) (Hymenoptera: Apidae), there is a need to consider alternate means of pollination (Adams 2001). *Osmia cornifrons* has been used successfully for the pollination of apple in Japan (Sekita and Yamada 1993), and since its introduction to the United States in 1976 (Batra 1998), it has been used for the pollination of spring blooming fruit trees (Batra 1998, Adams 2001) and vegetable crops (Wilson et al. 1999). Because the life cycle of the bee coincides well with blueberry flowering it is particularly attractive for blueberry pollination (West and McCutcheon 2005). Like other solitary hymenoptera, *O. cornifrons* is timid and rarely stings. In addition, it can be readily managed and propagated in commercial orchards (Batra 1998, Bosch and Kemp 2001). However, there are

several pests associated with *O. cornifrons* and *C. krombeini* is a major pest infesting the nesting sites of *O. cornifrons*. The large mite populations compete with developing larvae for pollen provisions, which leads to increased larval mortality causing reductions in *O. cornifrons* populations.

Objectives of Study

The goal of this study was to investigate two major problems facing *O. cornifrons* management and to develop possible solutions to utilize the bee for its maximum pollination potential. The objectives of this research were:

1. To model the relationship between temperature and diapause termination of *O. cornifrons* adults (Chapter 2).
2. To develop methods of controlling the pollen mite *C. krombeini* infesting *O. cornifrons* nesting sites (Chapter 3).

Literature Review

Life History of *O. cornifrons* and *C. krombeini*. *Osmia cornifrons* is native to Japan and introduced to the United States in 1973 (Batra 1998). It undergoes one generation per year. Adult bees spend only a few weeks actively engaged in pollination, which is typical for most solitary hymenopterans allowing the bee to spend only a few weeks actively engaged in pollination. Most of the life cycle is spent undergoing development and diapause as adult bees inside nests. *Osmia cornifrons* adults emerge from their nests in early April, mate immediately, and females then begin laying eggs and foraging. Female bees make pollen provisions for the eggs that have been laid inside nests. Adults die in early June and the eggs hatch and begin to

develop while feeding on the pollen provisions. Immature *O. cornifrons* complete larval and pupal development and remain in cocoons as adults inside nests from October until April of the following year (Fig. 1).

Under natural conditions, *Osmia* spp. (e.g., *O. cornifrons* and *O. lignaria*) can be found nesting in reeds. In commercial settings, however, it is commonly managed in small cardboard tubes used as nests (Batra 1998, Bosch and Kemp 2001). *Osmia cornifrons* is gregarious and the tubes facilitate their use in a targeted area. *Osmia cornifrons* constructs cells with mud partitions within the tubes which can be grouped together in bundles (Fig. 2). While this makes management easier it also provides an attractive habitat for natural enemies and pests such as parasitic wasps and mites (Krunić et al. 2005, Park et al. 2009).

One pest associated with *O. cornifrons* is *C. krombeini*, and it is of particular importance with respect to *O. cornifrons* propagation and management (Park et al. 2009). *Chaetodactylus krombeini* is a pollen mite that infests the tubes in which *Osmia* spp. nest (Bosch and Kemp 2001). The life cycle of *C. krombeini* has several stages including immature, reproductive, migratory, and cyst stages. As an immature protonymph, the mite can either continue to develop into the reproductive stage, change into the migratory (hypopus) stage or become dormant in the cyst stage. *Chaetodactylus krombeini* hypopi enters nesting tubes in a variety of ways. In general, a major dispersal mechanism of the mite is through phoretic behavior. When *C. krombeini* hypopi hitchhike on *O. cornifrons* adults, hypopi become entangled in the hairs of the bee. Park et al. (2009) found that *C. krombeini* hypopi were capable of walking from adjacent tube openings as well as through holes in tubes created by parasitic wasps. Once hypopi enter the tubes their population can increase to 170 to 5000 mites per cell in the nest (Park et al. 2009). *Chaetodactylus krombeini* populations are cleptoparasitic and compete with *O. cornifrons* larvae

for pollen provision in the tubes and have been reported to kill developing larvae (Bosch and Kemp 2001). Larvae that do survive may experience a reduction in size due to a decreased food supply during development (Bosch and Kemp 2001).

Modeling Temperature Dependent Diapause Termination. During the last several decades, temperature-dependent insect growth and development has been modeled with linear and non-linear regression (Wagner et al. 1984a and b). Such models provide predictions of insect life cycle (e.g. events, predicting emergence or peak population periods), which can be used for management of insects. Because diapause termination of *O. cornifrons* is also temperature-dependent (Yamada et al. 1971, Adams 2001) these regression approaches can be used to predict diapause termination of *O. cornifrons* adults. Linear regression can be used to determine the lower temperature threshold and non-linear regression can be used to determine the optimum and upper temperature thresholds. This study was conducted to provide a framework for manipulating *O. cornifrons* diapause termination so that growers can more effectively synchronize plant blooming period with flower visiting period of *O. cornifrons*.

Management of *C. krombeini*. Mites associated with honeybees have been effectively controlled with both formic acid and essential oils when used as fumigants (Amrine 2006, Amrine and Noel 2006). This method of fumigation is attractive as a control method because it does not disrupt the honeybees in the hive and the treatments are accepted as organic methods of control. Management of *C. krombeini* can be difficult because the mite has several different life stages, and is currently achieved both mechanically and chemically. Heat treatments have been used for *C. krombeini* control (Bosch and Kemp 2001). When nests of *Osmia* spp. infested with *C. krombeini* were exposed to high temperatures, the bees remained alive while the mites died. Another approach to control *C. krombeini* is the use of binder boards (i.e., boards with the

appropriate sized hole drilled through and then cut in half) allowing one to pry the two pieces of the board apart, gaining access to the cocoon inside (Strickler 2002). The binder boards can be used for *C. krombeini* control by opening the boards and removing the cocoons to separate those infested with *C. krombeini*. Cocoons that have been removed from binder boards also can be washed with a bleach solution (Strickler 2009). However, all of these methods may produce side effects. When chemicals are used, cocoons and nesting materials come into direct contact with the chemicals, which can cause chemical residue inside the nesting materials. Also, the physical removal of *O. cornifrons* from the binder boards generally results in releasing loose cocoons and may increase dispersal of pre-nesting female bees (Bosch and Kemp 2001); generally females leave their nesting tubes to feed for a few days and then return to lay eggs, while those females who have been released outside of their nesting tube have a greater chance of not returning to lay eggs.

