DRONE BROOD REMOVAL: A TOOL FOR MANAGING VARROA DESTRUCTOR IN APIS MELLIFERA COLONIES IN HAWAI‘I

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Tyler W.M. Ito

Dissertation Committee:

Ethel Villalobos, Chairperson
Mark G. Wright
Patricia Couvillon

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CHAPTER 1

VARROA DESTRUCTOR IN HAWAII: LOCAL BEEKEEPING PRACTICES AND CONTROL TREATMENT OPTIONS

1.1. Introduction

The arrival of Varroa destructor to the Hawaiian Islands in 2007 marked the beginning of widespread colony losses at a scale never observed since the introduction of Apis mellifera to the archipelago in 1857. The Varroa mite is an external parasite of honeybees which has been expanding its range, from its native origin in Java, Indonesia (first described in 1904), to Europe in 1962, the mainland USA in 1987, and New Zealand in 2000. This parasitic mite is among the most devastating pests beekeepers across the world have had to confront. The mites feed on the hemolymph of the adult and immature bees, which reduces the adult lifespan, can potentially kill larvae, and/or transmit deadly viruses among individual bees in the colony. V. destructor has been linked to colony losses in many parts of the world, and its arrival to Hawaii has also been linked to severe economic losses for the local beekeeping industry (Connor, 2011). The large impact of the Varroa mite invasion in Hawaii can be attributed to the combined effects of three main factors: 1) the tropical climate, which favors continuous mite reproduction, 2) the relatively small size of the islands, which allows for rapid and widespread parasite and pathogen colonization, and 3) the vulnerable European honeybee species (A. mellifera) that is used for commercial honey production and pollination services.

The rapid spread of V. destructor across Oahu and the Big Island, along with the high mite infestation levels observed (Villalobos, 2010), have precipitated major changes in the viral landscape of honeybee colonies in Hawaii (Martin et al., in preparation). The large parasitic
pressure of the mite and the now widespread distribution of *Varroa* transmitted bee viruses, such as the Deformed Wing Virus, threaten the sustainability of the Hawaiian beekeeping industry and have created an urgent need to examine which mite control options are most suitable for local conditions. In this document, we have gathered information on *Varroa* treatments, the pros and cons for each method, particularly with respect to chemical residue, and the suitability for use in tropical climates. In addition, we have undertaken an online beekeeper survey to assess the impact of *V. destructor* and document beekeepers’ management and treatment strategies. The results of this survey constitute a benchmark reference for Hawaii and will contribute to a better understanding of the practices and needs of the local beekeeping community.

1.2. Treatment Options

Treatment and control of *Varroa* mites is a serious concern for Hawaii’s beekeeping industry, however, practices commonly used on the mainland U.S. and in other countries may not be applicable in the islands. Under Hawaii’s subtropical climate, constant brood production may decrease the efficacy of certain treatments, and the warm climate may cause temperature dependent products to over-stress colonies. Treatment options can be categorized as synthetic chemicals, organic chemicals, stock improvement, biological control, and biomechanical methods. It should be noted that organic beekeeping is also very important in Hawaii, since the success of several honey producers depends on the “organic market”. To keep their organic status, beekeepers can only use treatments that are registered as organic, which limits their options. How each treatment functions and targets *Varroa* mites varies greatly, which can
influence the efficacy, limitations, and practicality. Described below are the treatment options for each category with particular emphasis on applicability in Hawaii.

1.2.1. Synthetic Chemicals

Beekeepers still rely heavily on the use of synthetic acaricides for controlling *V. destructor* due to their effectiveness, ease of application, relatively low cost compared to other treatments, and the minimal amount of stress to bees during treatment. However, there have been growing concerns about adverse effects on colony health due to the build-up of residues and the evolution of mite resistance to these chemicals. Some of the more commonly used chemicals include fluvalinate, coumaphos, and amitraz.

The chemical fluvalinate, commercially known as Apistan®, is a synthetic pyrethroid that was first released in 1988 as Apistan Queen Tabs (containing 1% tau-fluvalinate). Currently, the product Apistan consists of plastic polymer strips containing 10.25% fluvalinate. The strips are hung between the frames of the hive so that bees come into contact with the chemical as they move through the brood chamber, spreading the chemical throughout the hive. Fluvalinate use can result in mite mortality ranging from 80% to 100% (Elzen et al., 1999; Cabras et al., 1997). However, the increasing level of resistance in mites to fluvalinate has become a major concern for beekeepers (Pettis J. S., 2004). Resistance to fluvalinate was first reported in Lombardy (north Italy), where the average mite mortality was less than 70% in 7 of the 8 apiaries (Lodesani et al., 1995). Due to migratory beekeeping, resistant mites were able to spread at an accelerated rate throughout Italy and eventually to other parts of Europe (Milani N. , 1999). Several areas in the United States have also reported mites resistant to fluvalinate, with mite mortality as low as
Mite resistance has serious economic consequences for beekeepers due to increased frequency of treatments. Another cause for concern is the build-up of miticide residue within the hive. Fluvalinate is stable in nature and does not break down naturally in beeswax, consequently, repeated applications of Apistan could result in an accumulation of fluvalinate in beeswax over time (Lodesani et al., 1992). Haarmann et al. (2002) found that queens reared in hives containing high doses of fluvalinate weighed significantly less than queens from control (which for this purpose of this paper will be referring to untreated colonies) and low-dose colonies. When queen bees were exposed to Apistan Queen Tabs for 5 days in mailing cages, there were no significant differences in mortality, acceptance by the colony, brood viability, or longevity. However, there was a significant increase in the mortality of attendant worker bees (Pettis et al., 1991). It should be noted that the weight loss and mortality reported by Haarmann et al. (2002) and Pettis et al. (1991) probably resulted from the bees’ exposure levels exceeding the recommended treatment dose or treatment duration. While it is not likely that beekeepers would knowingly exceed the manufacturer’s recommendations, uninformed beekeepers compensating for mite resistance may lead to the misuse of fluvalinate, consequently harming their colonies.

In 1998, coumaphos, an organophosphate, was added to the list of synthetic chemicals approved for Varroa mite control. Coumaphos is commercially known as Checkmite+®, which comes in the form of plastic polymer strips, containing coumaphos that are applied the same way as Apistan, and Perizin®, which is a solution of coumaphos that is trickled directly over the bees inside the hive. Coumaphos can be very effective at controlling Varroa with mite mortality.
ranging from 80% to 100% (Pettis, 2004; Baxter et al., 1999; Milani et al., 1998). However, *Varroa* mites quickly developed resistance to this chemical; Pettis (2004) noted resistance in Maine and Florida, where mite mortality dropped to 13% and 7%, respectively.

Besides concern about resistances, one of the other main concerns regarding the use of coumaphos is its negative effect on queen bee health. When rearing queen bees in hives containing strips of coumaphos, researchers found several negative effects, such as reduced weight of the ovaries, lower total body weight of queen bees, and a reduction in stored sperm (Haarmann et al., 2002; Pettis et al., 2004). Similar to fluvalinate, coumaphos is a fat soluble substance that can accumulate in beeswax and honey. Pettis et al. (2004) found that the use of wax queen cells contaminated with coumaphos increased the rejection rate of grafted larvae. Queens reared in cups containing 100 mg/ka coumaphos, the U.S. tolerance level in beeswax (Federal Register, 2000), had a significantly higher rejection rate and were significantly lighter in weight compared to control queens (Pettis et al., 2004).

One of the earliest commercial chemicals tested for *Varroa* control was amitraz, belonging to the chemical class amidine. The product names are Apivar®, which consists of plastic polymer strips embedded with amitraz, and Mitac®, which applies amitraz as a spray. Similar to fluvalinate and coumaphos, mite mortality with use of amitraz can range from 80% to 99% (Baxter et al., 1999; Floris et al., 2001). However, as with fluvalinate and coumaphos, mite resistance has become a problem. Elzen et al. (2000) found mite mortality as low as 32.3% in Minnesota. In the former Yugoslavia, the efficacy of amitraz declined substantially by the fifth year of use (Dujin et al., 1991). Unlike fluvalinate and coumaphos, amitraz is unstable in honey and wax and therefore degrades quickly leaving very little residue (Lodesani et al., 1992; Floris et al., 2001). In a degradation study of amitraz in honey, Jimenez et al. (1997) found that amitraz
decomposes completely within 15 days. Lodesani et al. (1992) found amitraz (mean = 0.015 ppm, max = 0.061 ppm) to have the least amount of residue build up in wax from the brood chamber compared to fluvalinate (mean = 0.415 ppm, max = 4.65 ppm). Despite the very low levels of residue build up, amitraz was found to have some adverse effects on colony health. Bah (1999) showed an increase in mortality of 1-3 day old bee larvae. In addition, strips containing amitraz were shown to cause high adult bee mortality in packaged bees (Henderson et al., 1988).

Although synthetic varroacides are inexpensive, easy to apply, and initially achieve high mite mortality, beekeepers in Hawaii should be aware of their limitations. Synthetic varroacides are all contact treatments that target only phoretic mites (mites on adult bees) and therefore work best when the colonies are broodless. However, in Hawaii’s warm subtropical climate, where there is constant brood production, these chemicals need to be applied more frequently throughout the year. An increase in the frequency of application of synthetic chemicals is likely to result in adverse effects such as resistant mites and the build-up of residues within the hive. The use of these chemicals is not recommended during honey flows to prevent contamination. Therefore, the honey supers need to be removed before treatment, and doing so may interfere with Hawaii’s year round honey production. With studies showing the development of resistance and adverse effects on colony health due to residue build up, alternative products and methods need to be considered in a more sustainable approach to Varroa treatment.

