INTEGRATED MANAGEMENT OF *VARROA DESTRUCTOR* ANDERSON & TRUEMAN (ACARI: VARROIDAE) IN HONEY BEES, *APIS MELLIFERA* L. (HYMENOPTERA: APIDAE), IN WESTERN WASHINGTON STATE, USA

By

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To the faculty of Washington State University:

The members of the Committee appointed to examine the thesis of SAMUEL DAVID HAPKE find it satisfactory and recommend that it be accepted.

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Chair

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Abstract

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The ectoparasitic mite *Varroa destructor* (Anderson & Trueman) continues to threaten honey bee colonies worldwide. Emerging resistance in mite populations to available chemical acaricides coupled with fears of chemical contamination of hive products has illuminated the need to explore alternative options to control the mite. Formic acid is one of several "soft" alternatives being explored to replace synthetic acaricides. Five treatments for the management of *V. destructor* were tested to determine the efficacy and optimal timing of formic acid as an acaricide in western Washington State, USA. Treatment with formic acid led to no significant effects on bee population size, adult bee weight, or brood area. Mite levels were significantly diminished after August treatment, but rebounded by the following April. No significant differences in bee and mite population data were found between treatment groups during the following spring, but colonies treated with formic acid had higher survivorship than those untreated. The efficacy of the sticky board, which measures natural mite death in the colony, as an accurate predictor of total mite infestation was also examined. This study compared

counts from 48hr sticky boards with total mite populations estimated from infestations of adult bee and capped brood samples. A strong linear relationship between the two methods was found using regression analysis and a mathematical model for estimating total infestation from 48hr sticky board counts is reported. This model may have utility in integrated management programs.

TABLE OF CONTENTS

LIST OF FIGURES

Dedication

This thesis is dedicated to my mother's parents, Benjamin and Hannah Weinstein, who showed me how large the world is and how full life can be.

INTRODUCTION

Varroa destructor (Anderson & Trueman) is an ectoparasitic mite that feeds on the hemolymph of honey bees, *Apis mellifera* L. (Anderson & Trueman 2000). Parasitism by *V. destructor* is multi-faceted stressor on colony health and may be a key contributing factor in Colony Collapse Disorder (CCD) (Cox-Foster et al 2007, Oldroyd 2007). The mite exhibits both a phoretic phase, during which it clings to the abdomen of adult bees, and a reproductive phase, when it feeds on bee larvae and pupae inside capped brood cells (Martin 1994). The phoretic phase facilitates the spread of the mite both within and between colonies via adult bees (Rademacher 1991, Sammataro et al 2000). Feeding by reproductive mites and their offspring physically damages the developing host, leading to a weakened, unproductive adult bee that is characterized by deformed wings and legs (De Jong 1982). Mite populations increase exponentially within colonies (Martin & Kemp 1997) and thus feeding damage can rapidly affect colony performance. If left untreated, *V. destructor* infestations may lead to colony mortality in two years after introduction (De Jong 1997). Furthermore, the mites facilitate the spread of pathogens in colonies by weakening the immune systems of their hosts (Shen et al 2005) and, in some cases, directly vector viruses between individual bees (Bowen-Walker et al 1999).

On its natural host, *Apis cerana* Fabricius (Asian honey bee), *V. destructor* evolved a non-virulent host-parasite relationship, in which it infests and damages only drone brood. However, the mite is able to infest both drone and worker brood in *A. mellifera* colonies (Boot et al 1997), which impacts colony productivity. The host-shift from *A.*

cerana to *A. mellifera* appears to have occurred at least twice, once in Japan in the early 1950's and in eastern Russia in the late 1950's (Crane 1978, de Guzman et al 1997). The Russian haplotype of *V. destructor* exhibits higher virulence on *A. mellifera* than the Japanese haplotype (Anderson & Trueman 2000). Differential virulence between mite haplotypes may explain reports of bee colonies in the Americas surviving with long-term *Varroa* infestations in the absence of chemical treatment (Oldroyd 1999). After its introduction in the United States in 1987 (Anonymous 1987a), the Russian haplotype spread rapidly through managed honey bee populations (Wenner and Bushing 1996) and remains a ubiquitous threat today.

Commercial beekeepers rely heavily on chemical acaricides to control *V. destructor* populations in hives, but these compounds may negatively impact host health and the quality of hive products. Although chemical treatments typically keep mite populations under economically damaging levels, such practices have been shown to damage colonies. Treatment doses of Apistan® (tau-fluvalinate; Wellmark International, Bensenville, IL) decreased average body mass and lifespan of drones (Rinderer et al 1999). Residues of fluvalinate (a synthetic pyrethroid), amitraz (a formamidine) and coumaphos (an organophospate), the three most widely employed acaricides for *V. destructor* control, have been detected in wax, honey and other hive products (Bogdanov 2006, Chauzat and Faucon 2007). Sublethal effects, including anatomical and behavioral abnormalities, were observed in queens reared in wax containing high doses of fluvalinate (Haarman et al 2002). Coumaphos residues in queen cells led to significantly decreased acceptance rates and long-term performance of reared queens

(Haarman et al 2002, Collins et al 2004). Lodesani et al (1992) speculated that residues in the colony might prolong the exposure of mites to the acaricides and potentially induce selection for acaricide resistance. However, acaricides remain the most financially cost-effective treatments for *V. destructor*, but their use must be optimized to diminish detriments to colony health (Strange & Sheppard 2001).

The first report of fluvalinate-resistant *Varroa* mite populations was published in Italy in the 1990's (Lodesani et al 1995). Similar reports from the United States appeared in the late 1990's (Baxter et al 1998, Elzen et al 1999). In response to the reports of failing fluvalinate efficacy, the United States Environmental Protection Agency granted a limited-use permit for coumaphos in a slow-release strip form in 1998 (Pettis 2004). Coumaphos effectively controlled fluvalinate- and amitraz-resistant mite populations (Elzen et al 2000), however, reports of resistance to coumaphos surfaced in Florida in 2001 (Elzen and Westervelt 2002).

The emergence of mite populations resistant to acaricides, coupled with growing concerns over deleterious side effects of chemical treatments, necessitated the development of novel, integrated approaches to mite control. Integrated pest management (IPM) systems implement genetic, mechanical, and biological practices and bio-rational chemicals to reduce or replace the use of synthetic chemical treatments. Indeed, Caron (1999) reviewed a variety of alternative measures that have been explored to expand the options available for the control of *V. destructor* beyond synthetic acaricides. Pest population monitoring tools support control practices in IPM

programs by identifying critical pest outbreaks and facilitating the evaluation of treatment efficacy.

Genetic control measures in IPM strategies for honey bee pests rely on the isolation and propagation of heritable behavioral traits. Populations of honey bees have shown natural resistance to *V. destructor* (Ritter 1990, Anderson 1994, Eguaras et al 1995). In the United States, selection programs have explored three heritable mechanisms for behavioral resistance to *V. destructor*. The first resistance mechanism, known as hygienic behavior, involves the removal of damaged or diseased larvae and pupae by worker bees. Peng et al (1987) speculated that hygienic and grooming behaviors by Asian honey bee workers might help to maintain mite infestations below damaging levels in *A. cerana* colonies. Hygienic behavior has been observed in colonies of *A. mellifera*, where it effectively suppressed American foulbrood and chalkbrood and showed success in limiting population growth of *V. destructor* (Spivak & Reuter 2001). One test for the hygienic behavioral trait involves replacing freeze-killed brood in the colony and then monitoring the proportion of dead brood removed by workers over a set time period. Selection for hygienic behavior gave rise to the Minnesota hygienic stock (Spivak 1996). Researchers at Washington State University have also selected a hygienic stock using annual freeze-kill tests (W.S. Sheppard unpublished data).

The second heritable mechanism is the control of mite reproduction by increasing the proportion of phoretic mites in a population. Phoretic mites are unable to reproduce inside capped cells and are also more likely to be removed from host bees by worker

grooming behavior (Rinderer et al 2001). This mechanism was first detected in colonies originating from eastern Russia. *Apis mellifera* colonies imported into far eastern Russia in the 1800s have had approximately 150 years of exposure to *V. destructor* and may have coadapted with the mites during that time (Danka et al 1995). In the mid-1990's, the USDA imported bees from Russia and established a research and breeding program in isolation in the southern United States (Rinderer et al 1997). Russian bees bred by the USDA have demonstrated resistance to both *V. destructor* and *Acarapis woodi* (Rennie), the tracheal mite (Rinderer et al 2001), and produce honey at levels comparable to those observed in widely used Italian stocks (Ward et al 2008).

The third mechanism for heritable resistance is Varroa-sensitive hygiene (VSH), formerly referred to as Suppression of Mite Reproduction (SMR) (Harris 2007), in which bees selectively remove reproducing mites from capped cells (Harbo & Harris 2005). Researchers define VSH as the maintenance of low proportions of reproductive female mites and use that trait as the criterion for selection. Varroa-sensitive hygiene thus differs from the previously described hygienic behavior, which is identified by the removal of freeze-killed brood. The VSH line has demonstrated significant ability to suppress *V. destructor* reproduction, while still producing honey in volumes comparable to commercially available Italian colonies (Ward et al 2008).

Mechanical control measures for *V. destructor,* within the context of an IPM program, include drone and worker brood traps, combs with reduced cell size, pollen traps, screened bottom boards and inert dusts. Drone brood trapping exploits the preferential

infestation of drone cells by the mites. During periods of drone rearing, drone comb can be inserted into a colony to trap reproductive mites and then removed prior to emergence. Additionally, naturally occurring drone brood can be removed or destroyed to kill the mites inside it (Calis et al 1999, Calderone 2005). Infested worker brood can also be removed from the colony and treated with high temperature or organic acid to kill mites inside the cells. Trapping with worker brood has shown some efficacy and its use is not limited to periods of drone rearing (Calis et al 1998). Moreover, decreasing the size of brood cells might reduce the space available for successful mite reproduction. Piccirillo & De Jong (2003) concluded that the size of brood cells was significantly correlated to the rate of infestation by *V. destructor*. Worker bees will construct smaller cells if given foundation with smaller cell diameters (McMullan & Brown 2006), but the efficacy of this manipulation against mites has not been studied.