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Figure 1: Grouping of cardboard tubes (left) used for *O. cornifrons* nesting sites and cross-section (right) of *O. cornifrons* nests. Note that the tubes closed with mud (left) indicate that nested tubes by *O. cornifrons*.

Figure 2: Life cycle *O. cornifrons*. Adult bees emerge from diapause in April and are active until June. Development occurs from June through October and adult bees undergo diapause until the following April.

Figure 3

Figure 3: Generalized diagram of *C. krombeini* potential development. Note that at the protonymph stage, the mite can assume one of three different forms including regular duetonymph, migratory duetonymph (hypopus), or dormant duetonymph (cyst).

CHAPTER 2: Temperature-Dependent Diapause Termination of *Osmia cornifrons* (Hymenoptera: Megachilidae) Adults

Abstract: Japanese hornfaced bees, *Osmia cornifrons* (Radoszkowski), are key pollinators of spring blooming fruit crops such as apple, pear and blueberry. To maximize pollination of the fruit crops, diapause termination of overwintering *O. cornifrons* adults needs to be synchronized with the bloom of the target trees because adult bees are active as pollinators for only a few weeks. This study was conducted to determine lower temperature thresholds (LTD), optimum temperatures, and required degree-day accumulation for diapause termination of *O. cornifrons* adults. Patterns of temperature-dependent diapause termination of *O. cornifrons* adults at seven temperatures (4, 12, 19, 27, 30, 36 and 43 °C) were modeled and simulated with linear and non-linear regression analyses. Results showed that the required degree-days for diapause termination of male and female *O. cornifrons* adults were 125.2 with a LTD of 8.91 °C, and 179.8 with a LTD of 8.60 °C, respectively. The optimum temperatures for diapause termination were 35.5, 30.0, and 36.0 °C for male, female, and both sexes combined, respectively. This study provides a framework for manipulating diapause termination of *O. cornifrons* adults to synchronize their emergence with pollination periods of target fruiting crops.

Keywords: pollination, degree day, hornfaced bee

With the recent decline of honey bee populations, *Apis mellifera* (L.) (Hymenoptera: Apidae) (Stokstad 2007) it has become necessary to find alternate and supplemental methods of pollination in the United States (Winfree et al. 2008). A potential substitute or supplemental pollinator is the Japanese hornfaced bee, *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae), which is native to Japan. *Osmia cornifrons* has been used as a key pollinator of spring blooming fruit trees such as apple and pear in the United States (Batra 1998, Adams 2001, Bosch and Kemp 2002, Matsumoto et al. 2008) since it was introduced from Japan in the 1970's (Batra 1998). West and McCutcheon (2005) reported the potential use for pollinating small fruit crops such as blueberry. Although *O. cornifrons* is a solitary bee nesting in reeds and holes in the field, it exhibits gregarious behavior when managed commercially in bundles of artificial nests such as cardboard tubes and wood blocks with holes (Batra 1998, Wilson et al. 1999, Park et al. 2009). Like many solitary hymenoptera *O. cornifrons* is timid and rarely stings, it can be readily managed and propagated in commercial orchards (Batra 1998).

The life cycle of *O. cornifrons*, which is typical for most solitary hymenopterans, poses a problem because *O. cornifrons* spends only a few weeks actively engaged in pollination. Most of the life cycle is spent undergoing development followed by diapause as adult bees inside nests. As a result of the short pollination activity of *O. cornifrons* adults, growers need to ensure that adult bees are active blooming occurs to maximize pollination. Adding to the complexity of synchronization, target crops may have different pollination periods. For example, blueberry can have different bloom schedules, from April to June, depending on the variety (Pritts and Hancock 1992). One way to synchronize emergence with bloom is to manipulate diapause termination of adult bees. Adults undergo diapause inside cocoons during the winter, and diapause termination is temperature-dependent (Yamada 1971, Adams 2001). Adults break diapause and emerge from

their cocoons once the required heat units (i.e. degree-days) are accumulated. Therefore, it would be possible to manipulate *O. cornifrons* adult diapause termination to synchronize it with pollination periods of target crops.

During the last 20 years, temperature-dependent insect growth and development has been modeled with linear and non-linear regression (Wagner et al. 1984a and b). Such models provide predictions of insect life cycle (e.g. events, predicting emergence or peak population periods), which can be used for management of insects. Because diapause termination of *O. cornifrons* is also temperature-dependent (Yamada et al. 1971, Adams 2001) these regression approaches can be used to predict diapause termination of *O. cornifrons* adults. This study was conducted to predict diapause termination of *O. cornifrons* by modeling temperature-dependent diapause termination patterns at various temperature regimes. Linear regression was used to determine the lower temperature threshold and non-linear regression was used to determine the optimum and upper temperature thresholds. This study provides a framework for manipulating *O. cornifrons* diapause termination so that growers can synchronize plant blooming period with flower visiting period of *O. cornifrons*.

Materials and Methods

Colony source. Colonies of *O. cornifrons* were obtained from a local grower in Independence, West Virginia, who had been using *O. cornifrons* to pollinate blueberry for the last 10 years. The grower used cardboard tubes (7.9 mm. diameter, 0.8 mm thickness, and 152 mm length; Jonesville Paper Tube Co., Jonesville, MI, U.S.A.) as artificial nests for *O. cornifrons*. Prior to the experiment, the colonies of *O. cornifrons* were stored in the field during their developmental period and then stored at 4°C in a climate controlled container throughout their diapause period during the winter of 2007-2008.

Experimental design. The experiment was conducted in seven environmental chambers (I-60LVL, Percival Scientific, Boone, IA) maintained at 4, 12, 19, 27, 30, 36 or 42°C. These temperatures were chosen to expand on the temperature gradient used by Adams (2001). Temperature in each environmental chamber was monitored with a HOBO data logger (H08-007-02, OnSet Computer Corporation, Pocasset, MA) and photoperiod was kept constant at 9:15 (L:D) hr to simulate photoperiod conditions when bees would be active. In our preliminary study, we observed that some *O. cornifrons* adults returned back to their nests after emerging from the nests, so sometimes it was difficult to confirm if they emerged and subsequently returned back to their nests to hide. Therefore, we designed bundles of tubes to effectively monitor emergence of *O. cornifrons* adults from the artificial nests. A total of 25 randomly-selected cardboard nests with diapausing adults from the field were bundled and placed in a 0.3-liter clear water bottle that was cut in half (Fig. 1). The bottom portion of a water bottle was used to cover the bundle to prevent *O. cornifrons* from escaping from the nests. Tanglefoot® (Tanglefoot, Grand Rapids, MI, U.S.A.) was coated inside the bottom portion of a water bottle to prevent *O. cornifrons* adults from returning back to the nest after emergence from the nest. In each environmental chamber, 100 cardboard nests (i.e. four bundles with 25 cardboard nests per bundle) were placed and the number of emerged *O. cornifrons* adults was checked daily until all live bees emerged from the nests. The sex of emerged *O. cornifrons* adults was also determined.