1.2.2. Organic compounds

The problems and limitations of synthetic varroacides (e.g. mite resistance, wax and honey contamination, adverse effects on colony health) have led to the development of
alternative treatments using organic acids and oils. Organic compounds, specifically formic acid, oxalic acid, and thymol constitute the core of the arsenal of natural compounds presently used for control of *V. destructor*. Organic acaricides can be highly effective, relatively easy to apply (depending on the product and the delivery method), and have a low risk of wax contamination. In addition, there are no reports to date of mite resistance to these treatments. In contrast to synthetic miticides, the organic chemicals used for mite control do not leave residues in wax, although they can alter the taste of honey. Therefore, it is recommended that beekeepers remove the honey supers during treatment to avoid unusual taste in honey. One notable exception to this rule is the “Mite Away Quick Strips”, MAQS®, a new formic acid product, which appears to effectively fumigate the hive and then dissipate without altering honey quality (Villalobos et al., in preparation).

The effectiveness of organic compounds can vary depending on several factors, such as climatic conditions, application method, colony strength, and concentration of the chemical. However, the efficacy of organic acaricides that function as fumigants depends largely on ambient temperature. Therefore, it is important to evaluate the efficacy and secondary effects on colony health in subtropical regions, such as Hawaii, where ambient temperature is relatively high throughout the year.

Formic acid is an organic acid, which occurs naturally in the environment and foods (e.g. nectar and coffee) and is also a natural component in honey. It is used as a fumigant for the control of *V. destructor* as well as the tracheal mite (*Tropilaelaps clareae*). Formic acid was first used to control *Varroa* in Germany in the early 1980s (Ritter & Ruttner, 1980) and according to the European regulation 1804/99 on organic production is authorized in several regions as a natural compound for use in organic apiculture. To prevent honey contamination, synthetic
chemicals and the majority of organic compounds, are prohibited for use in colonies with honey supers. Therefore, during the nectar flow, beekeepers must decide whether to remove the honey supers prematurely to treat infested colonies immediately or delay treatment until after the nectar flow, with the increased risk of losing colonies. However, with the recent development of MAQS, beekeepers are now able to apply treatment without removing the honey supers and during the nectar flow.

The efficacy of formic acid has been comprehensively reviewed and found to be highly variable depending on environmental conditions, time of year, and method of application. Reported mite mortality rates range from 39.7% to 100% (Eguaras et al., 2003; Elzen et al., 2004; Satta et al., 2005). However, Ostermann et al. (2004) found mite mortality rates as low as 9.4% and 29.3% when using a formic acid slow-release and pour-on application method. Methods of application, such as absorbent pads, gel matrix, or evaporators, could affect the formic acid concentration profiles and total exposure time within the hive accounting for the variability in efficacy. Eguaras et al. (2003) found that the position of the formic acid dispenser within the hive can have an effect on mite mortality. Colonies treated with a formic acid gel matrix positioned at the top and bottom of the hive achieved higher mite mortality (94.5%), compared to colonies with the same doses of formic acid positioned only at the top of the hive (80.8%). Environmental conditions, including seasonal conditions, can also cause significant differences in efficacy. Depending on the release mechanism, high temperatures (above 26.6°C for some products) can cause the formic acid to evaporate at a higher rate resulting in possible negative effects on colony health due to the higher concentration of formic acid in the hive air. Negative effects may include mortality of adult and immature bees, delayed egg-laying, and queen loss. Low ambient temperatures (below 10°C based on manufacturer’s instructions of
Mite-Away II®) would result in poor vaporization of the formic acid, reducing mite mortality. Therefore, beekeepers must make sure that the methods of application of formic acid are suitable for the time of year and ambient temperature.

With the exception of formic acid, all varroicides only target the phoretic mites on adult bees. However, when brood is present, the majority of the mite population is located within the sealed brood cells. Thus, for varroicides to be effective, multiple applications or an extended application period is required to kill the mites that emerge with newly emerging adult bees.

Formic acid fumigation is unique in that it can reduce phoretic mites as well as mites in capped brood cells in a short time. VanEngelsdorp et al. (2008) found that a short-term fumigation treatment (17 hours) with 50% formic acid killed more than 60% of the mites in capped brood cells, which was also similar to the 55.6% mite mortality in capped brood of Africanized honeybees reported by Calderon et al. (2000).

The main concern regarding the use of formic acid products are the possible adverse effects on colony health, such as adult bee and brood mortality, interruption in egg-laying, and queen loss. Satta et al. (2005) found adult bee mortality was highest during the period when daily formic acid evaporation was at its peak. In addition, during a test of a slower release method (BeeVar® gel packets), interruption of brood rearing was observed but there was lower adult bee mortality. Elzen et al. (2004) reported similar findings, where a significant reduction in brood rearing and an interruption in egg-laying were observed during the first week of treatment when evaporation rates were high. VanEngelsdorp et al. (2008) found no negative changes in the number of frames of worker brood per hive during a 17 hour application of formic acid, however, they did reported queen loss in 10 of 51 treated colonies.
As mentioned earlier, formic acid occurs naturally in honey. Although the quantity of formic acid that accumulates in honey after treatment is low and safe, there are concerns regarding the taste of the honey being altered. The European food legislation does not allow additives in honey altering its natural flavor. The taste threshold (the lowest, correctly distinguishable concentration of an additive in honey) of formic acid in honey was found to range between 300 to 600 mg/kg (Bogdanov et al., 1999). However, formic acid levels in honey after treatment was only found to be 71 to 94 mg/kg (Bogdanov et al., 2002), therefore, altering the taste of the honey should not be an issue.

Another organic compound is oxalic acid also a natural constituent of honey that has been used to control Varroa mites by beekeepers in Europe and Canada, however, it is not registered as a pesticide in the United States. It is usually mixed in a sucrose solution and common methods of application include spraying or trickling the solution directly on the bees. Given that oxalic acid kills mites through contact rather than fumigation, ambient temperature is not a critical factor as it is for formic acid. Being a contact miticide also means that oxalic acid can only target phoretic mites, and therefore, it is most effective when no brood is present. When colonies were treated in early autumn when brood was still present, only moderate mite mortality rates of 21% to 65.3% were observed (Gregorc and Planinc, 2002; Bacandritsos et al., 2007; Gregorc and Poklukar, 2003). However, when colonies were treated during a broodless period, such as in late autumn, mite mortality rates were greater than 90% (Gregorc and Planinc, 2002; Mutinelli et al., 1997; Gregorc and Poklukar, 2003; Charriere and Imdorf, 2002). Adverse side effects, such as queen loss, bee mortality, reduced colony growth, and abnormal behavioral changes, were not observed in several oxalic acid studies (Mutinelli et al., 1997; Gregorc and Planinc, 2002; Bacandritsos et al., 2007). However, Higes et al. (1999) reported queen loss in
some hives treated with oxalic acid, while the remaining queens produced less brood than control hives. Studies have shown no residues or changes in concentration levels of oxalic acid in honey during and after treatments (Bogdanov et al., 2002; Mutinelli et al., 1997). The taste threshold of oxalic acid was found to range from 400 to 900 mg/kg (Bogdanov et al., 1999). However, oxalic acid levels in the honey of treated colonies only ranged from 18 to 33 mg/kg (Bogdanov et al., 2002). Therefore, beekeepers using oxalic acid should have no problem complying with the European food legislation banning foreign odors or tastes in honey.

Essential oils also have been studied extensively for the control of Varroa, however, only a few have proven to be effective. Thymol from thyme has proven to be the most effective of the essential oils. Commonly used thymol products are Apilife VAR®, which comes in the form of a vermiculite tablet (porous ceramic sponge), and Apiguard®, which consists of a gel matrix. Both products are placed on the top bars of the hive and are left in place for at least 4 weeks. Several efficacy studies have shown thymol to cause mite mortality greater than 90% (Chiesa, 1991; Floris et al., 2004; May-Itzá et al., 2007), suggesting that it, along with formic acid and oxalic acid, can be an effective alternative to synthetic chemicals. However, ambient temperature is a major factor affecting the evaporation of thymol; average daily temperature and average maximum daily temperature have been shown to be positively correlated with evaporation rate of thymol (Calderone, 1999). Therefore, it is recommended that thymol should be used when daily temperatures range between 15.5 to 40.5°C to achieve maximum efficacy while minimizing adverse effects on colony health (Chiesa, 1991; Calderone, 1999).

According to EU regulation No. 2377/90 thymol is in group II of the non-toxic veterinary drugs, which does not need an MRL (maximum residue limit). However, the European food legislation bans foreign odors or tastes in honey. Bogdanov et al. (1998) reported a sensory
perception threshold of thymol in honey between 1.1 and 1.6 mg/kg. However, even when thymol concentrations reached the threshold, test participants did not show a significant preference for control honey over the thymol-spiked honey. After a 4-week treatment with Apilife VAR and Apiguard, Floris et al. (2004) found honey thymol residues of only 0.62 and 0.96 mg/kg, therefore, reaching the taste threshold should not be problematic.

Organic compounds provide Hawaii’s beekeepers with a potentially more sustainable approach for controlling the Varroa mite if used appropriately. There are no known reports of resistance and no adverse effects on colony health due to residue build up as seen in synthetic chemicals. In addition, the recent development of a new formic acid product (MAQS) allows beekeepers to treat their colonies with the honey supers on, thus not interfering with Hawaii’s year round honey production (Villalobos et al., in preparation). However, not all organic compounds are suitable in Hawaii’s warm subtropical climate. With the efficacy of the majority of these chemicals being temperature dependent, application during the summer when temperatures are higher can over stress the colonies. Therefore, it is important for beekeepers to be aware of the limitations of organic compounds to prevent any unnecessary bee loss.

1.2.3. Stock Improvement

One of the main objectives in V. destructor management is to decrease the number of treatments and chemical applications needed to keep mite populations at non-threatening levels. To achieve this, researchers have been selectively breeding for Varroa resistant bees. These resistant bees may require fewer treatments to manage Varroa levels and therefore have the potential to be the foundation of Varroa IPM (Integrated Pest Management) programs involving alternative treatments, such as the biomechanical methods described below. At present, research
has produced three stocks of honeybees with documented Varroa resistance: MN hygienic stock, Russian stock, and Varroa sensitive hygiene (VSH) stock.