The three remaining mechanical methods separate phoretic mites from adult bees. Pollen traps force foraging bees through narrow spaces as they pass through the hive entrance, knocking pollen and mites off the body, thereby limiting mite infestations (Hart & Nabors 1999). Screened bottom boards prevent live fallen mites from reentering the hive (Pettis & Shimanuki 1999) and, thus, can augment the efficacy of chemical treatments that knock down large proportions of adult mites (Webster et al 2000). Inert dusts occupy the ambulacra (puvilli or tarsal pads) of mites and prevent them from adhering to adult bees (Macedo et al 2002). Dusting with finely ground confectioner sugar increased mite fall in laboratory studies and may be useful to remove *V. destructor* mites from package bees (Fakhimzadeh 2001). Although mechanical control

methods have shown success in reducing *V. destructor* infestations, none appear sufficiently effective to control an infestation alone. Therefore, their use is recommended in conjunction with other control methods (Delaplane 1997, Pettis & Shimanuki 1999).

Natural enemies of *V. destructor* have not been identified, but researchers have begun to explore the effects of biological agents that have impacted other mite species (Chandler et al 2001). The entomopathogenic fungi *Hirsutella thompsonii* Fisher and *Metarhizium anisopliae* Metschinkoff caused mite mortality in both laboratory and field studies without negatively impacting bee health (Kanga et al 2002, Kanga et al 2003). Meikle et al (2008) reported that mixtures of wax powder and conidia of *Beauveria bassiana* (Balsamo) Vuillemin increased mite fall without significantly affecting colony health. Other biological control agents, such as *Bacillus thuringiensis* Berliner, may show promise in *V. destructor* control but have yet to be explored (Chandler et al 2001).

Bio-rational alternatives to synthetic treatments include essential oils, sugar esters and organic acids. Essential oils are compounds derived from plant sources that may cause death in other species upon direct contact or ingestion (Imdorf et al 1999). Menthol, which can be obtained from peppermint oil, effectively controls the tracheal mite (Ellis & Baxendale 1997). Many essential oils cause *V. destructor* mortality in laboratory settings, but few of these compounds have been efficacious in field settings (Imdorf et al 1999, Lindberg et al 2000). Sucrose octanoate esters have effectively controlled *V. destructor* mites in temperate apiaries (Sheppard et al 2003, Stanghellini and Raybold

2004), but this efficacy was not reproduced in desert conditions (Sammataro et al 2008).

Organic acids, such as oxalic acid and formic acid, are additional bio-rational chemicals that have been demonstrated to control mites. Oxalic acid was highly toxic to *V. destructor* but exhibited little toxicity to honey bees (Milani 2001, Charriere & Imdorf 2002, Aliano et al 2006). It is not approved for use in the United States. Formic acid is a naturally occurring corrosive that inhibits a cytochrome oxidase complex in the mitochondrial electron transport chain, disrupting cellular respiration and leading to tissue death (Lieivuori & Savolainen 1991, NOD Apiary Products Ltd. 2008). Ritter and Ruttner (1980) first reported on the use of formic acid to control mites in honey bee colonies in Germany. Formic acid has since shown efficacy in controlling *V. destructor, A. woodi,* and *Tropilaelaps clareae* Delfinado and Baker, another brood mite of *A. mellifera* that naturally parasitizes the giant honey bee *Apis dorsata* Fabricius (Hoppe et al 1989). Early formic acid formulations consisted of absorbent materials, such as cardboard or cheesecloth (Liu & Nasr 1992), that had been soaked in diluted (≤ 65%) formic acid. The absorbed formic acid would evaporate when the soaked substrate was placed in a hive (Delaplane 1997). Absorbent pads soaked in diluted formic acid were sold commercially in Germany as “Illertissen mite plates” (IMP). Experimental evidence supported the efficacy of the IMP product in controlling mites (Wachendorfer et al 1985, Hoppe et al 1989), but also revealed queen mortality (Wachendorfer et al 1985) and worker clustering outside of the hive (Hoppe et al 1989) at doses recommended by the manufacturer. Bee disturbance was not observed when the IMP was placed on the

cooler bottom board contrary to label directions (Hoppe et al 1989). Soaked pad treatments relied on high temperatures in the hive to volatilize the acid and provide a brief fumigation. If brood were present, multiple treatments over several weeks were needed to account for mites that had been protected from fumigation inside capped brood cells (Bracey & Fischer 1989). Formic acid treatment was labor intensive, unreliable and potentially hazardous to beekeepers in early formulations (Delaplane 1997).

Modern formulations of formic acid reflect the efforts of researchers to decrease the number of treatments required per hive and reduce beekeeper exposure to the active ingredient. The goal is to develop a long lasting formulation that could be pre-packaged. Nelson et al (1994) fashioned a formic acid gel strip by mixing the acid with a colloid polymer, but the strips did not match the efficacy of liquid or IMP deliveries over a 23-day treatment. A second gel product, the Beltsville formic acid (BFA) gel packet, consisted of 65% formic acid gel sealed in a polypropylene bag. To treat colonies, four slits were made in the bag, which was then placed on the upper bars of the top brood chamber. In spring field tests, BFA packets had higher average efficacy against *V. destructor* than liquid formic treatments (Feldlaufer et al 1997). The BFA gel packet became Apicure™ (Apicure Inc.,Greenwich, NY), which was approved for use in hives (Anonymous 2000) until it was removed from the market after reports of leakage during transport (Elzen et al 2004). Mite-Away™ pads and their successors, Mite-Away II™ pads (NOD Apiaries Ltd., Frankford, Ontario), were another attempt to deliver pre-made formic acid treatments to beekeepers. Calderone and Nasr (1999) reported 56%

mite mortality from Mite Away™ pads, which was significantly lower than the 98% achieved with Apistan®. These researchers concluded that the pads were not releasing formic acid at a rate high enough to achieve mite control. Calderone (2000) later reported that fiber board soaked in a higher dose of formic acid (300 ml of 65% acid) matched the efficacy of Apistan® for fall mite treatment in temperate climates. However, the high dose fiber board treatment is not recommended for use in warmer climates where lack of efficacy and egg and brood death were reported (Elzen et al 2004). Stanghellini and Raybold (2004) reported mite mortality of ~70% using the MiteGone™ (MiteGone Enterprises Inc., Kelowna, BC) pad system, but the release rate (4-6g/day) was still below the recommended 7g/day (Imdorf et al 1996).

Single use, short term formic acid formulations were explored to provide treatment options that would not result in contaminated honey crops. The slow-release formulations of formic acid, such as gel packets and soaked fiber board bags, decreased the need for repetitive treatments, albeit they were shown to contaminate honey crops when used during nectar flows and thus were not viable treatment options at those times (NOD Apiaries Products LTD 2005, vanEngelsdorp et al 2008). If large mite populations were detected during the honey flow, beekeepers were forced to choose between treating the mites and ruining a honey crop or forgoing treatment and risking colony collapse the following fall or winter. Treatments requiring less than 24hr to work would allow beekeepers to treat *V. destructor* while only interrupting the honey flow for one or two days. Short term treatments would need to be strong enough to kill mites inside of capped brood to prevent quick mite population resurgence. Mortality of

mites in capped brood had been observed at high formic acid concentrations applied outside the colony (Calis et al 1998) and several researchers attempted to replicate this efficacy inside the hive. The “Flash” method from Quebec involved the saturation with formic acid of a sliding absorbent pad at the bottom of the hive box and has shown success in limited trials (Chapleau 2003). Armine and Noel (2006) reported 90-95% mite mortality with a single, 24-hr treatment of formic acid using their formic acid fumigator (FAF). However, fumigation with the FAF also caused 25% queen losses unless performed in the presence of Honey-B-Healthy® (HONEY-B-HEALTHY Inc., Cumberland, MD), a feeding stimulant containing water mixed with spearmint and lemongrass oils (Armine and Noel 2006). Later research involving 17hr fumigations resulted in 60% mortality of mites in brood cells and 43% mortality of mites on adult bees without evidence of harm to worker or queen bees (vanEngelsdorp et al 2008).

Fumigation with formic acid has also been explored as a method for treating overwintering colonies indoors. Significant reductions in *V. destructor* populations were observed in colonies fumigated for 48hr with three formic acid doses volatilized by fan. Queen losses were reported at formic acid concentrations above 25ppm of air in the room and 11ppm inside the hive (Underwood and Currie 2004). Further research found average mite mortalities of 93% after 53ppm fumigation for 9d with fewer than 10% worker losses at that concentration. Lower dose and longer-term fumigations fully reduced the effect on worker bees, but also decreased mite mortality to around 60% (Underwood and Currie 2005).

Despite numerous attempts to develop formic acid formulations, only two formulations have been registered by the United States Environmental Protection Agency (EPA) for use in honey bee hives against *Varroa* and tracheal mites. However, only one formulation was still marketed in 2008, namely, Mite-Away II™ pads (EPA Reg No 75710-1). This formulation was registered in 2005 (EPA 2008) for commercial beekeeping. For-Mite™ pads (Mann Lake Ltd., Hackensack, NJ) were registered in 1999 (EPA Reg No 61671-3), but are no longer marketed for commercial use.