Developmental distribution. Cumulative frequency distributions of diapause termination of *O. cornifrons* adults were quantified at each constant temperature to estimate median development time by using a Weibull function (Régnière 1984, Wagner et al. 1984a). We used a two-parameter Weibull function expressed as

$$P(x) = 100 - 100 \times \exp[-(x/\alpha)^\beta] ,$$

where $P(x)$ is the cumulative frequency (%) at time x in days, $\hat{\alpha}$ is scale parameter, and $\hat{\beta}$ is a shape parameter. Prior to fitting the frequency data to the Weibull function, the percentile cumulative diapause termination over time (d) for each temperature was calculated (Wagner et al. 1984a). Temperature-independent distribution curves were also generated by applying the Weibull equation against normalized time; the normalized time was calculated by dividing the time by the median time estimated at each temperature.

Development rate models. Rates of diapause termination (1/d) were modeled with the nonlinear biophysical model (Sharpe and DeMichele 1977) and the linear degree-day model (Campbell et al. 1974). The linear portion (excluding data collected from 4 and 42°C because of no emergence at these temperatures) of the rate of the diapause termination curve was modeled using linear regression (PROC REG, SAS Institute 2008). The lower temperature threshold for diapause termination was estimated by the x intercept of the regression fit, and the degree-day requirement for diapause termination of *O. cornifrons* adults was estimated from the inverse slope of the regression fit (Arnold 1959, Campbell et al. 1974).

The rates of diapause termination at seven different temperatures were fit with a four-parameter non-linear model (Sharpe and DeMicheal 1977, Rueda 1990) according to:

$$r(T) = \frac{RHO25 \frac{T}{298.15} \exp \left[\frac{HA}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right]}{1 + \exp \left[\frac{HH}{R} \left(\frac{1}{TH} - \frac{1}{T} \right) \right]},$$

where $r(T)$ is rate of growth at temperature T (° Kelvin), RHO25 is the development rate at 25°C, HA is enthalpy of activation by rate controlling enzyme, R is universal gas constant set as 1.987, TH is the ° Kelvin at which enzyme is one part active and one part inactive do to high temperature, and HH is the change in enthalpy associated with high-temperature inactivity. The

rate of diapause termination was modeled using non-linear regression (PROC NLIN, SAS Institute 2008) outlined by Wagner et al. (1984b) from which temperature thresholds and optimum temperatures for diapause termination were estimated.

Simulation of diapause termination pattern. The emergence of adults terminating diapause was simulated in relation to constant temperature (°C) and time (d) by combining the Weibull distribution and the biophysical rate models. The rate model determined the daily rate of diapause termination at a given temperature, and the distribution model determined the cumulative frequency in the population (Curry et al. 1978, Allen et al. 1995, Son and Lewis 2005). Daily rate of diapause termination is $R(T)$ at a certain constant temperature T , and the rate summed up to time x is calculated by multiplying the time x with the daily rate $R(T)$ (Wagner et al. 1985). The rate summed up to time is equal to $x \times R(T)$, where x is time (d). Therefore, the mathematical expression of the simulation model is,

$$F(x, T) = 100 - 100 \cdot \exp\left(-\left[\frac{xR(T)}{\alpha}\right]^\beta\right),$$

where $F(x, T)$ is the cumulative frequency (%) of the diapause termination at time x and constant temperature T , $R(T)$ is the temperature-dependent rate model; x is time (d), and α and β are the scale and shape parameters, respectively, from the Weibull function fit to normalized time. At each temperature, simulated values were demonstrated as frequency (%) of the adults completing diapause during day_{*i*}, for instance, which was calculated by subtracting the cumulative frequency value of the simulation result at day_{*i*} from that of day_{*i*+1}.

Results

Emergence patterns of *O. cornifrons* adults depended upon the temperature to which they were exposed (Table 1 and Fig. 2). No *O. cornifrons* adults emerged at the two extreme

temperatures (i.e. 4 and 42°C). Among the five temperature regimes where *O. cornifrons* adult emergence occurred, the slowest emergence was observed at 12°C when the first adult bees (males) emerged after 26 d. In contrast, the quickest adult emergence (males) was observed at 36°C where emergence occurred after as early as 3 d. The first female bees that terminated diapause at 30°C took 8 d longer than the first males. At these two extreme temperatures where emergence did occur, the number of *O. cornifrons* adults emerged was ≈ 17 fold lower than the number that emerged at 19°C, indicating that *O. cornifrons* emergence may be obtained quickly at high temperatures but with a decreased number of emerging adults.

Cumulative frequency distributions of diapause termination for the adults at each temperature were well described by the Weibull model (all $r^2 > 0.85$; $P < 0.01$) (Table 1). In 12 - 30.3°C where adults in both sexes emerged, it took longer for female *O. cornifrons* to terminate diapause than males (Fig. 2). The differences in the estimated median time for diapause termination between males and females became smaller as temperature increases, ranging from 2.1 d at 26.6 °C to 8.8 d at 12 °C.

The cumulative distribution against normalized time (d/median) was also well explained by the Weibull function for *O. cornifrons* males ($F = 400.6$; d.f. = 1, 41; $P < 0.001$, $r^2 = 0.91$), females ($F = 1323.1$; d.f. = 1, 28; $P < 0.001$, $r^2 = 0.98$) and the combined data ($F = 1899.0$; d.f. = 1, 53; $P < 0.001$, $r^2 = 0.97$) (Fig. 2 and Table 2). A higher value of the parameter (β) in the Weibull indicated the lower variability in the distribution; diapause termination of females ($\beta = 9.64$) exhibited less variation than that of males ($\beta = 5.92$).