The MN hygienic stock and VSH stock have been selected and bred for enhanced general hygienic behaviors, such as increased grooming and capped brood removal. In grooming, adult bees detect and remove phoretic mites from themselves or other bees. Adult bees may also have the ability to detect and remove mites under capped brood cells, disrupting mite reproduction (Rath & Drescher, 1990). These hygienic behaviors serve as important mechanisms of resistance against Varroa in the Asian honeybee (A. cerana) but, are exhibited to a lesser extent in A. mellifera (Peng et al., 1987).

The University of Minnesota has been selectively breeding their hygienic stock (MN hygienic stock) on the basis of the bees’ removal response to freeze-killed brood. The amount of time is recorded for bees to detect, uncap, and remove a 6 x 5.5 cm comb section containing freeze-killed pupae (Spivak, 1996). The time taken to remove the pupae is considered a measure of the bees’ ability to remove diseased or abnormal brood. Colonies that remove the freeze-killed pupae within 48 hours in two consecutive trials are considered hygienic (Spivak, 1996; Spivak & Reuter, 2001). To establish and maintain this hygienic line of bees, queen bees are raised from colonies that consistently remove 95% of the freeze-killed brood within 48 hours. Spivak and Reuter (2001) found that colonies with naturally mated queens raised from their hygienic lines had fewer mites on adult bees and fewer mites within worker brood cells than non-hygienic colonies for up to one year without treatment in a commercial beekeeping operation. They also found that hygienic colonies had a significantly smaller proportion of worker cells infested with multiple foundress mites. This finding is consistent with the finding that the bees clean infested cells at a higher rate when they contain more than one foundress (Spivak, 1996).
The USDA has developed and is assessing resistant stocks of bees, the Russian stock and VSH stock. Originating with bees from far-eastern Russia (Primorsky), the Russian stock was developed for improved resistance to both Varroa mites and tracheal mites (*Acarapis woodi*) and for good honey production. Rinderer et al. (2001) found that colonies with queens raised from imported Russian queens were able to suppress mite population growth throughout the year, while control colonies (commercial stocks used in the United States) were all lost within the year primarily due to varroosis. Several mechanisms in the Russian stock were found to provide substantial resistance to *V. destructor* (e.g. enhanced grooming), however, not all the contributing factors have been identified (Rinderer et al. 2001). The diversity of traits that act in concert to suppress *V. destructor* provides opportunities for further development of resistance through selective breeding.

For the VSH stock, selective breeding of their hygienic line is performed on the basis of the bees’ removal response to capped brood infested with *V. destructor*. The VSH trait can be quantified by introducing *Varroa* infested capped brood (with the infestation already known) into a colony and then comparing the infestation rate of the capped brood before and after exposure to honeybees (Harris J. W., 2007; Harris J. W., 2008). Ward et al. (2006) found that colonies produced by VSH queens had lower *Varroa* infestations and lower percentages of colonies that needed treatment than both control and Russian stock colonies. They also found no difference in the amount of honey produced by VSH colonies, suggesting that honey production is not hindered by fitness costs associated with enhanced hygienic behavior. In addition to the removal of infested brood cells, VSH colonies also show a higher frequency of non-reproducing mites. Previous studies found the prevalence of non-reproducing mites to be strongly correlated with reduced mite populations in VSH colonies, while the MN hygienic stock was not (Harbo &
Hoopingarner, 1997). This might be due to the findings that VSH colonies prefer not to remove mites that lay no eggs in the brood cell (Harbo & Harris, 2009). It may be that oviposition by the mites (or some cue associated with mite oviposition) stimulates VSH bees to remove infested brood.

Selectively breeding honeybees for mite resistant traits is still in early development but has already shown promise. It has the potential to be the foundation of IPM programs for controlling *V. destructor*. Studies have shown that all three resistant stocks (MN hygienic stock, Russian stock, and VSH stock) are able to tolerate *Varroa* better than honeybees not bred for resistance (Ward, Danka, & Ward, 2008; Spivak & Reuter, 2001; Rinderer, et al., 2001). In addition, the three resistant stocks needed treatments less frequently to keep mite populations at non-threatening levels. However, it should be noted that most of these studies were conducted at non-migratory apiaries in order to prevent large amounts of drift or interactions with honeybees not bred for resistance. Worker bees not bred for resistance that drift into resistant-bred colonies can be a detriment, since they may not possess the hygienic traits necessary to be efficient cleaners and may also bring in more mites. On the other hand, drone bees can be more detrimental than worker bees, because they have the potential to change the genetic quality of resistant-bred colonies. For instance, if a resistant-bred colony were to re-queen itself, the newly hatched queen may mate with drones from colonies not bred for resistance or with feral colonies nearby during their mating flights. Therefore, whether investing in *Varroa* resistant stocks of bees will be economically practical for beekeepers still remains unknown.
1.2.4. Biological Control

Several entomopathogenic fungi (*Metarhizium anisopliae*, *Hirsutella thompsonii*, and *Beauveria bassiana*) are currently being evaluated as possible biological control agents against *V. destructor* in managed honeybee colonies. These fungi could prove to be an effective tool for mite control, because they are able to infect *Varroa* directly through the exoskeleton and thus do not have to be consumed (Chandler et al., 2000). Furthermore, fungus-based products can be inexpensive to produce, and the registration process for microbial pesticides is usually less expensive compared to that for chemical pesticides. Recent studies have shown promise that entomopathogenic fungi could be an alternative to varroacides, however, the *Varroa* killing efficacy of the fungi, the infection rates pre and post application of the treatment, and the duration of the treatment effect on the colony, require further testing before the fungi is made available to beekeepers.

Kanga et al. (2003) found no significant difference in daily mite mortality during the treatment period between *M. anisopliae* and fluvalinate treated colonies. Achieving mite mortality levels comparable to fluvalinate required colonies to be treated with four plastic strips coated with a total of 15.6 g of *M. anisopliae* (≈3.9 g per strip) replaced three times at 10 day intervals. The number of mites per bee at the end of the experimental period (42 days) also showed no significant difference between *M. anisopliae* and fluvalinate treated colonies. Colonies that were treated with an initial higher dose of *M. anisopliae*, five strips coated with a total of 46.8 g of *M. anisopliae* (≈9.36 g per strip), but with no replacement and further augmentation of spores, did not display the same efficacy. This might have been due to the inability of *M. anisopliae* to establish itself throughout the treatment period after just one application. Therefore, multiple applications may be necessary to ensure the spread and
establishment of the *M. anisopliae* during the treatment period and for maximum efficacy. Similar results were also found for the fungus *H. thompsonii*, where infection levels did not increase over time without repeat applications (Kanga et al., 2002). However, *H. thompsonii* did display good persistence, because infected mites were still being recorded 30 days after the last fungal application.

Introducing any biological control agent into a new environment is always a cause for concern due to potential non-target impacts. Therefore, it is very important that the potential impact that entomopathogenic fungi on honeybee health is extensively assessed. Shaw et al. (2002) found that isolates of *M. anisopliae* and *H. thompsonii* caused significant mortality to caged honeybees sprayed with conidial suspensions (1 \( \times 10^8 \) ml\(^{-1} \)) up to 14 days after treatment. However, not all the morality could be attributed to fungal infection, since there was high mortality in the controls as well. Therefore, results from honeybee bioassays should be interpreted with caution given that bees may be maintained in conditions unfavorable to their survival. Recent studies have reported no significant difference in the daily mortality of adult honeybees between colonies treated with *M. anisopliae* or *H. thompsonii* and controls (Kanga et al. 2002; Kanga et al. 2003). There was also no difference in queen fecundity (number of eggs per day) between *H. thompsonii* treated colonies and controls (Kanga et al., 2002). Thus, fungi that cause adult honeybee mortality in laboratory bioassays may have minimal effects in whole-hive field experiments. Furthermore, Butt et al. (1998) reported no adverse effects on honeybee health when bees were used to deliver an isolate of *M. anisopliae* (which caused high adult bee mortality in a laboratory bioassay) to oilseed rape flowers for control of pollen beetles in the field. For these reasons, further studies assessing the impact different fungi isolates have on honeybee health are required.
1.2.5. Biomechanical Methods

Biomechanical methods involve using specific biological characteristics of the honeybee and *Varroa* mite in order to help manage mite population levels. When used alone, most of these methods are inadequate to achieve substantial *Varroa* control and therefore are usually combined with other treatments in an IPM program. Biomechanical methods offer a sustainable approach for controlling *Varroa*, however, the amount of time and labor required may make some of the methods impractical for large scale operations. Common biomechanical methods used by beekeepers include screen-bottom boards, small cell frames, sugar dusting, temporary queen confinement, and drone brood removal.

Beekeepers have used screen instead of wood as the floor in beehives for decades (Spear, 2002). *Varroa* mites will naturally fall to the bottom of the hive due to various reasons (i.e., loss of grip). With a screen floor, mites will fall through the screen and out of the hive rather than accumulate on a solid wood floor, from which they may be able to crawl back onto bees. Several studies have found that hives equipped with screen bottom boards tended to have fewer mites than hives with traditional wood floors, however, the differences were not statistically significant (Harbo and Harris, 2004; Rinderer et al., 2003). Screen-bottom boards can be a useful component in an IPM program, nevertheless, because they can be used to as a sampling tool to estimate and monitor mite population levels within colonies. Beekeepers can apply adhesive or sticky paper underneath the screen to catch the fallen mites, which can then be counted to give a rough estimate of the colony’s infestation level.