The specific conditions required to use formic acid are delineated on the label for Mite-Away II™ pads. The packaging suggests that the product be used as one component of an IPM program to "reduce the number of varroa mites for one season." Treatments should last 21 days and cannot be made while honey supers are on the hives. Temperatures exceeding 82°F (27.8°C) "can cause excessive brood mortality and absconding" if the formic pads are not removed from the hives during heat spells in the first week of treatment. Therefore, local weather conditions must be considered for every treatment location. Furthermore, treated colonies must contain at least 6 frames of bees or colony mortality may result. Brood mortality may result in any treated colony for up to 14 days after pad installation. The conditions outlined on the label should allow the active ingredient to vaporize at doses sufficiently high to kill mites, but low enough to prevent adverse effects on the bees (NOD Apiaries Products Ltd. 2005). Eischen (1998) speculated that treatment success depended on many factors, including hive temperature, position of the formic acid source within the hive, ventilation by workers, colony size, duration of treatment, and brood cycles.

Pest population monitoring is a critical supportive component to develop integrated management. By monitoring pest levels, applicators are able to establish economic threshold levels and restrict chemical treatment to times of need (Caron 1999). Monitoring tools also allow beekeepers to test and compare the efficacy of treatments. Several sampling methods exist to detect mites in colonies, including the sticky board, knockdown with acaricide, alcohol washes, ether rolls, brood uncapping and mite excreta. The sticky board, which consists of a paper card with the upper surface coated in adhesive material and a mesh screen to prevent contact with the bees themselves, allows researchers to track natural or induced mite fall and examine hive debris at the base of the colony. The board fits into the hive entrance and covers the entire bottom area. Sticky boards are relatively easy to use and have been recommended extensively in IPM literature (Anonymous 1987b, Delaplane 1997). Sampling with sticky boards can be done for any length of time to meet monitoring needs (Branco et al 2006). Although the use of sticky boards is itself a subsampling technique, several researchers have attempted to streamline the collection of data from individual sticky boards (Calderone & Turcotte 1998, Calderone 1999, Ostiguy & Sammataro 2000).

The presence of mites on sticky boards has been a reliable indicator of mite infestation (Anonymous 1987b), but the accuracy of using mite drop to predict total colony infestation levels is unclear. Mite population monitoring tools must be accurate to ensure the efficacy of IPM initiatives and lessen the number of unnecessary applications of acaricides. Branco et al (2006) found a strong linear correlation between

natural mite fall and total infestation using two-week sticky board counts in Portugal. The correlation existed only for infestations in broodright colonies not collapsing from varroosis; it had no predictive power for future mite levels. Mite fall rate was independent of total mite population density only in broodright, non-collapsing colonies. Branco et al (2006) reviewed several similar studies and attempted to explain contradictory results. In one of these other studies, Rademacher (1985) measured total infestation one month after sampling mite fall and reported high variability in fall measurements. He suggested that predictive models based on natural mite death might not be accurate. Branco et al (2006) responded to this conclusion by arguing that the variability arose during the month gap between samplings, when mite populations would shift. Milani (1990) reported weak correlations in highly infested colonies. Garza and Wilson (1994) found that sticky boards were the least precise method of tracking whole mite populations when compared to ether rolls and adult bee washes. However, during this study, sticky boards were left in colonies for only 24hrs, a time period that might have been too brief to account for variability in mite distribution throughout the brood (Branco et al 2006). Strange and Sheppard (2001) reported that 48hr sticky board counts exhibited a strong cubic correlation with total infestation levels in broodright colonies in the United States.

Other methods, including knockdown with acaricide, alcohol washes and ether rolls, can be used to sample the mites parasitizing adult bees. Apistan® (Mussen 1994), formic acid (Ellis & Baxendale 1994), and tobacco smoke (De Ruijter & Eijnde 1984) have been used to knock mites onto bottom boards to increase the likelihood of detecting an

infestation. If the Apistan® treatment is maintained throughout a complete brood cycle, a large proportion of the mites will die and the total infestation can be quantified (Stanghellini & Raybold 2004). Mites can also be dislodged from adult bees with fluid or aerosol ether (Anonymous 1987b). De Jong et al (1982) reported that agitation for 30 minutes in 25% alcohol dislodged 100% of phoretic mites from freshly killed adult bees. The solution can be filtered through wire mesh to separate the bees from the solution and loose mites. Later publications recommend shaking bees in 70% alcohol because the higher alcohol concentration preserves the bee bodies for other diagnostic procedures, such as tracheal mite dissections (Anonymous 1987b). The ether roll method involves brushing 300-500 adult bees into a jar and then spraying the bees with diethyl ether and shaking the jar. Mites are dislodged from the bees and stick to the sides of the jar, where they can be counted (Delaplane 1997). Ether roll tests can be performed directly in the field and are less labor-intensive than alcohol washes, sticky boards and brood uncappings (Delaplane 1997), but may not be an accurate predictor of total infestation (Delaplane & Hood 1997). Treatment thresholds for IPM protocols have been published for several regions using this method because it is relatively easy to perform (Mussen 1994, Delaplane & Hood 1997, Caron 1999, Strange & Sheppard 2001).

Brood uncapping and inspection of emerged brood cells for mite excreta are two methods to detect *V. destructor* infestation in bee brood. Uncapping brood cells and removing pupae can reveal reproducing and immature mites prior to bee emergence. Given that the mite preferentially infests drone brood, drone cells should be inspected

when looking for mites (Anonymous 1987b, Delaplane 1997). Erickson et al (1994) reported that white mite excreta, which can be found in every brood cell infested with mites and remains after bee emergence, could be used by beekeepers to quickly detect medium to large mite infestations without using chemicals or uncapping brood.

The following two chapters of this thesis address two aspects of IPM of *V. destructor*. The first study tested the efficacy of the sticky board sampling method as a predictor of total colony infestation. My second study evaluated several treatment regimens involving formic acid pads for control of *V. destructor* in western Washington State. Treatments varying in both timing and frequency of application were compared to determine the best protocol for use of formic acid in the temperate conditions of western Washington.

For Apidologie

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Efficacy of natural mite fall as an estimator of colony wide *Varroa destructor* (Acari: Varroidae) infestation level

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Abstract

Population monitoring is a cornerstone of any integrated pest management program. The sticky board is a popular non-destructive method for measuring the natural mortality rate of *Varroa destructor* (Anderson and Trueman) in honey bee, *Apis mellifera* L., colonies, but its accuracy as an estimator of total mite infestation is uncertain and may vary with climate. This study compared counts from 48hr sticky boards with estimates of total mite populations that were made based on infestations of adult bee and capped brood samples. A significant linear relationship ($r^2 = 0.36$; $F = 48.75$; $df = 1, 86$; $P \leq 0.001$) between the two methods was found using regression analysis and a mathematical model for estimating total infestation is reported.

Introduction

The ectoparasitic mite, *Varroa destructor* (Anderson and Trueman) (Acari: Varroidae), remains a ubiquitous threat to colonies of the honey bee, *Apis mellifera* L. Chemical acaricides are typically used to control mite infestations, but populations resistant to each of the three most widely used acaricides have been discovered (Lodesani et al 1995, Elzen et al 2000, Elzen and Westervelt 2002). To preserve the efficacy of failing chemical treatments for emergency use, beekeepers must adopt alternative control measures.

Population monitoring is a vital component of successful integrated pest management (Caron 1999). Beekeepers must be able to track mite populations to restrict chemical treatment to those infestations likely to cause economic injury to the colony (Delaplane et al 2005). *V. destructor* populations can be quantified by counting the mites killed by a chemical acaricide treatment. The method has been used to study the efficacy of novel chemical treatments (Ellis et al 1988, Stanghellini & Raybold 2004) and to gauge the spread of the mites through new territories (De Ruijter and Eijnde 1984). However, it is not useful for periodic IPM monitoring, because it requires the use of acaricides and is destructive to the mite population.

Several non-acaricide sampling methods can be employed to detect the presence of mites in colonies. The ether roll and alcohol wash methods separate phoretic mites from adult bees by shaking the pair in a medium (Anonymous 1987). Ether rolls can be

quickly and easily performed in the field, but the method may fail to dislodge all mites in a sample (Caron 1999) or to detect small or new infestations (Delaplane 1997). Alcohol washes are more labor intensive to perform, but will remove 100% of mites if shaking is maintained for 30 min (De Jong et al 1982). Brood cells can be uncapped and inspected for reproductive and immature mites, but this method is time consuming and may require large sample sizes to detect mites. Additionally, bottom boards collect hive debris and can be used to measure natural mite fall. Although bottom board sampling requires two trips to the apiary for each sample set, it is the least destructive method to sample mites (Anonymous 1987).

Each of the above methods has proven useful to detect *V. destructor* infestation, but the reliability of each as an estimator of total mite infestation requires further study. Previous research on the predictive value of sticky boards has yielded various conclusions and recommendations regarding the interpretation of sampling data (Branco et al 2006). It is imperative to the success of integrated management of *V. destructor* that beekeepers obtain reliable estimates of infestations inside their colonies (Delaplane 1998). In this study, the reliability of natural mite fall as an estimator of total mite infestation was examined. Data from sticky boards were compared to total mite population estimates that were extrapolated from samples of adult bees, brood cells and bee population data.

Materials and methods

An apiary of 60 uniform colonies was established in Puyallup, WA, in June 2007. Worker bees from 24 de-queened colonies were shaken into a population cage to homogenize worker and mite populations. Single story Langstroth hive bodies, each containing approximately two frames of brood, two frames of honey, one frame of pollen, three empty frames and a frame feeder, were set up around the apiary. The bees were distributed in 0.92 kg portions to each hive. Colonies were re-queened with 12 new queens from a commercial queen breeder and 48 new queens from the Washington State University queen breeding project, which selects queens based on several factors, including hygienic behavior, *V. destructor* resistance, and honey production.