Relationships between rate of diapause termination (y) and temperature (x) were $y = 0.007989 \times x - 0.07122$ ($F = 210.6$; d.f. = 1, 3; $P < 0.01$; $r^2 = 0.99$) for male bees and $y = 0.00562 \times x - 0.04784$ ($F = 99.9$; d.f. = 1, 2; $P < 0.01$; $r^2 = 0.98$) for female bees. The slope for male *O.*

cornifrons was greater than that of females, indicating male *O. cornifrons* broke diapause earlier than females. When male and female data were combined, the relationship was $y = 0.007927 \times x - 0.08326$ ($F = 39.2$; d.f. = 1, 3; $P < 0.01$; $r^2 = 0.93$). Based on these equations, lower temperatures for diapause termination were 8.9, 8.6 and 10.5 °C for male, female and both sex combined, respectively. Required degree-days for diapause termination of male and female *O. cornifrons* were 125.2 and 179.8, respectively. When male and female data were combined, degree-days required for diapause termination was 126.1 degree-days, which may be do, impart, because the sex ratio favored males over females.

Although the linear regression model can provide values for lower temperature thresholds and degree-day requirements for diapause termination, it does not indicate the upper threshold and the optimum temperature at which *O. cornifrons* adults break diapause. The non-linear models (Table 3 and Fig. 3) showed that the upper thresholds for *O. cornifrons* adult diapause termination were ≈ 41.2 , 37.1, and 42.3°C for male, female, and both sex combined, respectively. The optimum temperatures were 35.7, 30.2, and 36.5°C for male, female, and both sex combined, respectively.

Simulation models enable us to predict when the adult emergence upon diapause completion would occur over a full range of constant temperatures (Fig. 4). At optimum temperatures the emergence density curve exhibited lower variance and shortest time for the diapause termination. At very low and very high temperatures, the emergence time would be delayed with higher variation among cohorts. Female *O. cornifrons* would have a narrower range of temperature during their diapause completion than males, which is more evident at higher temperatures. For example, more than 99% of males would terminate diapause at 9.0 – 38.5 °C

within a period of 60 d while the same percentage of females would terminate diapause within 60 d at 13.5 – 32.5 °C.

Discussion

To maximize pollination, diapause termination of overwintering *O. cornifrons* adults must be synchronized with the bloom of the target crop. In this study we found that diapause termination of *O. cornifrons* adults could be manipulated by exposing them to specific temperatures. Although diapause of *O. cornifrons* adults could be terminated within 3 d after placing them at 36 °C, the largest number of bees emerged at 19°C. There was a 17-fold increase in total adult bee emergence at 19°C compared to 36°C, indicating a trade-off between expediting diapause termination and maximizing the number of emerged bees. This suggests that growers would need to place overwintering *O. cornifrons* adults at certain temperatures depending on how urgently they needed or how many they needed for crop pollination. In any case, excessively high or low temperatures should be avoided since we observed no emerging *O. cornifrons* at 4°C and 42°C. This knowledge of upper and lower temperature thresholds for diapause termination along with optimum temperatures for largest emergence and fastest diapause break can allow growers to release *O. cornifrons* adults in a timely manner.

A few previous studies attempted to determine the degree-day requirement for development and diapause termination of *O. cornifrons*. Yamada et al. (1971) conducted an experiment with five different temperatures and reported that 25°C was the optimum temperature for development from hatching to pupation. Adams (2001) reported that the lower temperature threshold for diapause termination was 6.04°C and the degree days required for emergence was 182, which was different from our results (i.e. a lower temperature threshold of 10.50°C and a degree-day requirement of 126.1). This may be due to different experimental procedures

between the two studies. Adams (2001) conducted the experiment for temperature-dependent diapause termination of *O. cornifrons* adults by removing them from their nests and placing them in plastic cups at five different temperatures. In our study, we attempted to simulate actual field conditions, so we allowed the *O. cornifrons* to remain in their nests in the growth chambers. In addition, we considered two extreme temperatures to model and simulate non-linear temperature-dependent diapause termination of *O. cornifrons* adults with seven different temperatures.

Since *O. cornifrons* adults spend a very short period of their life cycle engaged in pollination, it is crucial to synchronize the termination of diapause with the blooming period of the target plant to maximize pollination. There are three major applications of diapause termination of *O. cornifrons* adults for pollination. First, the lower and higher temperature thresholds can be used to determine temperatures for proper storage and termination of diapausing *O. cornifrons* adults during the winter. Diapausing *O. cornifrons* adults would need to be stored under the lower temperature threshold during the winter to maintain diapauses, and under the high temperature threshold to avoid mortality. Second, knowing the degree day requirements for *O. cornifrons* adult emergence allows for a more goal oriented release of *O. cornifrons* adults. For example, if a large number of bees are required over an extended period of time, then it might be advisable to expose *O. cornifrons* to optimum temperatures. However, when emergence of *O. cornifrons* adults is needed immediately and the number of emerged adults is less important, it would be necessary to expose the diapausing adult bees to higher temperatures. Likewise, if emergence needs to be delayed, then a temperature below the optimal temperature could be used to prolong diapause. However, prolonging diapause also decreases the total number of emerged adults. Third, growers need to consider sexual differences when manipulating diapause termination because female *O. cornifrons* are better pollinators (Bosch

and Kemp 2002). This study showed that females would emerge later than males with a shorter range of diapause termination period. Our simulation for female emergence would be a useful predictive tool and yet the actual emergence of males can be used as a reliable indicator of predicting female emergence (Fig. 5).

The results of this study indicated that diapause termination of *O. cornifrons* adults could be manipulated, based on models of diapause termination patterns, to synchronize adult bee emergence with the pollination periods of target fruit trees. Trade-offs between expedited termination of diapause and decreased bee abundance could be determined depending on various factors in each orchard such as the size of managing *O. cornifrons* colonies, climatic conditions, and fruit tree varieties varying blooming periods.