Another biomechanical approach to mite control involves altering the size of worker brood cells. In the United States, standard worker foundation for European honeybees is composed of cells 5.3 mm wide. However, using smaller cells (4.9 mm wide) has been
hypothesized to interfere with *Varroa* mite reproduction by possibly shortening the development time of worker brood. A shorter worker development time would make it difficult for *Varroa* mite offspring to mature in time before the worker bee emerges. A reduction of space within the cell may also be detrimental to the survivorship of male mites, some of which may become trapped in the upper part of the cell (Martin & Kryger, 2002). Although there have been some anecdotal reports of the effectiveness of small cells from hobbyists, research has shown otherwise. Ellis et al. (2009b) conducted field trials measuring the efficacy of small cell foundation and found no significant effect on total mites per colony, mites per brood cell, or mites per adult bee. Berry et al. (2009) actually found that small cell colonies had significantly higher mite populations in brood, a higher percentage of mite population in brood, and more mites per 100 adult bees compared to conventional sized cells. Therefore, using small cell frames or foundation to impede *Varroa* population growth does not appear to be an effective control method.

Dusting bees with powdered sugar also has been explored as a non-chemical method of control that targets the phoretic mites on adult bees. *Varroa* mites fall from bees at an accelerated rate, which may be due to the loss of grip as the sugar adheres to the mite’s tarsal pads (Fakhimzadeh, 2000) and the increased grooming of the bees (Macedo and Ellis, 2002). Studies measuring the efficacy of sugar dusting have produced mixed results. Laboratory studies have shown that sugar dusting was able to dislodge more than 90% of the mites on adult bees (Macedo and Ellis, 2002). To test the efficacy of sugar dusting under field conditions, Ellis et al. (2009a) dusted colonies every two weeks for 11 months with 120 grams powdered sugar per application. However, they found no significant difference in total number of mites per colony, number of mites per adult bee, or number of mites per capped brood cell between treated and
control colonies. Aliano and Ellis (2005) developed a method for using powdered sugar, which resulted in colonies dropping approximately 77% of their mites. However, their method requires forcing all the adult bees into a screened box to be dusted and is only recommended for treating broodless colonies. Therefore, this method would most likely be too time consuming and labor intensive for most beekeepers and cannot be used in tropical and subtropical climates where brood is produced year round.

Another biomechanical approach uses temporary queen confinement to interrupt the queen’s egg-laying, thus creating a broodless period. Without brood present, Varroa mites are unable to reproduce, preventing the mite population from increasing. However, this method can be time consuming to implement, labor intensive, and risky, since it involves finding and caging the queen without damaging her and then releasing her a few days later. It would not be practical for commercial scale operations to adopt this method and might realistically only be considered by experienced hobbyists.

Drone brood removal (DBR) is a method that takes advantage of the Varroa mite’s natural preference to invade drone brood. Studies have found Varroa infestation rates 8-11 times higher in drone brood than worker brood (Fuchs, 1990; Boot et al., 1995). Drone bees have a longer development time and larger cells, which allow foundress mites to have a higher reproductive output on drone brood (2.47 viable female offspring) compared to worker brood (1.41 viable female offspring) (Ghamdi & Hoopingarner, 2003). Beekeepers can take advantage of Varroa’s preference for drones by designating a frame solely for drone production, functioning as a Varroa mite sink which then can be removed along with a large portion of the colony’s mites. Field studies examining the efficacy of drone brood removal have shown promising results, suggesting its potential usefulness in IPM programs. Santas and Lazarkis
(1984) found that colonies under DBR were able to delay the need for acaricidal treatments for up two and a half years, whereas control colonies needed treatment after only one year. Damiani and Marcangeli (2006) were able to achieve a total efficacy of 84% but required three drone traps in each colony (each of which had a double-deep brood chamber), which also resulted in a decrease in honey production. On the other hand, studies shown DBR to be just as effective using two drone traps in each colony (double-deep brood chamber), with no disruption of colony development or honey production (Wantuch & Tarpy, 2009; Calderone N. W., 2005).

1.3. Beekeeper Survey Results

The online beekeeper survey (see appendix) was designed to gather information on Hawaii's apiculture industry and included questions to examine the profile of the typical Hawaiian beekeeper, details about the local style of colony management, the perceived impact of *V. destructor*, and the beekeepers’ treatment preferences. The online survey was created using Survey Monkey (an online survey website) and was conducted from August to October, 2010. The beekeeping community was alerted of the survey through email and word of mouth.

1.3.1. Beekeeper Profile

A total of 34 beekeepers responded to the survey, including 9 from Oahu, 15 from the Big Island of Hawaii, 2 from Maui, 7 from Kauai, and 1 from Molokai (who is the largest honey producer for that small island). Although the total number of respondents to the survey may seem low, these beekeepers currently own approximately 5,300 honeybee colonies and constitute a diverse representation of the local beekeeping community. In fact, the 2010 National Bee Survey, conducted by the USDA, characterized the beekeeping industry for the state of Hawaii
using a similar pool of respondents. The majority of the respondents classified themselves as hobbyists (38%) and small business owners (44%) (Table 1.1). However, a total of six large commercial beekeepers (including one with over 2,500 hives) also responded to our survey. On average, hobbyists owned 2.5 colonies, small businesses owned 28.7 colonies, and commercial operations had 819 colonies (range 60 to 2800). When asked to indicate the years of beekeeping experience, half of the respondents were relatively new and had 1 to 5 years of experience, while those with 6 to 10 years of experience made up only 15% of the total responses (Table 1.1). Around a third of the respondents (35%) have more than 10 years of beekeeping experience, the majority being small and large businesses. An unexpected finding from our survey was the significant number of beekeepers that had direct ties to the agricultural sector. A total of 12% of the respondents considered themselves primarily farmers, while 39% considered themselves to be both farmers and beekeepers. Expanding on this information, we asked participants to identify what their primary economic objective was in keeping bees. Of 34 respondents, 21 (62%) kept bees for both honey production and pollination, while 11 (32%) kept bees primarily for honey production (Table 1.1).

1.3.2. Colony Losses

The arrival of the Varroa mite on Oahu (April 2007) and the Big Island (August 2008) constitute defining points where major colony losses occurred across the two islands. We estimated, based on informal interviews with Oahu beekeepers conducted during the first year of Varroa, that beekeepers lost approximately 65% (274 out of 419) of their colonies (unpublished data). Our online survey was conducted about 3 years after the initial invasion by V. destructor and included a larger beekeeper sample and more detailed questions. This recent survey
provided the beekeepers the opportunity to record their colony losses by indicating their total number of colonies pre and post Varroa; and to select among several factors that may have contributed to their losses. Respondents could check one or more parasites and pathogen agents, including Varroa, common bee diseases, and the recently arrived honeybee parasite, the small hive beetle (*Aethina tumida*). Beekeepers were also asked to consider the role of environmental conditions, such as climate and flowering resource availability, on colony losses. Because respondents could select multiple factors, the summary results for the colony loss categories can add up to more than 100%.

The nine Oahu beekeepers that responded to the survey owned 378 colonies before the arrival of Varroa compared to 296 colonies at the time of the survey, this constitutes a 22% colony reduction in 3 years since the mite’s introduction. Beekeepers on the Big Island (15 respondents) owned 5450 colonies pre-Varroa compared to 4686 colonies during the survey, a 14% reduction in 2 years since the mite’s arrival to the island. The largest commercial honey producer in Hawaii (located on the Big Island) owned approximately 3,800 colonies pre-Varroa compared to 2,800 colonies at the time of the survey, this constitutes a 26% reduction in hive numbers. This commercial beekeeper also indicated that his annual pre-Varroa colony losses rate was approximately 10-15%. On the islands where the mite is not present (Kauai, Maui, and Molokai), beekeepers experienced minimal annual colony losses of approximately 3%. The perceived reasons for colony loss varied between islands, however, the more severe colony losses were attributed to both *V. destructor* and *A. tumida*. All of the Oahu beekeepers attributed some colony losses to the Varroa mite, however, an additional thirty three percent of the Oahu respondents also cited diseases as a contributing factor for colony decline. Fifty five percent of the Big Island beekeepers attributed their losses to the small hive beetle (SHB). An additional
forty five percent of the respondents on the Big Island named *Varroa* as a factor in colony loss, and thirty six percent attributed colony losses to disease. On the islands of Kauai, Maui, and Molokai where *Varroa* and SHB were not present, beekeepers attributed colony losses to starvation (10%) and unknown factors (20%).

### 1.3.3. Colony Management

Beekeeping practices and hive set-up can have profound effects on treatment efficacy against honeybee pests. For example, the use of a queen excluder, a plastic or metal screen that restricts the queen to the "brood box", permits beekeepers to create a physical separation of bee brood and honey storage areas. This separation, in turn, facilitates the removal of the honey supers, as indicated for some *Varroa* treatments, and also allows beekeepers to strategically place mite treatment products near the brood, thus improving their effectiveness. Our results show that nearly half (47%) of the Hawaiian beekeepers had incorporated queen excluders in their hive setup (Table 1.2). However, the proportion of beekeepers that reported using queen excluders varied with island, and only 23% of the respondents on the Big Island used this hive set up compared to 62% of the beekeepers on the other islands. Screen-bottom boards are also an useful component in IPM programs, originally praised as a control method, are now used mostly as a sampling tool for *Varroa*. Our survey indicated that almost half of the respondents across all islands (47%) have equipped their hives with screen-bottom boards instead of traditional solid wood floors (Table 1.2). The great majority of the users were found on Oahu and the Big Island, 89% and 54% respectively.

Beekeeper attentiveness to pest levels can also have a great impact in colony survival. We documented the frequency at which beekeepers check their brood box, since changes in bee
health and colony strength will be more likely detected by beekeepers that examine the brood frequently. On Oahu and the Big Island, where colony health needs to be monitored closely due to the parasitic pressure from *Varroa* and SHB, 45% of the beekeepers checked their brood box at least once a month. On the islands of Maui, Kauai, and Molokai (all *Varroa* and SHB free), only 10% of the beekeepers checked their brood box once a month.