Sampling

Estimates of the mite population size in each colony were derived by three sampling methods: (1) counts of mites in samples of adult bees and brood cells extrapolated to the colony level based on bee population measurements; (2) natural mite drop; and (3) the number of mites knocked down by formic acid treatment. The sampling of each colony began by weighing the hive equipment while bees were present with a hanging scale on a tripod. The queen was caged and removed during sampling. All other bees in the colony were shaken into a population cage for holding. A sample of approximately 300 adult bees was collected from the cage with a scoop into a dry, pre-weighed 250ml polypropylene bottle. Bees in the cage had been shaken from all

frames in the colony and thus a sample from the cage represented the full age spectrum of adult bees. Total brood area was measured in 25 cm^2 square units using a clear clipboard marked with a grid of 5 cm X 5 cm squares. One frame of capped brood was chosen at random and a horizontal transect was set across the frame. Two-hundred purple-eyed worker pupae were removed with forceps from brood cells along the transect and examined for mites. All frames were then returned to the hive box and the empty equipment was weighed. The bees and queen were then replaced into the colony. The sample bottles containing the adult bees were later weighed in the laboratory. Ethanol (70%) was then added to the bottles, which were inverted and mechanically shaken for 1hr over mesh screens to separate mites from the bees. Finally, mites and bees were counted for each sample.

Total adult bee weight was calculated by subtracting the weight of empty equipment from the weight of the same equipment filled with bees. The weight of the bees in a bottle divided by the number of bees in the bottle gave the average worker bee weight. Total bee weight was divided by the average worker weight to estimate the total number of adult workers in the colony. The number of mites counted during the alcohol wash was divided by the number of bees in the bottle to obtain a mite-to-adult bee ratio, which was then multiplied by the total number of adult bees to estimate the total number of mites on adult bees in the colony. Total brood area was calculated by multiplying the number of 25 cm^2 square units found in each hive by 25 cm^2. The number of capped brood cells in the colony was calculated by multiplying the total brood area by 4.285 cells/cm^2 (Strange & Sheppard 2001). Dividing the number of mites per 200 cells by

200 and multiplying that mite-per-cell figure by the total number of cells calculated the total number of mites in capped cells. Adding the mite totals for adults and brood gave the total infestation estimate. The ratio of mites per 100 bees was calculated by dividing the estimated total number of mites in the colony by the estimated total number of bees in the colony and then multiplying that figure by 100.

Natural mite fall and mite fall induced by formic acid treatment were estimated using sticky boards to collect hive debris (Delaplane & Hood 1997, Strange & Sheppard 2001). Litter fell from the colony onto a 32 by 38.5-cm paper card with a 28 by 33-cm green grid printed on the top. The grid area was smeared with petroleum jelly (Vaseline®, Unilever United States, Inc., Englewood Cliffs, NJ). An 8-mesh screen, held by a wooden frame placed on top of the card, prevented bees from accessing the card surface and becoming stuck or removing dead mites. Sticky boards collected hive debris for 48hr periods prior to removal and analysis. The rate of mite mortality was determined by dividing the natural mite drop level by the total mite population estimate for each colony. Formic acid treatment was applied to eight colonies in late July. Sticky boards were used to measure mite mortality for 48hr after the application of Mite-Away II™ formic acid pads (NOD Apiaries Ltd., Frankford, Ontario) according to label directions.

Colonies were monitored and sampled from July 2007 through May 2008. The mite and bee populations were sampled and estimated five times (July 1 2007, July 27 2007, October 10 2007, April 10 2008, May 15 2008) during the experiment. On the first

sampling date, all colonies were sampled. Fifteen colonies were randomly selected and sampled on each of the next two dates. All remaining colonies were sampled on the final two dates. Affected colonies were removed from the experiment upon the discovery of any of the following signs of brood interruption: presence of American foulbrood infection, queen loss, queen supersedure, and colony collapse. Twenty-one colonies were removed during the first month of the experiment. By May 2008, only seven of the original sixty colonies remained in the experiment. Natural mite fall was measured for each sampled colony at all five dates.

Statistical Analyses

Distributions of the raw estimated total mite populations, raw natural mite fall counts, raw induced mite fall counts, and raw estimated mites-per-bee ratios were non-normal. Therefore, log transformation was used to normalize all four sets of data. Regression analysis was used to test the relationship between sticky board sampling counts and both total mite population estimates and mite-per-bee ratios estimated from adult bee and brood infestation levels (SPSS 1998). Regression relationships were considered significant at the $\alpha \leq 0.05$ level.

Results

The absence of brood rearing in colonies resulted in a sharp increase in mite mortality rate. Data taken from broodless colonies were identified as outliers and their removal increased the fit of regression analyses. Eighty-eight observations were analyzed after the removal of data from four broodless, queen-right outliers. For natural mite fall to be

a useful linear predictor of total mite population, the rate of natural fall must be independent of the mite population density (Branco et al 2006). Correlations between natural mite death rate and both total mite population and the number of mites detected per 200 brood cells were tested. In both cases, the weak correlation provided evidence that the rate of mite death was independent of mite population density. The Pearson correlation coefficient between death rate and total population and between death rate and the ratio of mites per cell was –0.305 (r^2 = 0.09; F = 8.84; df = 1, 86; $P \leq 0.004$) and –0.194 (r^2 = 0.04; F = 3.53; df = 1, 86; $P \leq 0.071$), respectively.

Figure 1 depicts the significant linear relationship (r^2 = 0.36; F = 48.75; df = 1, 86; $P \leq$ 0.001) between 48hr sticky board counts and total mite population estimates. The linear regression model log (mite drop) = 0.0501 log (mite drop) + 4.24 was generated to estimate total mite population from 48hr mite mortality counts. The residuals for the model were normally distributed and independent with an autocorrelation estimate of r = 0.185. The standard errors and p-values for the coefficients in the model were 0.291 and 0.001 for the slope ($ß_1$) and 0.072 and 0.001 for the intercept ($ß_0$).

A significant linear relationship (r^2 = 0.114; F = 11.07; df = 1, 86; $P \leq 0.001$) was also found between 48hr sticky board counts and the ratio of mites per 100 bees for broodright colonies (Fig. 2). Residuals for the regression were normally distributed and independent with an autocorrelation estimate of r = 0.163.

Natural mite fall measured for 48hr prior to treatment with formic acid was compared to mite fall measured for the 48hr-post-treatment period in eight colonies. The linear regression between the two variables (r^2 = 0.74; F = 16.93; df = 1, 6; $P \leq 0.006$) is described by the equation log (mite drop post-tx) = 2.213 log (mite drop pre-tx) + 0.910, but the intercept estimate ($ß_0$) was not found to be significant ($P \leq 0.059$). The residuals for this model were independent and normally distributed with an autocorrelation estimate of r = -0.085 (± 0.137 SE). Figure 3 depicts the comparison between 48hr mite mortality and the mites killed by formic acid treatment.

The number of mites killed in 48hr by formic acid treatment was also compared to the total mite population estimated from adult bee and brood samples in the eight treated colonies. The regression analysis (Fig. 4) between the two variables exhibited a poor linear relationship (r^2 = 0.18; F = 1.32; df = 1, 6; $P \leq 0.294$) with insufficient evidence (p = 0.294) to reject the null hypothesis that $ß_1 = 0$.

Discussion

Sampling adult bee and capped brood infestation has proven to be a precise method for estimating *V. destructor* populations (Strange & Sheppard 2001, Branco et al 2006), but requires a large amount of labor for each colony sampled and is destructive in nature and is thus impractical for periodic use by beekeepers. A significant linear relationship between natural mite fall and the estimated total colony infestation was demonstrated in this study. These findings should enable beekeepers to use a relatively simple method, namely sticky boards, to more accurately monitor total mite infestations in their colonies.

Furthermore, if properly protected with a mesh screen, sticky boards are non-destructive to bee populations. The mathematical relationship reported here is only valid for use in colonies rearing brood at the time of sampling and under the assumption that mite death rate is independent of total mite infestation. These conditions match the findings of previous studies making similar comparisons (Strange & Sheppard 2001, Branco et al 2006).

The weak relationships observed between the number of mites killed by formic acid exposure and both natural mite fall and total infestation estimates can be explained by errors in the experimental design. Formic acid pads have shown low efficacy as acaricides relative to synthetics, such as Apistan® (tau-fluvalinate; Wellmark International, Bensenville, IL). There is no acaricide that will kill 100% of mites at each application, but formic acid may kill as few as ~60% of mites (Calderone & Nasr 1999). Low treatment efficacy would lead to mite drops that would underestimate the total mite infestation. Formic acid delivered on an absorbent pad was a less sensitive indicator of *V. destructor* populations than Apistan® in an earlier study (Ellis & Baxendale 1994). The sampling period of 48hr was also insufficient to measure the proportion of mites in capped brood, which later would be exposed to the full effects of the formic acid treatment over 21 days. Sticky boards should have been used to track mite drop throughout the entire tenure of the formic acid pads in the colonies as was done by Branco et al (2006) using Apistan® for the same purpose. Finally, increasing the sample size beyond the eight colonies tested for the relationship might elucidate trends undetected by the analysis presented here.

Branco et al (2006) previously reported a strong linear relationship between natural mite fall and total mite populations estimated from infestations of adult bee and brood cells samples. This study differed from the previous one in the sampling methodology for the total populations and in the duration of sticky board sampling. Our study measured brood area directly in the field using 25cm^2 units, while Branco et al (2006) measured the area from video recording of brood frames. The authors estimated total adult bee population by comparison of photographed frames to calibrated photographs, while we used hive weight to estimate bee population. The most important difference between the studies was the duration of sticky board measurements. Branco et al (2006) used week long sticky board samples, which were five days longer than the 48hr regime used in this study. They also claimed that a week was necessary to account for daily variability in natural mite fall. However, this study indicates that a shorter monitoring period can produce accurate results. Using 48hr sticky board monitoring will allow beekeepers to receive accurate information about mite infestation in a shorter period of time.