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Table1. Parameter estimate ($\pm$ SE) of the Weibull model used to estimate median time (d) for diapause termination of overwintering *O. cornifrons* adults at constant temperatures

Temp. (°C)	Sex	Weibull Model			
		α	β	r^2	Median (d)
4	Male	- *	-	-	-
	Female	-	-	-	-
	Combined	-	-	-	-
12	Male	33.54 $\pm$ 0.69	9.15 $\pm$ 2.60	0.851	32.2
	Female	42.33 $\pm$ 0.40	11.86 $\pm$ 1.76	0.961	41.0
	Combined	36.41 $\pm$ 0.51	5.34 $\pm$ 0.61	0.953	34.0
19	Male	13.51 $\pm$ 0.19	4.89 $\pm$ 0.43	0.979	12.5
	Female	20.10 $\pm$ 0.17	9.45 $\pm$ 0.94	0.982	19.3
	Combined	17.87 $\pm$ 0.13	4.33 $\pm$ 0.18	0.993	16.4
27	Male	8.30 $\pm$ 0.16	4.62 $\pm$ 0.54	0.981	7.7
	Female	10.24 $\pm$ 0.03	8.95 $\pm$ 0.34	0.999	9.8
	Combined	9.73 $\pm$ 0.05	5.51 $\pm$ 0.22	0.998	9.1
30	Male	5.97 $\pm$ 0.19	3.11 $\pm$ 0.45	0.971	5.3
	Female	7.76 $\pm$ 0.03	13.41 $\pm$ 0.93	0.999	7.6
	Combined	7.14 $\pm$ 0.17	4.12 $\pm$ 0.54	0.977	6.5
36	Male	4.12 $\pm$ 0.20	2.98 $\pm$ 0.64	0.968	3.6
	Female	4.12 $\pm$ 0.20	2.98 $\pm$ 0.64	0.968	3.6
	Combined	4.12 $\pm$ 0.20	2.98 $\pm$ 0.64	0.968	3.6
43	Male	-	-	-	-
	Female	-	-	-	-
	Combined	-	-	-	-

*, no adults were emerged

Table 2. Parameter estimate ($\pm$ SE) of the Weibull model to describe variability of diapause termination rate for the overwintering *O. cornifrons* adults against normalized time (d/median)

Parameter	Male	Female	Combined
α	1.04 ± 0.01	1.04 ± 0.01	1.08 ± 0.01
β	5.92 ± 0.60	9.64 ± 0.59	4.58 ± 0.21
r^2	0.909	0.980	0.973

Table 3. Parameter values ($\pm$ SE) and associated statistics for non-linear model (Rueda et al. 1990) for temperature-dependant diapause termination of *O. cornifrons* adults

Sex	Parameter	Estimates (SE)	r^2	d.f.	F	P
Male	RHO25 ¹	0.0926 (0.00181)	0.99	3, 6	187.7	<0.001
	HA ²	16823 (544.1)				
	HH ³	195751 (38663.8)				
	TH ⁴	310.7 (2.09)				
Female	RHO25	0.0827 (0.00131)	0.97	3, 6	109.2	<0.001
	HA	17862 (498.5)				
	HH	241510 (54325.4)				
	TH	305.0 (1.95)				
Combined	RHO25	0.1017 (0.00286)	0.97	3, 6	81.6	<0.001
	HA	13802 (856.9)				
	HH	272984 (60875.7)				
	TH	311.2 (3.29)				

¹ development rate at 25°C

² enthalpy of activation by rate controlling enzyme (cal/mol)

³ change in enthalpy associated with high-temperature inactivation of enzyme (cal/mol)

⁴ Kelvin temperature at which enzyme is one part active and one part inactive due to high temperature

Fig. 1. Device to capture *O. cornifrons* adults after emergence to prevent them from escaping or returning back to the nests. Tanglefoot was coated inside the bottom portion of a water bottle to capture emerged *O. cornifrons* adults.

Fig. 2. Temperature-dependant diapause termination of *O. cornifrons* adults fitting with non-linear model described by Ruena et al. (1990). See text and Table 3 for mathematical equation and parameter values for non-linear modeling.

Fig. 3. Cumulative frequency (%) of development completion for *O. cornifrons* adults fit with Weibull function to the cumulative frequency of normalized age (day/mean development duration).

Fig. 4. Simulated temperature-dependent patterns of diapause termination of *O. cornifrons*.

Fig. 5. Median time (d) required for diapause termination of overwintering *O. cornifrons* males and females at constant temperatures. Note that time difference in diapause termination between males and females becomes smaller as temperature increases.

CHAPTER 3: Bioassay of Potential Fumigants to Control *Chaetodactylus krombeini* (Acari: Chaetodactylidae) Associated with *Osmia cornifrons* (Hymenoptera: Megachilidae)

Abstract: With the recent decline of honey bees, *Apis mellifera* (L.) (Hymenoptera: Apidae), alternative insects are needed for supplemental pollination. One candidate supplemental pollinator, the Japanese hornfaced bee, *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae) is associated with the hairy-footed mite, *Chaetodactylus krombeini* Baker (Acari: Chaetodactylidae) that can impede the propagation of *O. cornifrons*. This study was conducted to evaluate the efficacy of formic acid and wintergreen in controlling *C. krombeini*. This study was conducted in two bioassays: to investigate *O. cornifrons* tolerance of formic acid and wintergreen and to find the lethal concentration of formic acid and wintergreen needed for mite control. We found that *O. cornifrons* could tolerate low doses of both formic acid and wintergreen for extended periods of time. The time and dosage required to induce *C. krombeini* mortality with either formic acid or wintergreen was significantly less than the time required to produce *O. cornifrons* mortality. Estimates of the LC₅₀ and LC₉₀ for *C. krombeini* hypopi treated with wintergreen were ca. five times lower than those with formic acid, indicating that wintergreen was a more effective mite control tactic. Mortality of *O. cornifrons* was low when exposed to either formic acid or wintergreen for up to 20 minutes. This study provides evidence that *C. krombeini* can be managed effectively without inducing mortality in *O. cornifrons*.

Keywords: formic acid, essential oil, wintergreen, hornfaced bee, hairy-footed mite

Crop and fruit tree pollination is a 15 billion dollar industry each year in the United States (Morse and Calderone 2000). With recent events such as parasitic mites (Finley 1995), Africanized honeybees (Sammataro and Avitable 1998) and Colony Collapse Disorder (Stokstad 2007) contributing to the decline of honeybees there is a need to consider alternate means of pollination, such as with the Japanese hornfaced bee, *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae) (Adams 2001). *Osmia cornifrons* has been used successfully for the pollination of apple in Japan (Sekita and Yamada 1993), and since its introduction to the United States in 1976 (Batra 1998), it has been used for the pollination of spring blooming fruit trees (Batra 1998, Adams 2001) and vegetable crops (Wilson et al. 1999). Because the life cycle of the bee coincides well with blueberry flowering it is particularly useful in blueberry pollination (West and McCutcheon 2005). *Osmia cornifrons* is also timid and rarely stings, which makes using the bee in commercial orchards attractive to growers (Batra 1998, Bosch and Kemp 2001).