The genetic characteristics of the colony can influence the degree of resistance to pathogens and parasites and will consequently impact overall colony health. Beekeepers in Hawaii can only buy queens from local queen breeders, as importation of US mainland honeybees is not legal. Alternatively, beekeepers can use their own stock by raising their own queens or allowing their hives to re-queen themselves naturally, and/or they can add “feral” bee stock through swarm catching. Through the online survey, we gathered information on bee stock selection on the different islands (Table 1.2). The majority of beekeepers (62%) prefer natural re-queening, while 14% re-queen their colonies once a year, which is considered standard frequency of queen replacement among mainland US beekeepers. The majority of beekeepers in Hawaii (75%) prefer re-queening with their own stock of bees, however, many beekeepers on Oahu (50%) rely on imported commercial queens from the Big Island of Hawaii.

1.3.4. Treatment Preferences

Our survey focused mainly on treatment preferences against *Varroa*, thus only respondents from Oahu and the Big Island contributed to this section (control methods used by beekeepers are listed in Table 1.3). The most commonly used *Varroa* mite treatment in Hawaii was formic acid (76%), followed by powdered sugar (29%), fluvalinate (24%), thymol (19%), and drone brood removal (19%). Fluvalinate use was only reported by Oahu beekeepers,
although we know of producers in the Big Island that have and continue to use this product but that did not participate in the survey (pers. com). Thymol use was nearly three times as common among Big Island beekeepers compared to Oahu (30% vs. 11% respectively), while the use of powdered sugar was more common among Oahu respondents compared to Big Island beekeepers, 40% to 20% respectively. Finally, drone brood removal was used approximately by 20% of respondents on both islands.

In addition to treatment preference, the survey also gathered information about the individual beekeeper application strategies and frequency of pest control treatments. When asked if they alternated between different control treatments, less than half of the beekeepers (39%) answered affirmatively. When asked whether they modify the treatment dose of products according to hive condition, approximately half of the respondents (56%) reduced the amount of product if mite levels were low or if colony was deemed small. It is interesting to note that the tendency to alternate between treatments and to modify the dosage was much higher among Oahu beekeepers, who have been dealing with the mite for longer than the Big Island respondents. Survey results also indicated that the great majority of beekeepers (76%) do not treat their apiaries on a schedule but instead treat when they perceived the hives are not doing well. Nevertheless, 80% of the respondents said that they sampled their hives for mites before treating and the most commonly used Varroa sampling methods included sticky boards (68%), inspection of capped brood cells (55%), and examining the ratio of mites to adult bees (18%).

1.3.5. Discussion

The arrival of V. destructor to Hawaii resulted in extensive losses for many island beekeepers. However, following the first year of Varroa introduction, the reported colony losses
began to decline slightly, in part due to beekeeper experience and the availability of new mite control products. In fact, the 2nd year post-*Varroa* colony losses reported for Hawaii were lower than the reported overwintering losses in the mainland US, according to the congressional Honey Bee Colony Collapse Disorder report (Johnson, 2010). Our data suggest that beekeepers in Hawaii were able to re-populate their apiaries through improved mite management, even though the local conditions required frequent monitoring and more treatments per year than the colonies in the mainland US (Villalobos, 2010). Another possible factor that has contributed to the observed recovery trend derives from the intense efforts of the local beekeepers to box honeybee swarms and to conduct frequent colony divisions, or "splits", to further increase their hive numbers (personal observation).

The respondents from Oahu and the Big Island attributed the bulk of the colony losses to SHB and *Varroa*. In comparison, the respondents on the other islands listed diseases and starvation as potential causes for colony loss. It is conceivable that beekeepers are more likely to attribute losses to larger, visible parasites, compared to microscopic pathogens, such as *Nosema*. However, the minimal colony losses reported from Kauai, Maui, and Molokai suggested that infectious microscopic diseases had less of an impact on bee health than did *Varroa* and *Nosema*. Much research is needed to understand the role played by bee diseases in the weakening of hives and how honeybee colonies respond to attack by multiple pathogenic agents. Unfortunately, the recovery trend detected by our survey has since been compromised with the arrival of the small hive beetle to Hawaii, and a follow up study on colony survivorship should be undertaken in the near future.

The effectiveness of synthetic *Varroa* treatments in the mainland US and Europe has been greatly diminished by the development of pesticide resistance by the mite and the reports of
damaging side effects on colony health. Hawaiian beekeepers, although introduced initially to synthetic chemicals for mite control, now rely heavily on alternative treatment products, especially thymol and formic acid. The comparatively low interest in synthetic chemicals exhibited by Hawaiian beekeepers is not the result of a decrease in the efficacy of the pesticides, as the mite in Hawaii has not developed the levels of resistance reported for other localities (unpublished data), but reflects rather the local need to maintain organic standards and/or a personal choice to minimize the chemical introductions to the hive. In addition, the commercial release of MAQS, greatly facilitated the transition to organic treatments by the local beekeepers. Also notable is the use of drone comb removal by Hawaiian beekeepers, especially since this technique is time consuming, and its effectiveness depends on the seasonal drone production levels of the colonies. Nevertheless, the local environment creates enough of an "extended drone season", with peak drone from June to September, which allows beekeepers to effectively include this technique as part of an Integrated Pest Management strategy against *V. destructor* (Ito et al. in preparation).

Based on our survey, it seems that Hawaii's beekeepers have adopted a number of management techniques that were originally offered to them by the Hawaiian Department of Agriculture (HDoA) and the University of Hawaii. For example, the most commonly used *Varroa* sampling method were sticky boards, which are less intrusive, but more expensive than brood or adult bee samples. This choice probably reflects the initial distribution, free of charge, of screened-bottom boards and sticky boards by the HDoA to the local beekeepers. The choice of mite treatments has also been influenced by cost and local availability of the product, and in the case of the MAQS, by the participation of the local beekeepers, along with the UH Honeybee Project, in the testing of this new mite control product.
Our survey results also indicated that, in spite of the recent pest problems, there are a relatively high number of new beekeepers in the islands. This renewed interest in keeping bees seems to parallel the recent increase in backyard beekeeping that is taking place across the mainland US and Europe (Keshner, 2010). The resurgence of small scale beekeeping in Hawaii, possibly due to more public awareness of the importance of bees and alternative pollinators, should be encouraged, especially because many of the tropical fruits and nuts planted locally are dependent on insect pollination for fruit production, and their yields are greatly improved by increased honeybee visitation. The results of the online survey confirm that there is already a close tie between farming and beekeeping interest in Hawaii. Historically, small scale farmers relied on feral bee populations to pollinate their crops, while only the large scale farmers had established arrangements with beekeepers. Since the arrival of *V. destructor* and the subsequent demise of feral bee colonies, the demand for managed pollinators in Hawaii has increased. However, many of the small scale vegetable farmers do not have the financial resources to pay for bees (personal observation). The local need for training has been met in part via workshops and conferences designed to educate the public about pollinators and provide practical assistance with honeybee colony management and pest control. On Oahu, the staff of the University of Hawaii Honeybee Project held six workshops at local farms and hosted a Honeybee Pollinator Expo on the University of Hawaii, Manoa, campus that drew approximately 200 attendees. On the Big Island of Hawaii, established beekeepers have received grant support form USDA agencies such as SARE and are now teaching members of the community how to box swarms and providing basic training in beekeeping. The popularity of these events is evidence of the recent public interest and need for more educational outreach programs.
Table 1.1 Beekeeper survey background results

<table>
<thead>
<tr>
<th>Island</th>
<th>Type of business</th>
<th>Years of experience</th>
<th>Primary use of the bees</th>
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<td></td>
<td>Hobbyist</td>
<td>Small business</td>
<td>Commercial</td>
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<td>Total</td>
<td>38%</td>
<td>44%</td>
<td>19%</td>
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<td>Oahu (n=9)</td>
<td>22%</td>
<td>67%</td>
<td>11%</td>
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<td>Big Island (n=15)</td>
<td>54%</td>
<td>31%</td>
<td>15%</td>
</tr>
<tr>
<td>Kauai, Maui, and Molokai (n=10)</td>
<td>30%</td>
<td>40%</td>
<td>30%</td>
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</table>
Table 1.2 Beekeeper survey hive maintenance and management results

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<th>Island</th>
<th>Queen excluder</th>
<th>Screen-bottom board</th>
<th>Opens brood box at least once a month</th>
<th>Re-queening frequency</th>
<th>Prefers commercial queens</th>
<th>Prefers own stock</th>
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<td>50%</td>
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<tr>
<td>Big Island (n=15)</td>
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<td>46%</td>
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<td>67%</td>
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<td>18%</td>
<td>82%</td>
</tr>
<tr>
<td>Kauai, Maui, and Molokai (n=10)</td>
<td>70%</td>
<td>10%</td>
<td>10%</td>
<td>0%</td>
<td>20%</td>
<td>80%</td>
</tr>
</tbody>
</table>

1 Any device with openings to permit the passage of worker bees but excluding the passage of drones and queen bees. Prevents the queen from entering honey supers.
2 A framed screen used instead of a solid bottom board to improve air circulation. Also, allows Varroa mites and other pests and debris to fall through and out of the hive.
3 Re-queening can be done either by intentionally introducing a queen to a queenless colony or allowing the colony to naturally re-queen itself. Although it is recommended to re-queen a colony once a year, colonies in Hawaii tend to re-queen themselves within a year (personal observation)
Table 1.3 Beekeeper survey treatment preference and practices results

<table>
<thead>
<tr>
<th>Island</th>
<th>Varroa mite treatment(s) used</th>
<th>Treatment practices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formic acid</td>
<td>Thymol</td>
</tr>
<tr>
<td>Total</td>
<td>76%</td>
<td>19%</td>
</tr>
<tr>
<td>Oahu (n=9)</td>
<td>78%</td>
<td>11%</td>
</tr>
<tr>
<td>Big Island (n=15)</td>
<td>90%</td>
<td>30%</td>
</tr>
<tr>
<td>Kauai, Maui, and Molokai (n=10)</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>
1.4. Appendix

1.4.1. UH Honeybee Online Survey

Beekeeper/grower information:

1) Name
2) Telephone
3) Address
4) Email

Background:

1) Do you consider yourself primarily a beekeeper or a farmer?
   a) Beekeeper
   b) Farmer
   c) Both
2) Number of years of beekeeping experience:
   a) Less than 1 year
   b) 1-5 years
   c) 6-10 years
   d) More than 10 years
3) If you consider yourself a beekeeper, which would best describe your business?
   a) Hobbyist (less than 5 hives)
   b) Small business (5-50 hives)
   c) Large business (more than 50 hives)
4) Location(s) of hives (check all that apply):
   a) Oahu
   b) Big island
   c) Kauai
   d) Maui
   e) Lanai
   f) Molokai
5) What do you keep bees for?
   a) Honey
   b) Pollination
   c) Both
6) If you consider yourself a farmer, what types of crops do you have? (check all that apply)
   a) Tropical fruits (example: lychee, rambutan, mango, avocado)
   b) Tropical nuts (example: macadamia)
   c) Vegetables (example: watermelon, cucumber, pumpkin)
   d) Herbs (example: basil, mint)
7) Number of hives before the Varroa mite arrived:
8) Number of hives currently:
9) Number of hives lost per year:
10) What do you think is the cause of your losses? (check all that apply)
   a) Mites
   b) Small hive beetle (SHB)
   c) Starvation
   d) Disease (example: nosema, chalkbrood)
   e) Unknown

Colony setup, dynamics, and management:

1) Hive setup (check all that apply):
   a) Single deep brood box
   b) Double deep brood box
   c) Western deep brood box
   d) Deep + western brood box
   e) Queen excluder
   f) Solid bottom board
   g) Screen bottom board
2) Honey flow months:
3) Swarm season months:
4) Drone season months:
5) How often do you re-queen?
   a) More than once a year
   b) Once a year
   c) Every 2 years
   d) Every 3 years
   e) Never, only natural re-queening
6) Do you prefer to use your own stock or to buy commercial queens?
   a) Own stock
   b) Commercial
7) How often do you work your hives in general?
   a) More than once a month
   b) Once a month
   c) Once every 2 months
   d) Once every 3 months
   e) Less than once every 3 months
8) How often do you check your honey supers?
   a) More than once a month
   b) Once a month
c) Once every 2 months  
d) Once every 3 months  
e) Less than once every 3 months  

9) How often do you go into your brood box?  
a) More than once a month  
b) Once a month  
c) Once every 2 months  
d) Once every 3 months  
e) Less than once every 3 months  

**Varroa** mite sampling and treatment:  

1) Types of **Varroa** mite treatment(s) used (check all that apply):  
a) Apistan  
b) Checkmite+  
c) Apiguard  
d) Formic acid  
e) Sucrocide  
f) Drone brood removal  
g) Powdered sugar  
h) Other (please specify)  

2) Do you alternate between different types of treatments or do you use a single product?  
a) Alternate  
b) Single product  

3) Do you modify the application according to the hive condition? For example do you use half the dose if you think the mite numbers are low or if colony size is small?  
a) No, I always use the same dose independent of the mite levels and colony size  
b) Yes, I reduce the amount of product if the mite levels are low or if colony size is small  

4) Do you treat in a schedule or when you sense the hives are not doing well?  
a) Yes, I treat all the colonies in my apiary in a set schedule  
b) No, I treat hives only when I feel they needed it  

5) How much do you estimate you spend on **Varroa** mite treatment on each hive per year?  
a) Less than $5 per hive  
b) $5-$10 per hive  
c) More than $10 per hive  

6) Do you sample for mites before treating?  
a) Yes  
b) No  

7) Method(s) used to sample for **Varroa** mites (check all that apply):  
a) Sticky boards  
b) Adult bee samples (alcohol/soap washing or sugar dusting of bees to dislodge the mites)
c) Capped brood samples (opening cells in search of mites)

8) What Varroa mite treatment(s) would you recommend to other beekeepers?
9) What makes this treatment(s) practical and appealing to use?

Small hive beetle (SHB) treatment:

1) Types of SHB treatment(s) used (check all that apply):
   a) Beetle traps
   b) Guard star
   c) Checkmite+
   d) Other (please specify)

2) Do you alternate between different types of treatments or do you use a single product?
   a) Alternate
   b) Single product

3) How much do you spend on each hive per year?
   a) Less than $5 per hive
   b) $5-$10 per hive
   c) More than $10 per hive

4) What SHB treatment(s) would you recommend to other beekeepers?
5) What makes this treatment(s) practical and appealing to use?
1.5. References


Villalobos, E.M. (2010) Treatment Recommendations Q1-2010, online publication by the UH Honeybee Project, last accessed on June 2011.

2.1. Abstract

The reproductive rate of *Varroa destructor* was measured in the drone brood of European honeybees (*Apis mellifera*) in Hawaii. A total of 601 infested sealed drone brood cells containing 1,146 foundress mites were analyzed. Of these cells, 327 were infested by a single foundress, while 274 were infested by two or more foundress mites. The number of infertile (non-reproductive) single foundresses was relatively high in this study; among singly infested drone cells, 18% had no evidence of egg laying. Viable offspring was recorded in 55% of the cells infested by a single foundress, while non-viable offspring occurred in 27% of the cells. The percentage of viable offspring in cells infested by multiple foundresses was higher at 85%, while 13.5% of the cells contained non-viable offspring. The fecundity of foundress mites (number of viable female offspring produced/foundress) in singly infested cells was higher than in cells infested by multiple foundresses. Drone cells infested by one foundress produced 3.28 viable female offspring/foundress, however decreased to as low as 1.10 when more than four foundresses infested the same cell.

2.2. Introduction

The *Varroa* mite, *Varroa destructor*, is a serious ectoparasitic pest of the European honeybee (*Apis mellifera*) that feeds on the hemolymph of adult and immature bees and reproduces exclusively within the cells of honeybee brood (Samataro et al., 2000). In *A.
*mellifera* colonies, adult *Varroa* females utilize drone and worker cells for reproduction. "Foundress" mites invade brood cells just before they are capped by the worker bees and begin to sequentially lay eggs within 5 days after cell invasion (Ifantidis, 1983). *A. mellifera* workers are capable of detecting and removing the bee brood from mite infested cells, thus interrupting the mite reproductive cycle (Spivak, 1996). However, such strong hygienic tendencies are present in only a small percentage of colonies within an apiary (Spivak & Gilliam, 1993), therefore, the great majority of invading mites have access to the brood for the whole length of their developmental cycle. Upon emerging from the brood cells, adult mites enter the phoretic stage and feed on adult worker bees. Mite parasitism debilitates immature and adult bees and contributes to the transmission of viruses among workers and drones (Carreck et al., 2010).

*Varroa* reproduction on its original host, the Asian honeybee, (*Apis cerana*), differs from that described above for *A. mellifera*. Reproduction in *A. cerana* is limited to drone brood (Koeniger et al., 1983), thus the reproductive success of foundress mites is directly linked to the availability of drones in the colony. In addition, *A. cerana* workers are able to detect and remove mites from infected sealed worker cells and groom themselves and other workers in the colony to remove mites (Rath & Drescher, 1990). The restriction to drone brood only, combined with behavioral adaptations against *Varroa* exhibited by *A. cerana* workers, typically prevents mite populations from reaching damaging levels in these colonies (Rath, 1999). In contrast, the host expansion to the European honeybee (*A. mellifera*) has greatly increased the potential population growth and impact of this parasite. *Varroa destructor* foundress mites attacking *A. mellifera* colonies have a higher reproductive output in drone brood compared to worker brood (Martin, 1995a; Ghami & Hoopinggarner, 2003; Calderon et al., 2007; Mondragon et al., 2006; Medina & Martin, 1999). Based on the studies cited above, foundress mites produce an average of 2.2 - 2.6
viable female offspring/cell in drone brood compared to, 0.7 - 1.0 viable female offspring/cell in worker brood. As a result, the Varroa mite has developed a stronger preference (8-11 fold higher) for drone cells over worker cells (Fuchs, 1990; Boot et al., 1995).

Despite the mite's higher reproductive output in drone brood, drone production in temperate climates is highly seasonal and drones constitute an abundant resource for mite reproduction only during a short part of the year. In Hawaii, however, the warm subtropical climate results in production of drone brood year-round, thus allowing Varroa mites to utilize drone brood even during the winter season. Nonetheless, population growth of V. destructor depends not only on brood availability, but also on the mite’s reproductive success within the brood cells. The objective of the present study was to describe the reproductive success of V. destructor on A. mellifera under subtropical conditions. The chapter is divided into 3 sections. In the first, a brief overview of Varroa mite reproductive biology is presented. Then, I report the results of original research on the reproductive success of the Varroa mite in A. mellifera drone brood in Hawaii. In the third section, I discuss the impact of the mite's reproductive success combined with the year-round drone brood production typical of Hawaii's honeybee colonies.

2.3. Varroa destructor biology and ecology

Parasites have a variety of adaptations to exploit their hosts, including specialized morphology, physiology, and behavior, as well as chemical mimicry (Vander Meer et al., 1989). V. destructor is a highly adapted bee parasite with morphological and physiological modifications including body shape, host chemical recognition, and synchrony of reproductive development with its host. Perhaps the most obvious morphological modifications of this parasitic mite is the absence of eyes, which is most likely related to the darkness of the inside of
the hive, and the subsequent need to rely on chemical signals for host selection. Researchers have reported that *V. destructor* females utilize semiochemicals to distinguish between different aged bees and preferentially attach themselves to nurse bees over foragers (Pernal et al., 2005). *Varroa* females are also known to use chemical cues to locate and invade brood cells immediately before they are sealed and to differentiate between drone and worker brood (Donze et al., 1998; Le Conte et al., 1989).