Strange and Sheppard (2001) reported a strong relationship between 48hr sticky board counts and mite per bee ratios in broodright colonies, but their model was cubic. Although a cubic model can statistically analyze the strength of a relationship between two variables, the practical biological application of such a model is unclear (Delaplane & Hood 1999). A non-linear model implies the rate of mite death changes with the density of the mite population. The model formulated in this study is linear in nature and

assumes a density independent mite death rate. The assumption of independence is supported for broodright colonies by weak Pearson correlation coefficients between mite death rate and both total mite population and the ratio of mites to capped brood cells.

The mean daily mite fall in this study was calculated to be 0.043 (± 0.010 SE) (day $^{-1}$), which is larger than the rate of 0.011 (day $^{-1}$) reported by Branco et al (2006). The authors of that study suggest that variations in mite drop rate might be explained by differences between the climates and honey bee races involved in various studies. Hygienic behavior may also be contributing to the increased rate of mite fall observed in this study. The number of samples taken from colonies with selected queens outnumbered that taken from colonies with commercial queens, 71 to 17. Hygienic behavior involves the uncapping and removal of damaged and diseased larvae and pupae (Spivak 1996) and should increase the rate of mite drop in the colony. When data from colonies with commercial queens was separated from those colonies with selected queens, the average mite drop rate differed significantly ($t = 3.64$, $P \leq 0.001$) between the groups. Mean mite drop rate for the commercial and selected groups was 0.028 ± 0.013 (day $^{-1}$) and 0.048 ± 0.011 (day $^{-1}$), respectively.

Integrated pest management (IPM) regimens have shown success in decreasing levels of *V. destructor* infestations (Delaplane et al 2005) and likely hold the solution to the emerging problem of acaricide resistance. This study demonstrated that sticky boards reliably estimate total mite infestation levels when used to measure natural mite fall for

48hrs inside colonies. Sticky boards should be used in IPM programs to monitor mite populations and to delay the application of pesticides until necessary to prevent economic damage (Delaplane & Hood 1997, Strange & Sheppard 2001).

Acknowledgements

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Figure captions

Fig. 1. Comparison of 48hr natural mite fall in colonies measured by sticky board and total mite population estimated by sampling adult bee and capped brood infestation. Both variables have been log transformed.

Fig. 2. Comparison of 48hr natural mite fall in colonies measured by sticky board and the ratio of mites per 100 bees those colonies. Both variables have been log transformed.

Fig. 3. Comparison of 48hr natural mite fall in colonies measured by sticky board and the number of mites killed by formic acid treatment in those colonies. Both variables have been log transformed.

Fig. 4. Comparison of mites killed by formic acid treatment measured by sticky board and the total mite population estimated by sampling adult bee and capped brood infestation. Both variables have been log transformed.

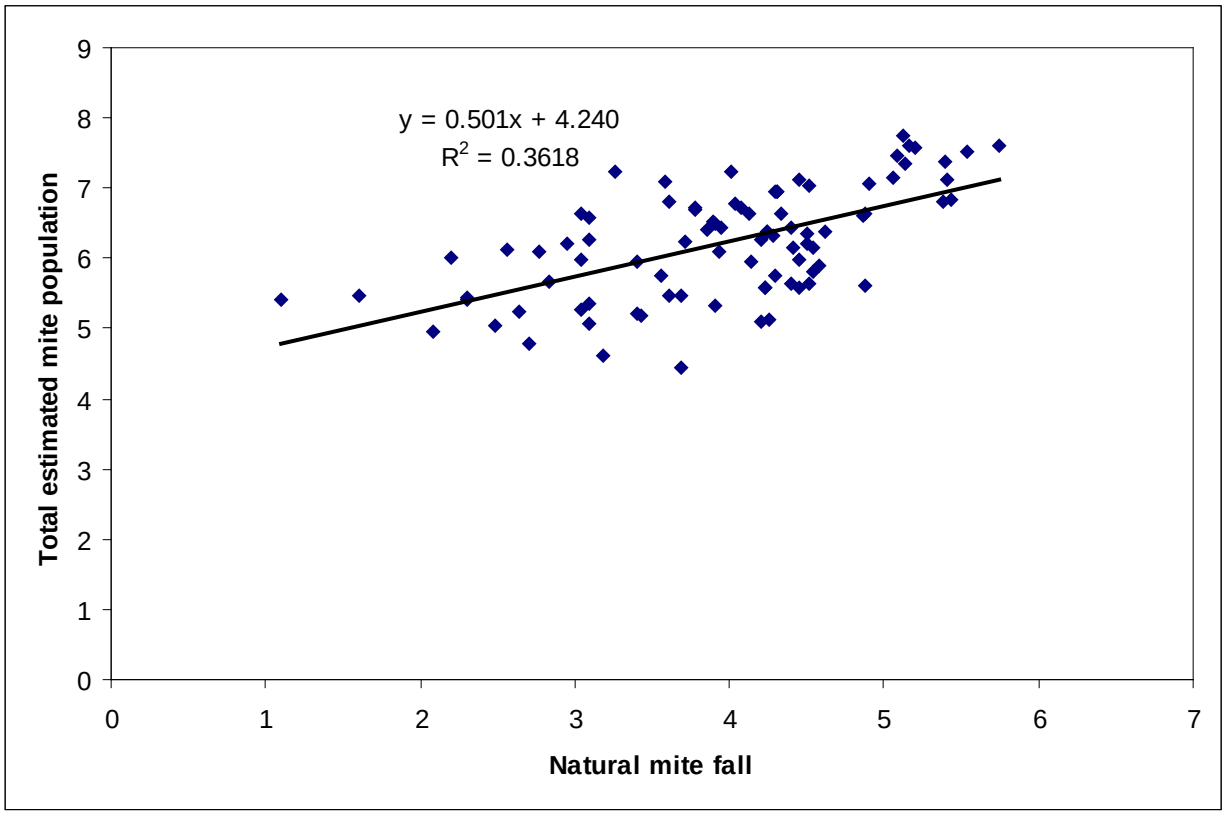
y = 0.501x + 4.240
R2 = 0.3618
Total estimated mite population
Natural mite fall
0
1
2
3
4
5
6
7
8
9

Figure 1.

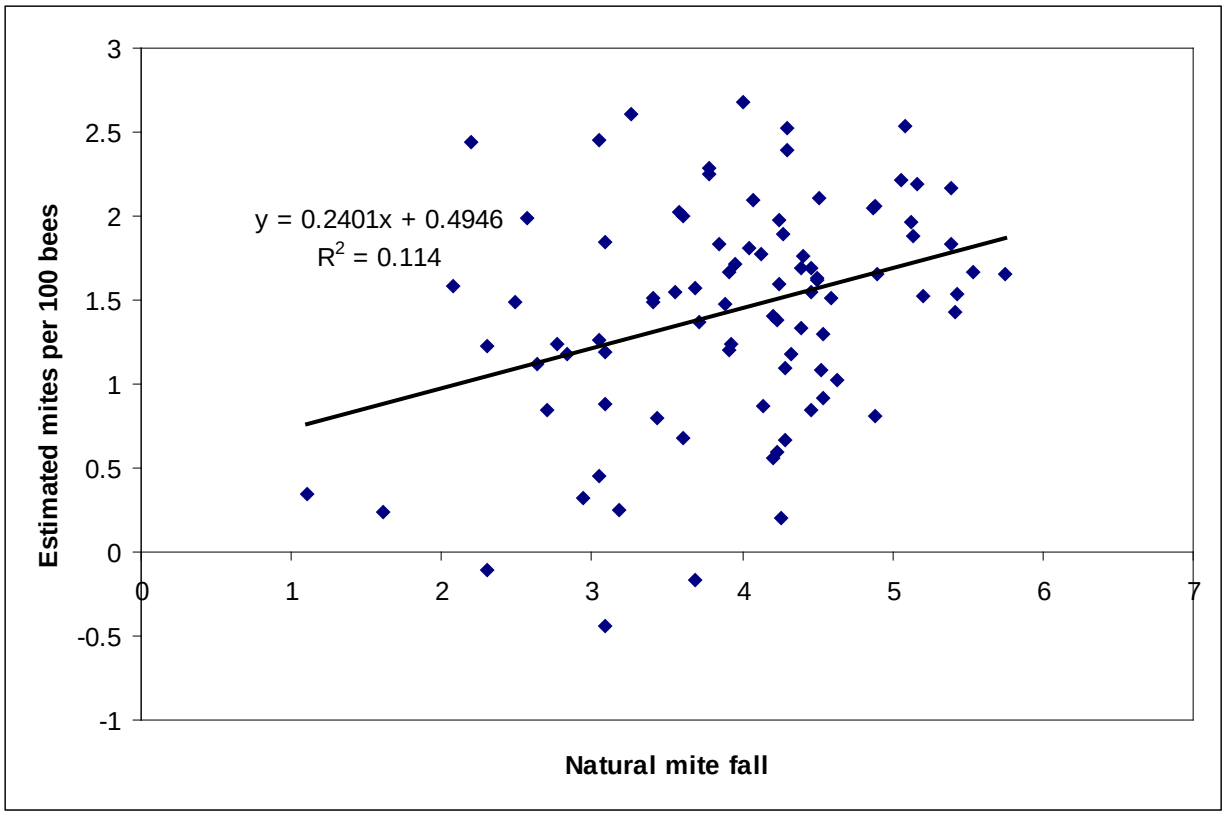
y = 0.2401x + 0.4946
R2 = 0.114
Estimated mites per 100 bees
Natural mite fall
3
2.5
2
1.5
1
0.5
0
-0.5
-1
0
1
2
3
4
5
6
7

Figure 2.