Under natural conditions, *Osmia* spp. (e.g., *O. cornifrons* and *O. lignaria*) can be found nesting in reeds. In commercial settings it is, however, commonly managed in small cardboard tubes used as nests (Batra 1998, Bosch and Kemp 2001). *Osmia cornifrons* is gregarious and the tubes facilitate their use in a targeted area. *Osmia cornifrons* constructs cells with mud partitions within the tubes and these tubes can be grouped together in bundles. While this makes management easier it also provides an attractive habitat for pests such as parasitic wasps and mites, by increasing bee density it in turn provides an increase in density of pests (Krunić et al. 2005, Park et al. 2009).

One pest associated with *O. cornifrons* is the hairy-footed mite, *Chaetodactylus krombeini* Baker (Acari: Chaetodactylidae), and it is of particular importance with respect to *O.*

cornifrons propagation and management (Park et al. 2009). *Chaetodactylus krombeini* is a pollen mite that infests the tubes in which *Osmia* spp. nest (Bosch and Kemp 2001).

Chaetodactylus krombeini hypopi enters nesting tubes in a variety of ways. Park et al. (2009) found that *C. krombeini* hypopi were capable of walking from adjacent tube openings as well as through holes in tubes created by parasitic wasps, and a major dispersal mechanism is through phoretic behavior. When *C. krombeini* hypopi hitchhike on *O. cornifrons* adults, hypopi become entangled in the hairs of the bee. We observed that *O. cornifrons* adults are visibly irritated when infested by *C. krombeini* and some adults had difficulty flying because of the number of mites on the bee. Once hypopi enter the tubes their population can increase to 170 to 5000 mites per cell (Park et al. 2009). *Chaetodactylus krombeini* populations will compete with *O. cornifrons* larvae for pollen provision in the tubes and have been reported to kill developing larvae (Bosch and Kemp 2001). Larvae that do survive may experience a reduction in size due to a decreased food supply during development (Bosch and Kemp 2001). A reduction in larval size may also cause a corresponding reduction in pollinating efficiency as adults.

Currently *C. krombeini* is controlled by cultural methods such as physically removing *O. cornifrons* cocoons (Krunic' et al. 2005) and cleaning them with bleach (Bosch and Kemp 2001), or with heat treatments (Sekita and Yamada 1993). However, these methods have several disadvantages. Physically removing cocoons disturbs the bees' natural nesting behavior. Cleaning with bleach requires the removal from the nesting tube and presents further complications when the cocoons are exposed to bleach treatment. Thus, additional, less invasive control methods that allow *O. cornifrons* to remain undisturbed would benefit their use as a supplemental pollinator (Park et al. 2009).

Mites associated with honeybees have been effectively controlled by both formic acid and essential oils (Amrine 2006). A non-intrusive method, such as fumigation, would be best suited for pest control of *O. cornifrons* because disturbances to the bees may cause a reduction in the number of females returning to nesting tubes to lay eggs (Bosch and Kemp 2001). This study was conducted to evaluate formic acid and wintergreen to determine if the volatile nature of these compounds would effectively control *C. krombeini* hypopi without impacting *O. cornifrons*.

Materials and Methods

Experimental animals. *Osmia cornifrons* and *C. krombeini* hypopi were obtained from a local grower in Independence, West Virginia who has been using the *O. cornifrons* to pollinate blueberry for the last 10 yr. The grower has used cardboard tubes (7.9 mm diameter, 0.8 mm thickness, and 152 mm length, Jonesville Paper Tube Co., Jonesville, MI) as artificial nests for *O. cornifrons*.

Bioassay of formic acid and wintergreen on *O. cornifrons*. Mortality of *O. cornifrons* adults was determined by exposure to various volumes of 50% formic acid and wintergreen oil. Both 50% formic acid and wintergreen oil have been shown to kill mites associated with honeybees while not producing harmful side effects to the honeybees (Amrine 2006). Bioassays were conducted under ambient environment ($23\pm 2.1^{\circ}\text{C}$ and $58\pm 6.2\%$ RH). For the bioassay arena, we used 56.6-cm³ Petri dishes (3-cm radius with 2-cm depth, Lab-Tek® 4036, LAB-TEK Division Miles Laboratories, Inc., Naperville IL) with 55-mm-diameter filter paper (Whatman® 1001-055, Whatman International Ltd, Maidstone England) taped onto the lid of the Petri dish. Seven different volumes (0, 5, 10, 20, 40, 60 and 80 µl per 56.6 cm³) of 50% formic acid

(Chemical and Solvents Inc., Roanoke, VA) or artificial wintergreen oil, Methyl Salicylate Synthetic (Lorann Oils, Lansing, MI) were applied to the filter paper using micropipettes. One *O. cornifrons* adult female who had emerged from a cleaned cocoon was placed inside the Petri dish and then the Petri dish was wrapped with Parafilm® (American National Can™, Neenah, WI). Time required to cause death of each *O. cornifrons* adult was recorded and the bioassay was replicated five times for each amount and chemical. Data were analyzed with ANOVA and comparisons among treatments were based upon Tukey's HSD test at 0.05% error rate (PROC GLM, SAS Institute 2008).

Bioassay of formic acid and wintergreen on *C. krombeini*. Mortality of *C. krombeini* hypopi exposed to various amounts of 50% formic acid and wintergreen was conducted using the same design as previously mentioned. A total of 28 ± 11.7 *C. krombeini* hypopi were placed in a Petri dish with a fine brush. Four different amounts (0, 5, 10, and 20 μl per 56.6 cm^3) of 50% formic acid or wintergreen were applied to the filter paper using micropipettes. The Petri dish and lid were sealed with parafilm and mortality was recorded at intervals of 0, 5, 10 and 15 min. We set these time intervals and amounts based on our preliminary study that showed that both formic acid and wintergreen were effective at $< 20 \mu\text{l}$ when applied under 20 min. However, because the size of *C. krombeini* hypopi is ca. $\approx 0.3 \text{ mm}$ (Park et al. 2009) and they are generally very active when they are out of the nest, accurately counting the number of dead and live mites can be very difficult and time consuming and prone to observation error. Therefore, we developed an image analysis approach as a more practical and effective method of recorded mite mortality.