Another important morphological modification for bee parasitism is body size and shape. Adult female *Varroa* mites measure about 1.1 mm long x 1.6 mm wide and are reddish brown in color, while adult males are smaller, 0.75 - 0.98 mm long x 0.70 - 0.88 mm wide, and are light brown in color (Sammataro, 1995). Although female *Varroa* can attach themselves to the thorax (usually between the wings) where they are protected from the bee’s own grooming behavior, the mites’ dorsoventrally flattened body shape allows them to fit between the tergites of the worker's abdomens. Adult females are most often found feeding between the 3rd and 4th tergite in a ventro-lateral position on the left side of the bee's body (Figure 2.1) where they can best access the bee's ventricles with their mouthparts, (Bowen et al., 1997).

The life cycle of a parasite is often finely tuned to correspond to that of its host (Price, 1980) and the reproductive cycle of *V. destructor* shows a high degree of synchronization with its *Apis* hosts. The life cycle of *V. destructor* includes a phoretic stage and a reproductive stage. During the reproductive stage, a fertile adult female mite (foundress) will usually enter a prepupal brood cell one to two days before it is capped. The mite resides in the larval food at the bottom of the cell presumably to avoid being removed by nurse bees. Once the cell is capped, the mite attaches to the larva as it spins its cocoon. During the pupal stage, the foundress creates a feeding site on the 2nd abdominal segment of the bee (Calderon et al., 2009). This feeding site
will be the only one established, since the opening of a wound requires a high investment of energy (Donze et al., 1998). The foundress mite will begin egg laying 60 hours after the cell has been capped, the first egg usually being a haploid male (Ifantidis, 1983). The subsequent eggs will develop into diploid females and are laid sequentially one every 30 hours (Ifantidis, 1983). The emerging mite offspring will continue to feed from the same feeding site the foundress mite initially created. Female mites mature in 6.5 to 6.9 days, while male mites mature in 5.5 to 6.3 days and then mate with their mature sisters in succession (Donze & Guerin, 1994). The original foundress mite and her mature daughter offspring emerge with the newly developed adult bee and may be phoretic for a period of time (four to five days on average) before invading a new prepupal brood cell (Sammataro et al., 2000). Each foundress mite can undergo two to three reproductive cycles (Martin & Kemp, 1997). On the other hand, male Varroa mites have a short life span and are never seen feeding on adult bees. The main role of the male mite is to fertilize mature female offspring before the developing bee emerges from its cell. During the males’ final molt, their piercing-sucking mouthparts are modified solely for sperm transfer, and males die shortly after mating within the brood cell of a developing bee (Donze & Guerin, 1994).

2.4. Materials and Methods

2.4.1. Study Site and Hive Setup

The study was conducted on Oahu, Hawaii, from November 2008 to October 2009. A total of 19 colonies were utilized in this study, and hives were kept in a macadamia nut grove at the University of Hawaii Waimanalo Research Station. Each colony was kept in standard Langstroth hives, consisting of a single full-depth hive body with 10 full-depth brood combs. The study colonies were managed using standard apiculture techniques, including swarm
management, adding and removing honey supers, and providing sugar syrup and pollen patties as food during early stages of colony growth.

To encourage drone production, study colonies were equipped with either a Pierco plastic drone frame or a handmade half-frame, which consists of a Langstroth frame divided horizontally by a thin wooden bar with the top half of the frame containing worker wax foundation and the bottom half without foundation. The empty space provided below the foundation is then used by worker bees to draw comb for rearing drone brood. Pierco frames and half-frames were placed in the 3rd position (third frame from the right of the hive) within the study colonies and were replaced each month (except twice in August), totaling 13 drone comb removal dates. However, not all the study colonies received drone combs simultaneously at the beginning of the experiment. The study group initially started with five colonies and then increased to nineteen colonies by June 2009 as our apiary expanded through colony splits and captured honeybee swarms.

2.4.2. Drone Brood Sampling and Reconstruction of Mite Families

Drone combs were removed from hives as described above, and frozen at -20°C to kill the developing bees and mites within the cells. The total number of capped drone cells on each frame was recorded. A total of 50 capped drone cells from each drone frame (25 cells per side) was randomly sampled. The developing drones were carefully removed from each cell, and the infestation level and the developmental stage of the bee were recorded. The age of the drone brood sampled was determined by pupal eye color (Martin S. J., 1994). If mites were present, they were removed from the bee’s body and/or cell wall, placed on a microscope slide using dissecting forceps and a needle, and then examined through a stereo zoom microscope. Mite
family histories were reconstructed by counting the total number of foundress mites, male mites, mature daughter mites, deutonymphs, protonymphs, and eggs per cell. An example of a reconstructed mite family extracted from a drone cell is shown in figure 2.2. Because foundress mites and their mature daughters are similar in appearance, the number of foundress mites was inferred by counting the molt of each daughter mite (foundress mites leave no molt). Foundress mites trapped in the cocoon of the honeybee as well as dead mite offspring (evidenced by misshapen or shriveled bodies) were recorded as well. Data collection of “mite families” for the Waimanalo apiary was not adopted until January 2009.

To increase the accuracy of the mite family data, only infested cells containing drone brood 19 days (purple eye stage) or older were analyzed. Determining the age of the developing bee based on the color of the eyes and body has been widely used in similar studies (Martin, 1994; Medina & Martin, 1999; Martin, 2001; Ghamdi & Hoopingarner, 2003). To calculate the number of viable female offspring, deutonymphs were considered for drone brood 19-21 days old and protonymphs were considered only for drone brood 19 days old. The reason for those cut-offs is that the drone cells would have remained capped long enough for both mite stages to reach adulthood before the bee emerges. Drone cells containing a single foundress were analyzed separately from cells containing multiple foundress mites, for comparisons to be made with the results of published studies in which only brood cells containing a single foundress were examined.

Comparisons among existing studies are difficult, due largely to the different interpretations of mite family data that have been applied by various authors. The definition of reproductive variables including what constitutes a viable offspring and the quantification of female mite fecundity also appears to vary between studies. In this study, reproductive success
of foundress mites was estimated via dissection of cells containing honeybee pupa in the purple
eye stage and older. Because female mites lay their eggs sequentially over a period of days,
examining earlier honeybee pupal stages does not accurately reflect the reproductive potential of
the mother mite, as she might still lay more eggs, and it is also more difficult to estimate how
many of the immature mites will complete development before the bee emerges. Capped cells
with purple eye pupa and older were considered to contain viable mite offspring if the cells
contained at least one adult male (needed for fertilization) and at least one daughter mite that can
be expected to complete development prior to bee emergence (Martin, 1994). Non-viable
offspring include those brood cells containing only male offspring, only female offspring due to
male mite mortality or absence of a male, and female offspring that will not complete
development before the bee emerges.

The fertility of single foundress mites was based on the individual’s ability to produce at
least one offspring. Thus, foundress mites were characterized as infertile (non-reproductive) if
no offspring were found within the capped cell. Fecundity of single foundresses was defined as
the total number of viable offspring/cell. In those instances where multiple females occupy a
cell, an estimate of female mite fecundity was calculated based on the number of viable
offspring/number of total foundresses/cell. The fecundity of single foundresses provides
information on the reproductive potential of females, while the per capita fecundity rate obtained
for cells infested by multiple females provide important clues of female-female interactions
within cells. The data collected on mite fertility, the proportion of foundresses in single versus
multiple cells, and their respective fecundity rates/cell will contribute to our understanding of the
population dynamics of *V. destructor* under in Hawaii conditions.
2.4.3. Statistical analysis

The relationships between the number of capped drone cells and i) the percentage of infested drone cells and ii) the percent of infested cells containing multiple foundresses were described using a simple linear regression analysis (SigmaStat, 2006). To detect significant differences in the means of viable female offspring produced between singly and multiply infested cells containing two, three, four, and more than four foundresses, a Kruskal-Wallis one way analysis of variance on ranks was conducted (SigmaStat, 2006).

2.5. Results

Of the 69 drone frames sampled and 3,132 drone cells dissected during the course of this study, 1,545 cells (average 51% per frame, SD = 19.8%) were infested by 2,758 foundress Varroa mites. Mite infestation levels varied with the number of drones available for invasion by the female mites. The strong preference of foundress mites for drone cells resulted in a "crowding effect" of foundress mites when drone production was low, and the number of capped drone cells/frame was negatively related to mite infestation levels ($F_{1,67} = 18.24; R^2 = 0.214; P < 0.001$, Figure 2.3). Seasonal variation in drone production was observed throughout the year and will be discussed in detail in Chapter Three, however, drone production varied greatly among colonies independent of season, and mite family data can be best interpreted by considering the drone abundance in individual colonies rather than the time of year.

Among the drone cells dissected, 601 (17%) contained pupa that were old enough (purple eye stage or approximately 19 days old) to provide accurate data for mite family construction. These older drone cells produced a total of 1,146 foundress mites, which occurred either singly in a cell, in which case the daughter mites could only mate with their own brother, and as multiple foundress mites, in which case more than one male was usually present. Over 50% of
these cells (327/601) were invaded by a single foundress mite, and the remainder (274/601), had more than one foundress mite. The results also showed that cells with either two or three or more foundresses were equally common (24% and 22% of cells, respectively) (Figure 2.4). As with percent infestation, there was a significant negative relationship between drone production, and the proportion of multiple foundress mites/cell ($F_{1.58} = 5.29; R^2 = 0.08; P = 0.02$, Figure 2.5).

Mite fertility was calculated only for singly infested brood cells, since multiply infested cells could contain both fertile and non-reproductive foundress mites. The number of infertile (non-reproductive) single foundresses was relatively high in this study; among drone cells found to contain a single foundress, 18% (60/327) had no evidence of egg laying (Table 2.1).