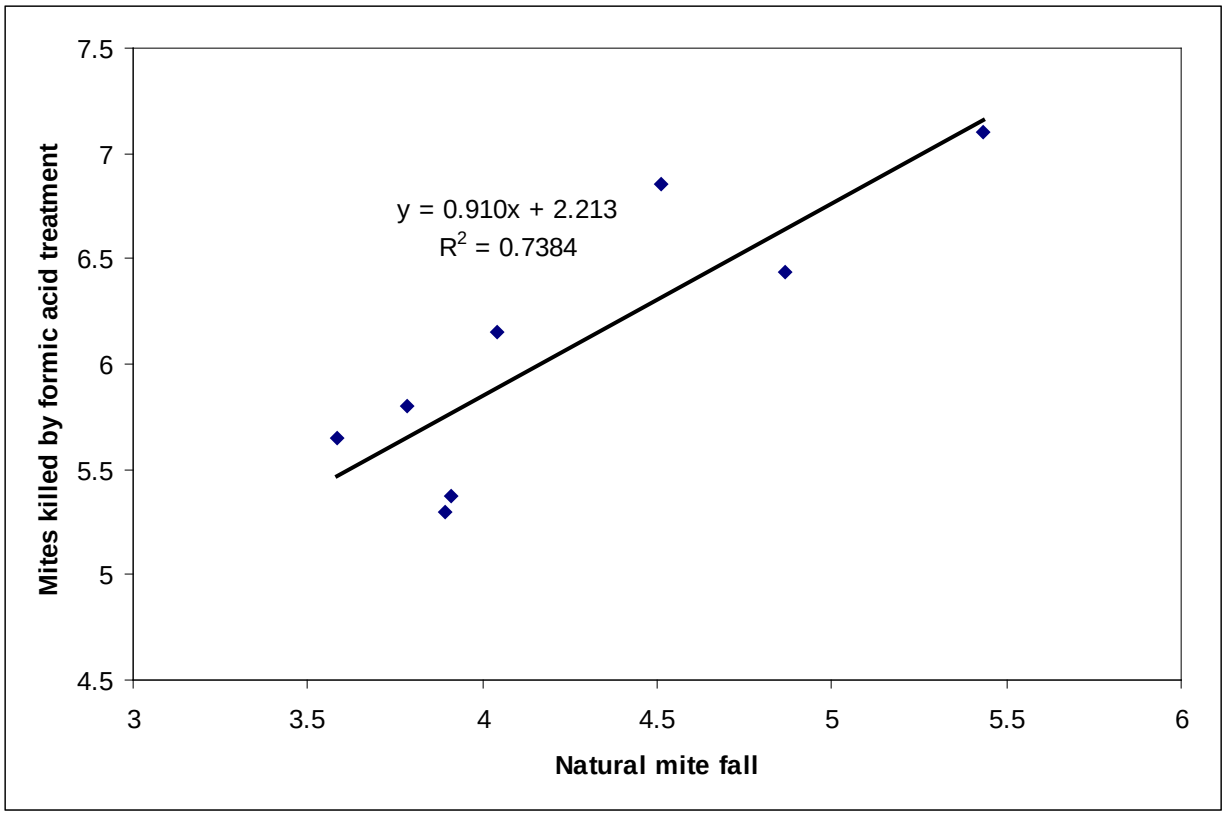
7.5
7
6.5
6
5.5
5
4.5
3
3.5
4
4.5
5
5.5
6
Mites killed by formic acid treatment
Natural mite fall
y = 0.910x + 2.213
R2 = 0.7384

Figure 3.

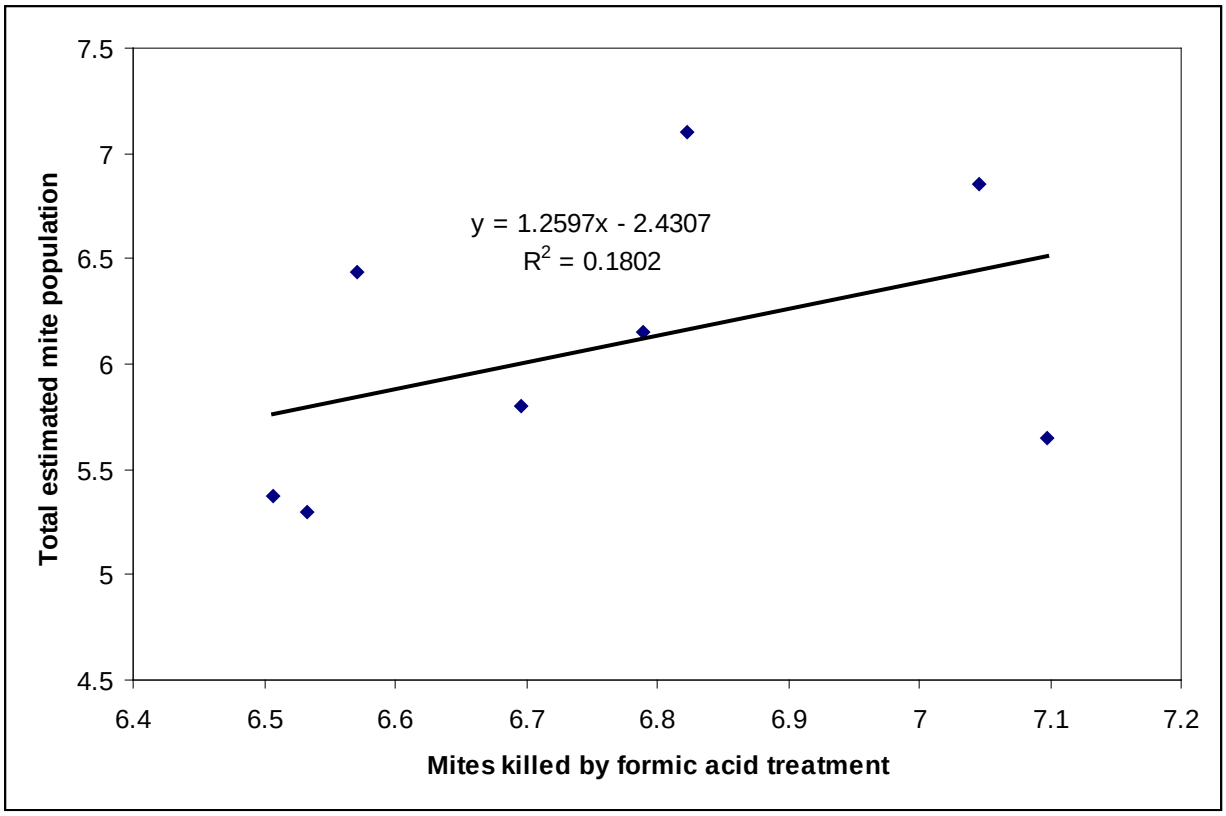
7.5
7
6.5
6
5.5
5
4.5
6.4
6.5
6.6
6.7
6.8
6.9
7
7.1
7.2
Total estimated mite population
Mites killed by formic acid treatment
y = 1.2597x - 2.4307
R2 = 0.1802

Figure 4.

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Control of *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) with formic acid in western Washington State, USA

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Abstract

Formic acid has been explored as a bio-rational alternative to synthetic acaricides for the control of *Varroa destructor* (Anderson & Trueman) in honey bee colonies. Formic acid treatments for the management of *V. destructor* were tested in western Washington State, USA. The treatments were: (1) formic acid in August; (2) formic acid in August and October; (3) formic acid delivered when mite populations exceeded published economic thresholds; (4) control (commercial stocks) - no treatment; (5) control (WSU stocks) - no treatment. Treatment with formic acid had no significant effects on bee population size, adult bee weight, or brood area. Mite levels were significantly diminished after August treatment, but rebounded 7-8 months later. No untreated colonies survived the winter. No significant differences between treatment groups in bee and mite population parameters were found in April after the colonies had overwintered, but treated colonies had higher survivorship than those not treated.

Introduction

Varroa destructor (Anderson and Trueman), an ectoparasitic brood mite, is a serious pest of managed honey bees, *Apis mellifera* L., worldwide. Mite infestation is lethal to most colonies if control measures are not implemented (De Jong 1997). Two synthetic acaricides, Apistan® (tau-fluvalinate; Wellmark International, Bensenville, IL) and Checkmite+™ (coumaphos; Bayer, Shawnee Mission, KS), are widely used, often prophylactically, to control mite infestations. However, populations of the mite in Europe (Lodesani et al 1995, Colin et al 1997, Spreafico et al 2001, Thompson et al 2002), the United States (Elzen et al 1999, Elzen & Westervelt 2002), Israel (Mozes-Koch 2000), and New Zealand (Goodwin et al 2005) have exhibited resistance to one or both of these compounds. Alternative control methods must be implemented to preserve the efficacy of these acaricides for emergency situations.

Several integrated management techniques have been developed to control *V. destructor*, namely: mechanical measures, such as screened bottom boards (Pettis & Shimanuki 1999), pollen traps (Hart & Nabors 1999) and dusts (Fakhimzadeh 2001); genetic controls, such as selection for behavioral resistance to the mite (Harbo & Harris 1999, de Guzman et al 2007, Ibrahim et al 2007); and biological control, involving entomopathogenic fungi (Kanga et al 2003, Meikle et al 2008). Additionally, many “soft chemicals” and natural products, such as essential oils, sugar esters and organic acids, have been tested as replacements for synthetic chemical control products. Numerous essential oils and other botanical extracts have shown acaricidal properties in laboratory settings, but few have proven useful for control of *Varroa* in field studies (Imdorf et al

1999, Lindberg et al 2000). Sugar esters offer an effective, albeit labor-intensive alternative to synthetics (Sheppard et al 2003). Organic acids, such as citric acid (Milani 2001), lactic acid (Kraus & Berg 1994), oxalic acid (Aliano et al 2006, Rademacher & Harz 2006) and formic acid (Calderone 2000) have also been considered as replacement chemicals.