Images were taken with a digital camera (Sony® Model DCS-W50, Sony Corporation of America, New York, NY) at intervals of 0, 5, 10 and 15 min. These images were processed and

analyzed with Photoshop CS3 Extended[®] (Adobe Systems Inc., San Jose, CA) to count the number of dead and live hypopi (Figure 1). Images taken from each Petri dish at each time interval were overlaid and any movements of hypopi were detected with the ‘difference’ blend mode function in Photoshop CS3[®]. This function made it possible to align two images and look at the differences in pixels between the two images. When movement of hypopi was detected between two images, the function detected the difference in pixels, and the hypopi were considered alive; otherwise, they were considered dead. These steps were repeated with successive images in the time series. When live hypopi were detected, the mites were digitally marked and automatically counted with the ‘analysis’ function in Photoshop CS3 Extended[®]. Mortality of hypopi for each chemical amount and time interval was calculated.

Total numbers of dead individuals for each treatment were corrected against the control using Abbott’s formula (Abbott 1925). Corrected data were subjected to logit analysis to determine the main effects of dose and treatment, and their interaction (PROC GENMOD, SAS Institute 2008). Overdispersion in data was corrected using the scaled deviance parameter in SAS. Significance of effects was based upon the likelihood ratio chi-squared. Logit analysis was used to estimate LC₅₀ and LC₉₀ (Robertson et al. 1984, Stokes et al. 2000). In addition, the two chemical treatments were compared for treatment effects using survival analysis (PROC LIFE TEST, SAS Institute 1999) to determine interactions among treatment, dose and time.

Results

Bioassay of formic acid and wintergreen with *O. cornifrons* adults. When formic acid was directly applied to *O. cornifrons*, the dense hairs of the body seemed to considerably prevent the chemical from coming into contact with the body of the bee. Consequently there was no

mortality. In contrast, wintergreen quickly soaked into the dense hairs on *O. cornifrons* resulting in death (Fig. 2). Bees that came into direct contact with wintergreen became highly irritated and were observed to shake and twitch. When formic acid and wintergreen were applied as volatiles, *O. cornifrons* adults could survive for 111.5 hr with low-dosage applications of wintergreen (Table 1). *Osmia. cornifrons* lived much longer in the presence of wintergreen than in formic acid at doses of $< 20 \mu\text{l}$ per 56.6 cm^3 . However, at higher doses ($> 60 \mu\text{l}$ per 56.6 cm^3), *O. cornifrons* lives slightly longer when in the presence of formic acid than wintergreen.

Bioassay of formic acid and wintergreen with *C. krombeini* hypopi. The logit analysis showed significant effects of dose ($\chi^2 = 14.31$; $\text{df} = 1$; $P < 0.001$), treatment ($\chi^2 = 19.46$; $\text{df} = 1$; $P < 0.001$), and their interaction ($\chi^2 = 17.28$; $\text{df} = 1$; $P < 0.001$). Highly significant differences between formic acid and wintergreen was also confirmed by survival analysis ($\chi^2 = 1355.7$; $\text{df} = 1$; $P < 0.0001$). When homogeneity among doses and time was tested for wintergreen only, the survival of *C. krombeini* hypopi at 10, 15, and $20 \mu\text{l}$ per 56.6 cm^3 was statistically the same across all time intervals ($\chi^2 = 2.6$, $\text{df} = 2$, $P = 0.2758$); however, when these amounts were grouped together, they as a group were significantly different from the control ($\chi^2 = 465.4$, $\text{df} = 1$, $P < 0.0001$) (Fig. 3). When testing formic acid alone, the concentration of $10 \mu\text{l}$ was not significantly different from the control ($\chi^2 = 3.4$; $\text{df} = 1$; $P = 0.0644$). However, this group was significantly different than the $15 \mu\text{l}$ concentration ($\chi^2 = 63.9$; $\text{df} = 1$; $P < 0.0012$), which was in turn significantly different than the $20 \mu\text{l}$ concentration ($\chi^2 = 10.5$; $\text{df} = 1$; $P < 0.0012$) (Fig. 3). Estimates of the LC_{50} and LC_{90} for *C. krombeini* hypopi treated with wintergreen were ca. five times higher than those with formic acid (Table 2). Survival analysis also showed that *C. krombeini* survival subjected to wintergreen decreased rapidly at 5 min. while survival was sustained twice as long when subjected to formic acid (Fig. 3).

Discussion

Formic acid and wintergreen have been used to control parasitic mites associated with honeybees such as the varroa mite, *Varroa destructor* (Mesostigmata: Varroidae) and the tracheal mite, *Acarapis woodi* (Prostigmata: Tarsonemidae). Amrine et al. (2006) showed that formic acid and essential oils killed the mites but did not affect the honeybee. They also showed that formic acid could be applied as a fumigant and has been used as a low cost method that achieves 90-95% mortality of the mites. This study showed that *O. cornifrons* adults generally tolerated the fumigant properties of formic acid and wintergreen (< 5 min. with applications of < 20 μl per 56.6 cm^3) that successfully killed hypopi, indicating the potential for *C. krombeini* control. In addition, we also observed that *O. cornifrons* adults reacted differently when physical contact of 50% formic acid and wintergreen was made on the body of the adult bee.

Chaetodactylus krombeini is currently managed in several different ways, both mechanically and chemically. Heat treatments have been used for *C. krombeini* control (Bosch and Kemp 2001), and when nests of *Osmia* spp. infested with *C. krombeini* were exposed to high temperatures, the bees remained alive while the mites died. Another approach to control *C. krombeini* is the use of binder boards (i.e., boards with the appropriate sized hole drilled through and then cut in half) allowing the user to pry the two pieces of the board apart, gaining access to the cocoon inside (Strickler 2002). The binder boards can be used for *C. krombeini* control by opening the boards and removing the cocoons to separate those infested with *C. krombeini*. Cocoons that have been removed from binder boards also can be washed with a bleach solution (Strickler 2009). Binder boards and nesting materials can also be treated directly with endosulfan (Kronic' et al. 2005). However, all of these methods may produce adverse side

effects. Cocoons and nesting materials could come into direct contact with any chemicals used, which can cause chemical residue to remain inside the nesting materials. Also, the physical removal of *O. cornifrons* from the binder boards generally results in releasing loose cocoons and may increase unwanted dispersal of pre-nesting female bees (Bosch and Kemp 2001); generally females will leave their nesting tubes to feed for a few days and then return to oviposit, while those females who have been released outside of their nesting tube have a greater chance of not returning to oviposit. This study showed that formic acid and wintergreen were very effective in killing *C. krombeini* hypopi in a short time period and could be used to reduce the side effects associated with current *C. krombeini* management tactics. Formic acid and wintergreen can be used as a volatile and thus they would allow the bees to remain undisturbed in the nest or binder board during application. This can reduce pre-nesting dispersal by females and potentially increase *O. cornifrons* populations in the subsequent year (Bosch and Kemp 2001). It would also allow the bees to avoid direct contact with the substance, which would reduce the likelihood of residual effects.