Formic acid has been the most efficacious of the organic acids in mite control and has been shown to kill *V. destructor, Acarapis woodi* (Rennie), and *Tropilaelaps clareae* (Delfinado and Baker) (Hoppe et al 1989). However, successful control of *Varroa* mites with formic acid has not been consistently demonstrated. Reported mite mortalities vary widely between studies and several authors suggest that multiple formic treatments are required to match the control power of a single Apistan® or Checkmite+™ application (Feldlaufer et al 1997, Eischen 1998, Calderone & Nasr 1999, Elzen et al 2004).

Two formulations of formic acid are registered for use in the United States, but only one, Mite-Away II™ (NOD Apiaries Ltd., Frankford, Ontario), is currently available for commercial use. The Mite-Away II™ formulation consists of an absorbent pad impregnated with formic acid and wrapped in a perforated plastic bag. The pads resemble early experimental formic acid applicators, but are shipped prefabricated and packaged to minimize beekeeper exposure to the acid. To accommodate the pad, a 3.5cm (1.5”) rim is placed between the top box and lid of the hive and the pad is elevated 1cm above the frames using two sticks. The pad fumigates the hive for three weeks.

Mite-Away II™ is marketed as a single-use application to “reduce the number of varroa mites for one season” as “part of an Integrated Pest Management (IPM) program” (NOD Apiaries Products Ltd. 2005), rather than to “control” mites as is Apistan® (Wellmark International 2002). Treatment efficacy and bee safety in formic acid use depend on a variety of conditions, but appear particularly sensitive to changes in temperature. Mite-Away II™ may cause brood damage at temperatures above 82ºF (27.78ºC) (NOD Apiaries Ltd. 2005). The contingency of successful use of this product to climatic conditions necessitates that it be evaluated for reliability and safety under conditions in which it may be employed.

The objective of this study was to evaluate the efficacy of slow-release formic acid pads for control of *V. destructor* in honey bee colonies in western Washington State. Mite and bee populations were periodically monitored over the course of one year to track the effects of formic acid delivered in late summer, fall or at both times. Winter survivorship and spring colony health indicators were compared between treatment groups.

Materials & Methods

An apiary of 60 uniform colonies was established in Puyallup, WA in June 2007. The worker bees from 24 de-queened colonies were shaken into a population cage to homogenize worker and mite populations. Combinations of approximately two frames of brood, two frames of honey, one frame of pollen, three empty frames and a feeder

frame, were placed into single-story Langstroth hive bodies each with a bottom board, lid, and hive stands. The hives were grouped into five circles of twelve. Within each circle, hives were approximately 2m apart and roughly 6m separated adjacent circles to minimize bee drift between groups. Each hive box received 0.92kg of bees shaken from the population cage. Sixty newly mated queens were used to queen the colonies. Queens were placed into the hive boxes in corked queen cages and released 72 hours later.

Each of the five circles was randomly assigned one of five treatments. Three circles received formic acid treatment for the control of *V. destructor*. Formic acid was delivered using Mite-Away II™ pads in accordance with label directions. The two groups not treated with formic acid were established to compare the performance of different genetic lines against *V. destructor* mites in the absence of chemical treatment. One of the two untreated circles was queened with Washington State University (WSU) selected queens, while the other was given commercially-available queens. The WSU Queen Breeding Project annually selects and breeds queens based on multiple criteria, including hygienic behavior, mite resistance and honey production (W.S. Sheppard unpublished data). All three formic acid treated groups had queens from the WSU program. The five treatments were: (1) control (no chemical treatment) using WSU queens (CW); (2) formic acid in August or October based on monitoring mite populations (IM); (3) control (no chemical treatment) using commercially-available queens (CC); (4) formic acid delivered in August and October (AO); (5) formic acid in August only (AU). Mite populations were monitored in IM colonies using sticky boards.

Individual colonies were treated with formic acid if sticky board counts exceeded the treatment thresholds for Washington State published by Strange and Sheppard (2001).

Sampling

Bee and mite populations were monitored using the methodology described in Chapter One of this thesis. Calculations of total mite infestations and mite-per-bee ratios also followed those outlined in Chapter One. Colonies were maintained from June 2007 through May 2008. The mite and bee populations were sampled and estimated five times (July 1 2007, July 27 2007, October 6 2007, April 15 2008, May 22 2008) during the experiment. On the first sampling date, July 1 2007, all colonies were sampled. A subsample of fifteen randomly selected colonies was taken on each of the next two dates, in late July and early October 2007. All remaining colonies were sampled on the final two dates, in April and May 2008. Colonies were removed from the experiment and analyses if they experienced queen mortality or supersedure, succumbed to varroosis, or showed signs of American foulbrood.

Analyses

Analysis of variance (ANOVA) was used to compare the effects of treatment on bee and mite population variables between groups at each sampling date. Effects were analyzed for a completely random design ($\alpha \leq 0.05$) (SPSS 1998). Data from colonies removed from the experiment were discarded from analysis. Colonies that had not surpassed their initial weight of 0.92 kg by the following April were considered to be economically damaged. A colony weight of 0.92 kg correlated with a brood area of 943

cm^2 in the spring. Mite levels that correlated with spring colonies with less than 0.92 kg of bees or 943 cm^2 were considered damaging (Strange & Sheppard 2001). For survivorship calculations, the initial number of colonies in each group was taken as the number of colonies present at the end of July. Colony losses (21) prior to July were attributed to queen supersedure and American foulbrood. The number of experimental colonies (39) remained constant between sampling dates in early and late July.

Results

Bee population, average bee weight, and brood area

No significant differences among groups were found in total bee weight or estimated total bee populations at the outset of the experiment or at any subsequent sampling date. Average bee weight did not differ significantly between groups at any sampling period. Brood area (cm^2) was not significantly different between treatment groups at any of the sampling dates. Mean brood area in the April 2008 was higher in groups AU and AO than in the other three groups, but the difference was not significant.

Colony V. destructor populations

At the first sampling date, CW colonies had a significantly higher mite-per-bee ratio than did IM colonies. However, IM colonies were not significantly different from any of the other three treatment groups at that date. No significant difference in mean mite-per-bee ratios was found between groups in subsample data taken four weeks later. At the October sampling date, colonies that received formic acid treatment in August had a significantly lower mean mite-per-bee ratio and mean mite drop when compared to the

colonies not treated. These differences in mite population estimates were not accompanied by significant differences in mean brood area, mean individual bee weight, or mean estimated total bee population. The mean mites-per-100 bees ratio in treated colonies was 1.64 ± 0.32 eight weeks after treatment with formic acid. No significant effects of formic acid treatment in 2007 were found on 2008 spring mite populations or mite-per-bee ratios.

Winter survivorship and percentage of colonies falling below damage threshold

The damage threshold for colonies in the spring was set at a total bee weight less than the starting weight of 0.92 kg or a brood area of less than 943 cm^2. No colonies (0%) from the CW group were above the damage threshold at the April sampling date. Of the seven colonies initially in the CW treatment group, two colonies died before the October sampling date, three more died over the winter, one was queenless in the spring and one had a brood area below the damage threshold in the spring. In the IM group, which had nine colonies in July 2007, four died by October, four more died by April, one was queenless in April and one was broodless in April, leaving no colonies (0%) above the damage threshold in the spring. The CC group had eight colonies in July. By the following April, only one CC colony (12.5%) was above damage thresholds. Group AO had ten colonies in July, four of which (40%) were above damage levels in the spring. The final group, AU, had five colonies in July, but only two of them (40%) were above damage thresholds in the following spring.

Discussion

Large-scale winter losses in groups CW, CC, and IM constrained the statistical analysis of spring data and the calculation of fall treatment thresholds. The assertions about spring populations were based on calculations that used these small sample sizes. No correlation was detected between fall sticky board counts and the likelihood of colonies surpassing damage thresholds in the spring. Therefore, we were unable to make recommendations for fall treatment thresholds (Strange & Sheppard 2001). Furthermore, this experiment provided no insight into the relative performance of the WSU-selected queens. The untreated control groups (CW and CC) did not differ significantly in any measured variable throughout the experiment. Again, the low winter survivorship limited our ability to evaluate relative performance between the control groups.

The complete demise of the IM group would seem to indicate that an integrated management approach to treatment was harmful to the bees. However, such a claim makes little sense in light of the methodology used to treat the IM colonies. The IM group exhibited lower survivorship than groups AO and AU, despite not having significantly different initial bee or mite populations and receiving formic acid treatments identical to those administered to AO and AU colonies. Five of the nine colonies in this group received treatment on the same day in August and with the same formic acid product as colonies in groups AU and AO. Of the five IM colonies treated in August, three were dead by October and two were dead by April. A sixth IM colony received treatment in October and this colony was considered damaged in April. Of the three

untreated colonies, one died before October and the other two were dead by April. Contrary to our findings, Delaplane et al (2005) demonstrated that using population monitoring to time treatments controlled *Varroa* populations in the field.

The fate of the IM group along with the losses observed in the other groups, suggested that factors other than *V. destructor* infestation affected the experimental colonies. Along with the CW group, IM lost every colony. Groups CW and IM were located at the southern side of the apiary. Other pathogens may have weakened the experimental colonies. *Nosema sp.* spores were detected retroactively in the surviving colonies (M. Smart unpublished data) and may have contributed to the high winter losses. Furthermore, many of the colonies were weak entering the winter. More intense feeding and management in the fall may have bolstered the colonies to levels more apt to survive the winter.

Despite the lack of spring data for groups CW, CC and IM, we were able to examine the effects of formic acid treatment on AU and AO colonies. No significant differences were found between the AU and AO treatment groups for spring bee or mite populations. The percentage of colonies above spring damage thresholds was identical between groups AO and AU. This suggests that a single application in August was sufficient to control mites over the winter. However, such a recommendation must be taken with caution. Both of these groups lost 60% of their colonies. If treated colonies from the IM group are also incorporated into the analysis, then 75% of treated colonies were either dead or damaged by the spring. Formic acid treatment did provide increased colony

survival compared to lack of treatment, but this observation is not practically helpful as losses in the treated groups were still unacceptably high.

Mite-Away II™, which uses a slow-release packet formulation, may be best marketed to suppress rather than control *V. destructor* populations in western Washington State. Formic acid has been demonstrated to have both equivalent (Calderone 2000) and lesser (Eischen 1998) efficacy relative to fluvalinate. The results of this study suggest that formic acid may not be as effective as fluvalinate in killing *Varroa* in Washington State. The mean mites-per-100-bees ratio eight weeks after formic acid treatment was 1.64 ± 0.32 and was 6.42 ± 1.12 at the April sampling date. These figures were higher than the values of 1.22 ± 1.04 twelve weeks after August fluvalinate treatment and 2.49 ± 2.40 in April reported by Strange and Sheppard (2001).

Temperature likely did not confound formic acid treatment efficacy in the experimental apiary. The average temperature during the August formic acid treatment was 17.2ºC (63.0ºF). In Puyallup, WA the average temperature in August ranges from 10.1ºC (50.1ºF) and 25.6ºC (78.0ºF) (Western Regional Climate Center 2008), which is almost identical that recommended for treatment on the label, 10ºC (50ºF) to 26ºC (79ºF) (NOD Apiaries Ltd. 2005). Average maximum and minimum temperatures in Puyallup, WA respectively range from 7.9ºC (46.3ºF) and 0.0ºC (32.0ºF) in January to 25.7ºC (78.2ºF) and 10.2ºC (50.3ºF) in July. Temperatures exceeding 37.8ºC (100ºF) have been recorded in Puyallup. Average rainfall is 85.19 cm (33.54”) (Western Regional Climate Center 2008). Formic acid was applied on July 31, 2007 and remained in the colonies

for 21 days until August 20, 2007. During that time, the temperature exceeded the label removal limit of 82ºF (27.78ºC) on two days during the first week of treatment (Weather Underground 2008). This oversight potentially caused damage to the treated colonies (NOD Apiaries Ltd. 2005), although none was detected at the October sampling date. Rain fell on the apiary during three days of treatment (Weather Underground 2008).

In conclusion, formic acid appeared to decrease *V. destructor* infestations in western Washington State, but may be inadequate to control them. Formic acid is a popular alternative to synthetic acaricides and should be useful for western Washington beekeepers when coupled with other IPM tools (Webster et al 2000). Beekeepers in this area must be wary of temperature spikes when using Mite-Away II™ pads in July and August. The losses observed in the IM group are likely attributable to factors other than the IPM strategy employed and thus, we encourage beekeepers to time formic acid treatments based on mite population monitoring.

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Discussion

The regression analysis of sticky board counts versus estimated total mite population demonstrated that sticky boards are reliable tools to monitor mite infestations. We were able to report a significant linear relationship using 48hr sticky boards. Previous reports presented non-linear relationships (Strange & Sheppard 2001) or required the use of week-long sticky board samples (Branco et al 2006). The sticky board is a relatively easy monitoring tool that requires little training or time to implement.

One of the initial goals of this experiment was to establish economic treatment thresholds for *V. destructor* in western Washington State. Thresholds have been published for areas of Washington State east of the Cascade Mountains (Strange & Sheppard 2001), but the climate differs between the two sides of the state. Delaplane (1998) proposed that *V. destructor* population dynamics and thus economically damaging infestation levels were likely to vary with climate. We followed much of the experimental methodology used by Strange and Sheppard (2001) to establish the eastern Washington thresholds, but were unable to attempt similar analyses due to incomplete fall data and small sample sizes in the spring. It is imperative that these levels be established to support IPM programs for western Washington State beekeepers.

The lack of a sample taken in November, after all treatments had been given, limited the analysis of this work. Strange and Sheppard (2001) sampled all remaining colonies in

November to collect population data before the winter for comparison to values measured in the following spring. The November sample allowed the authors to gauge the efficacy of treatments within the same season they had been given and set up correlations that led to numerical treatment thresholds. We had planned on taking an equivalent sample, but were unable to do so given the weather conditions in Puyallup during November 2007. The air temperature was too low to sample the colonies without damaging the bee populations.

Future experiments in western Washington State should be conducted by individuals with easier access to the apiaries. We drove five hours across Washington State to interact with the experimental colonies and thus could not sample on short notice. The weather conditions in western Washington State, which are often rainy and cool, necessitate that work be done when the weather permits, instead of when schedules allow. Several of our sampling dates fell during rain. The immense disturbance inherent in the sampling regime used may have had negative impacts on colonies sampled on cool, wet days.

We suspect that the outcome of this experiment was also confounded by pathogens, such as American foulbrood (AFB) and *Nosema sp.*, as well as poor queen acceptance. Prior to this experiment, colonies at the Puyallup apiary had been more susceptible to AFB relative to those kept in apiaries in Pullman, WA (W.S. Sheppard unpublished data). We detected and removed four cases of AFB one month into the experiment. No

subsequent cases were diagnosed during the experiment, but weak, undetected infections may have lowered the defenses of some colonies. *Nosema sp*. spores were detected retrospectively in several of the surviving colonies (M. Smart unpublished data). *Nosema* infections have been associated with adult bee malnutrition, winter dwindling and queen supersedure (Hornitzky 2005), and may have contributed to the inadequate colony buildup observed during the experiment. Despite numerous feedings with sugar syrup, many of the colonies were not using the food or storing honey. Although most of the experimental colonies continued to grow throughout the summer, many were not adequately prepared to survive the winter.

Poor queen acceptance at the onset of the experiment led to brood gaps and colony deaths and removal of colonies from the experiment. Early queen rejection decreased the sample sizes of all treatment groups. To decrease the negative impact on sample sizes, the formic acid treatment research should have been separated from the comparison between breeding lines. In an experiment examining only formic acid efficacy, strong established colonies could be sampled throughout a season without being disrupted by queen replacement. Such an experiment would lose the uniformity established at the onset of the experiment described in this thesis, but initial variation could be accounted for using covariation analysis. It would be valuable to repeat this experiment with larger initial colony and treatment group sizes in addition to more frequent monitoring.

Future work should also explore the efficacy of formic acid relative to fluvalinate in western Washington State. Sticky boards could be used to monitor total mite drop throughout the entire treatment period. Our data on the mite fall induced by formic acid were limited to a 48hr sample, which likely underestimated the number of mites killed during the twenty-one day treatment. This design error could easily be corrected for future trials. We know that formic acid did kill mites here, but we have little knowledge of the degree of its impact on the total population or how it would compare to fluvalinate under these specific weather conditions.

This work yielded no indication of the progress of the WSU queen selection program. It would be valuable to run another experiment similar to this one, but perhaps larger in size, comparing WSU-queened colonies against those with commercially available queens. Resistant stocks might be able to tolerate larger populations of *Varroa* without being damaged (Spivak & Reuter 2001). Economic injury levels for the mite pests might differ for selected queens.

Formic acid is a soft or bio-rational alternative to the synthetic acaricides for the control *V. destructor* mites in honey bee hives (Delaplane 1997). Formic acid has been efficacious in treating tracheal mites (Hoppe et al 1989) and has been shown to decrease populations of *Varroa* mites both on adult bees (Calderone 2000) and in capped brood cells (Calis et al 1998). However, the efficacy of formic acid treatments has been shown to be variable (Eischen 1998) and to be contingent on environmental

factors (Bracey & Fischer 1989). Reports have also elucidated the potential for damage to brood and queens by formic acid vapors (Underwood & Currie 2004). We documented significant suppression of *V. destructor* populations after a single formic acid treatment, but this decrease did not appear to be sufficient to control the mites over the winter. We observed queen loss and colony collapse in experimental colonies, but these phenomena were not isolated to colonies treated with formic acid. Thus, formic acid was likely not the cause of the observed damage.

Despite the shortcomings that were illuminated in this work, formic acid remains a valuable tool for IPM of *Varroa* mites and should not be abandoned. Chemical treatments for *Varroa*, whether natural or synthetic, must meet a rigorous set of criteria to be considered for registration and use. Treatments must be effective against the mite, but not harm any life stage of the host bees. Additionally, treatments must be strong enough to penetrate beeswax or last long enough to encompass whole bee brood cycles without lingering in hive products. Moreover, treatment formulations must be effective in the hive, but be safe for shipping and for use by beekeepers. And finally, treatments must not require large inputs of labor or time or they likely will not be considered cost-effective for large-scale commercial operations. Formic acid meets the above criteria and thus expands the options available for beekeepers. The Mite-Away II™ product is a safe, easy-to-use formic acid formulation that does not harm the host bees if used according to label directions.

In conclusion, sticky boards appear to be a useful tool to estimate and monitor *V. destructor* infestations in honey bee colonies. Pest population monitoring is a critical component in integrated pest management because it allows the applicator to restrict treatments to times of need. By replacing acaricides with natural alternatives and adopting integrated management techniques, beekeepers can decrease the frivolous use of acaricides and preserve the efficacy of synthetic chemical treatments for emergency situations (Delaplane et al 2005). Formic acid has the potential to augment control of *V. destructor* in IPM programs, but its use requires thorough consideration of local climate conditions.

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