This study suggests two considerations on the utilization of formic acid and wintergreen to control *C. krombeini*. First, formic acid and wintergreen need to be used as fumigants because *O. cornifrons* adults can be killed by the direct contact with them. In parasitic mite control in honeybee hives, formic acid volatiles are circulated throughout the bee hive by wing fanning, penetrating the brood cells, and thereby killing the parasitic mites (Amrine et al 2006). Wintergreen for mite control in honeybee hives is generally used as an ingredient to grease patties, on which the honeybees feed on and slowly emit volatiles to kill mites. Honeybees will feed on grease patties and as they do the wintergreen acts as an irritant to the varroa mite causing the mite to fall off the bee and through the frames of the hive to the bottom board (Amrine 2006).

However, this method may not be directly applicable to the case with *O. cornifrons* because *O. cornifrons* adults do not ventilate air inside the nest that is partitioned with mud. Also, old nests that are intended for use in the future need to be fumigated with formic acid or wintergreen to control *C. krombeini* when bees do not inhabit the nests. When binder boards are used, the boards could be spread open allowing the fumigant to contact the mites. Due to the short application period required, active bees may be treated outside of their nests as they emerge. However, further studies are needed to investigate practical and effective methods of applying both formic acid and wintergreen as a volatile for control of *C. krombeini*.

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Table 1. Required hours to death of *O. cornifrons* adults with formic acid and wintergreen treatments

Dosage Amount (μl per 56.6 cm^3)	Formic acid	Wintergreen
0	$21.9 \pm 2.6a^*$	$132.5 \pm 42.7a$
5	$10.1 \pm 2.1a$	$111.5 \pm 81.1b$
10	$8.3 \pm 2.9b$	$18.5 \pm 4.8b$
20	$8.0 \pm 3.3b$	$9.9 \pm 5.3b$
40	$6.2 \pm 2.4b$	$5.5 \pm 2.5b$
60	$5.2 \pm 2.8b$	$3.5 \pm 0.7c$
80	$3.7 \pm 0.7b$	$3.1 \pm 0.7c$
100	$5.7 \pm 2.6b$	$2.6 \pm 0.5b$
120	$6.2 \pm 2.2b$	$2.7 \pm 1.0b$
140	$4.8 \pm 0.5b$	$1.8 \pm 0.9c$

*Means within each variable in each column followed by the same letter are not significantly different ($P > 0.05$; Tukey's standardized range test).

Table 2. Estimates of lethal concentration (LC_{50}) of wintergreen (WG) and formic acid (FA) in *O. cornifrons*

	LC_{50}	LC_{50} -UCL ¹	LC_{50} -LCL ²	LC_{90}	LC_{90} -UCL ¹	LC_{90} -LCL ²
WG	3.1	10.9	0.9	5.7	20.2	1.6
FA	15.4	15.8	14.9	23.4	24.2	22.7

¹UCL, Upper confidence limit

² LCL, Lower confidence limit

Figure 1: Procedures of image analysis to count numbers of *C. krombeini* hypopi using Adobe Photoshop CS3 Extended. (A) Digital camera used to take 4 images of mites in Petri dish, one image per interval at 0, 5, 10 and 15 minutes. (B) 4 images combined and layered in 1 Photoshop document. (C) Levels and curves adjustment layers used to add additional contrast to images. (D) 4 blank layers added over original images and mites were marked on each new layer with paintbrush tool. Blank layers were set to Difference blend mode to allow changes in movement to appear in each subsequent image. (E) Final processed images show mite movement at each interval. (F) Magic Wand tool used to select the mites that have been marked on each layer. Measurement Log used to count the number of selected mites and export the data. (G) Exported data was opened in Microsoft Excel and analyzed.

Figure 2: Mortality of *O. cornifrons* adults when wintergreen was applied directly to the bee.

*, means within each variable in each column followed by the same letter are not significantly different ($P > 0.05$; Tukey's standardized range test).

Figure 3: Survivor analysis of *C. krombeini* hypopi when fumigated with (A) wintergreen and (B) formic acid at three different amount of application: 0 (control), 5, 10, and 20 μl per 56.6 cm^3 .

CHAPTER 4: CONCLUSION

Osmia cornifrons adults spend a very short period of their life cycle engaged in pollination. Therefore, it is crucial to synchronize the termination of *O. cornifrons* adult diapause with the blooming period of the target plant to maximize pollination. Currently, *C. krombeini*, a pest associated with *O. cornifrons*, has been of particular importance with respect to *O. cornifrons* propagation and management.

The result of this study (Chapter 1) indicates two major applications of diapause termination of *O. cornifrons* adults for pollination. First, the lower and higher temperature thresholds can be used to determine temperatures for proper storage and termination of diapausing *O. cornifrons* adults during the winter. Diapausing *O. cornifrons* adults would need to be stored under the lower temperature threshold during the winter to maintain diapause, and under the high temperature threshold to avoid mortality. Second, knowing the degree day requirements for *O. cornifrons* adult emergence allows for a more goal oriented release of *O. cornifrons* adults. With the use of a model of diapause termination patterns, a greater synchronization of adult bee emergence with the pollination periods of target fruit trees can be achieved. However, trade-offs exist between expedited termination of diapause and decreased bee abundance. Depending on various factors in each orchard such as the size of managing *O. cornifrons* colonies, climatic conditions, and fruit tree varieties varying blooming periods diapause manipulation can be goal-oriented to achieve the desired response.

This study (Chapter 2) also considers the implications of management strategies for *C. krombeini*. Formic acid and wintergreen need to be used as fumigants because *O. cornifrons* adults can be killed by the direct contact with them. Old nests that are intended for use in the future need to be fumigated with formic acid or wintergreen to control *C. krombeini* when bees

do not inhabit the nests. When binder boards are in use, the boards could be spread open allowing the fumigant to contact the mites. Due to the short application period required, active bees may be treated outside of their nests as they emerge. However, further studies are needed to investigate practical and effective methods of applying both formic acid and wintergreen as a volatile for control of *C. krombeini*.

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