Within brood cells containing a single foundress, viable offspring were recorded in 55% (181/327) of the cells; non-viable offspring occurred in 27% of the cells. Of the single foundress cells containing non-viable female offspring, 9% (29 cells) was due to male mortality and 10% (32 cells) was due to the absence of a male. A total of nine cells (3%) occupied by a single foundress produced only male offspring and did not contribute female offspring. Sixteen single foundress cells (5%) produced mature males and daughter mites, however the daughter mites’ development did not coincide with the age of the pupa, and as a result these mites would not be expected to mature before the bee brood would emerge. Consequently, these cells were also included in the non-viable offspring category under delayed egg laying.

In multiply infested brood cells, the percentage of cells containing viable female offspring was 85% (233 cells). Of the cells containing non-viable female offspring, 6.5% (18 cells) was due to male mite mortality and 6.5% (18 cells) was due to the absence of a male. Although records of fertility for individual foundresses could not be calculated from cells containing more than one mother mite, four cells (1.5%) with a total of 10 foundress mites had
no evidence of reproduction, suggesting that in those cells all 10 females were infertile. Cells with male offspring only were rare (0.5%) in multiply infested cells (Table 2.1).

The number of viable female offspring produced, which reflects the fecundity of the mites, was approximated in cells containing a single foundress and multiple foundress mites (Figure 2.6). Infested cells containing single foundress mites that produced viable offspring produced an average of 3.28 ± 0.11 (mean ± SE) viable females. However, when taking into account all adult foundresses (i.e., including non-reproductive mites and foundresses producing non-viable offspring), the mean number of viable female offspring in singly infested cells decreased to 2.15 ± 0.11 (mean ± SE). Having multiple foundress mites infest the same cell can negatively impact the reproductive potential of each mite due to overcrowding and competition for the same feeding site. As shown in figure 2.6, the production of viable female offspring decreased as the number of foundress mites per cell increased. In singly infested cells, each foundress mite produced significantly more viable offspring than multiply infested cells containing two, three, four, or more than four foundresses (all $P < 0.05$).

2.6. Discussion

Mite fertility (production of any offspring) was calculated only in cells containing a single foundress, since multiply infested cells could contain both fertile and non-reproductive foundress mites. In this study, the mite fertility in singly infested drone cells was 82%, a value lower than reported in other studies (Table 2.1). Calderon et al. (2007) reported mite fertility of 95.1% in drone brood of Africanized honeybees (AHB) in Costa Rica. Ghamdi and Hoopingarner (2003) and Martin (1995a) reported mite fertility of 93% and 96.3%, respectively, in drone brood of European honeybees. As a result of the relatively low mite fertility found in
this experiment, the percentage of non-reproductive foundresses was substantially higher in comparison with the other studies. According to the literature, 3.3% to 7% of the foundress mites were non-reproductive compared to the 18% found in our colonies (Calderon et al., 2007; Ghamdi & Hoopingarner, 2003; Martin, 1995a). Although it is unclear why mite fertility was lower than what was previously reported in other areas, factors such as climate, honeybee race, and Varroa haplotype have been found to influence Varroa infestation and reproduction (Martin et al., 1997; Correa-Marques et al., 2003), and may be important in Hawaii as well.

As stated earlier, viable offspring is defined in this study as the mite progeny found in capped cells (purple pupa eye stage and older) that contain one adult male and daughter mites that would complete development prior to bee emergence. This reproductive variable is crucial because these potentially mated females have a direct impact on the growth of the total mite population. Within singly infested cells, 55% of the foundress mites produced viable female offspring, which was considerably lower than reported by Martin (1995a) (64%), in European honeybees in southern England. Interestingly, the percentage of viable female offspring found in this experiment was closer to that reported in Africanized honeybees in the tropics (53%) (Calderon et al., 2007).

Non-viable offspring included cells that contained only male offspring (no female offspring produced), only female offspring due to male mite mortality or absence of a male, and female offspring that would not complete development before the bee emerges. Non-viable mites are unable to reproduce and, therefore, cannot contribute to the growth of the total mite population. Nineteen percent of singly infested brood cells contained only female offspring (9% male mite mortality and 10% absence of a male), which was nearly twice that reported by Martin (10%, 1995a). On the other hand, Calderon et al. (2007) reported a substantially higher number
of cells containing only female offspring (32.6%) in Africanized honeybees, with the majority being due to the absence of a male (26.5%). The higher percentage of non-viable offspring seen in the drone brood of Africanized honeybees compared to European honeybees has also been documented in the worker bee brood as well. Studies on Varroa mite reproduction in Africanized honeybee worker brood reported that nearly half of the cells (48%) contained non-viable offspring (mainly due to male mite mortality) compared to only 16% in European honeybee worker brood (Medina & Martin, 1999; Martin, 1994). The high percentage of non-viable females in both worker and drone brood of Africanized honeybees plays an important role in limiting the growth rate of the mite population, a characteristic not seen in European honeybee colonies.

Among multiply infested cells, 85% contained viable female offspring, while 13% contained non-viable offspring. The higher percentage of cells containing viable offspring was to be expected, since having multiple foundresses within a cell increases the chance of having a live male and live adult female present.

The mean number of viable females produced was 3.28 when only foundresses producing viable offspring were included (method one), which was relatively high compared to other studies using the same method. Both Ghamid and Hoopingarner (2003) and Calderon et al. (2007) reported foundress mites producing around 2.60 viable offspring, a difference of 0.68 from the value reported here. However, when all foundress mites are taken into account (method two), the mean number of viable females produced decreased to 2.15. This was consistent with the majority of findings (1.9 to 2.4 viable females) reported by researchers evaluating their data in the same manner (Martin, 1995b; Ghamid & Hoopingarner, 2003; Martin, 1995a). When comparing viable offspring produced within cells containing multiple foundress mites, there was
a negative correlation. As the number of foundresses within a cell increased, the number of viable female offspring per foundress decreased. This was to be expected, since multiple foundress mites would create more competition for the feeding site located on the honeybee pupae, thus, resulting in an increase of offspring mortality (Martin, 1995a; Martin, 1995b).
Figure 2.1. Adult female Varroa mite feeding on the abdomen of an *Apis mellifera* worker bee.
Figure 2.2. A reconstructed mite family extracted from a drone cell. F - foundress mite, M - male mite, D - mature daughter mites, Du - deutonymphs, P - protonymph.
Figure 2.3. The relationship between the number of capped drone cells/frame and infestation rate. The regression equation is: infestation = -0.0002(capped drone cells) + 0.611 and the relationship is significant ($F_{1,67} = 18.24; R^2 = 0.214; P < 0.001$).
Figure 2.4. The percentage of infested brood cells containing one, two, three, four, and more than four foundress mites. Total number of cells in parentheses.
Figure 2.5. The relationship between the number of capped drone cells and the percent of infested cells containing multiple foundress mites of each drone frame. The regression equation is: multiple foundress mites = -0.0001(capped drone cells) + 0.513 and the relationship is significant ($F_{1,58} = 5.29; R^2 = 0.084; P = 0.025$).
Figure 2.6. Average number of viable females produced per foundress producing viable offspring within cells containing single and multiple foundress mites.
Table 2.1. Reproductive parameters of *V. destructor* foundress mites in singly and multiply
infested drone brood of *A. mellifera*

<table>
<thead>
<tr>
<th>Reproductive Parameters</th>
<th>Single Foundress (n=327)</th>
<th>Multiple Foundress (n=274)</th>
<th>Martin, 1995a</th>
<th>Calderon et al., 2007*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent cells sampled (n= cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infertile (no reproduction)</td>
<td>18% (60)</td>
<td>1.5% (4)</td>
<td>3.3%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Viable offspring (mature male present and females can complete their development before bee emerges)</td>
<td>55% (181)</td>
<td>85% (233)</td>
<td>64%</td>
<td>53%</td>
</tr>
<tr>
<td>Only male offspring (no female offspring produced)</td>
<td>3% (9)</td>
<td>0.5% (1)</td>
<td>9.8%</td>
<td>7.6%</td>
</tr>
<tr>
<td>Non-viable due to male death</td>
<td>9% (29)</td>
<td>6.5% (18)</td>
<td>7%</td>
<td>6.1%</td>
</tr>
<tr>
<td>Non-viable due to male missing</td>
<td>10% (32)</td>
<td>6.5% (18)</td>
<td>4.1%</td>
<td>26.5%</td>
</tr>
<tr>
<td>Non-viable due to delayed egg laying (mature male present but female offspring will not complete development before bee emerges)</td>
<td>5% (16)</td>
<td>0%</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

*Africanized honeybees*
2.7. References


CHAPTER 3

IMPACT OF DRONE SEASONALITY ON THE EFFICACY OF DRONE BROOD REMOVAL FOR VARROA DESTRUCTOR CONTROL IN APIS MELLIFERA COLONIES IN HAWAII

3.1. Abstract

The drone brood removal method (DBR) is a biomechanical control option used to help manage Varroa destructor population levels in Apis mellifera colonies. Since Varroa mites have a stronger preference to reproduce in drone brood (developing male bees), it is possible to utilize drone brood as a trap to remove mites from the hive. Hawaii’s subtropical climate allows brood to be produced year-round, however, to date there have been no studies quantifying drone production. Seasonal drone patterns varied between our two study sites; the Waimanalo apiary exhibited a peak (June – September) and low (October – May) drone season, while the Manoa apiary had consistent drone levels throughout the year. During optimum drone conditions when colonies produced over 1,000 drones per frame, nearly half of the total mite population was trapped and removed. However, because the production of drones is a high investment for colonies, drone levels were considered low during the majority of the year. The results of this study suggest that DBR can be a useful component in an integrated pest management program by reducing the number of chemical treatments needed.

3.2. Introduction

Considered the most serious pest of honeybees, the Varroa mite (Varroa destructor) is an ectoparasite that feeds on the hemolymph of adult and immature bees, causing shortened life
span, disease, death, and ultimately weakened honeybee colonies. The mite was first detected in Hawaii in April 2007 on the island of Oahu, and then on the Big Island in August 2008, posing a serious threat to Hawaii’s honey and queen bee industry. Since the mite’s introduction to Hawaii in 2007, beekeepers have experienced an increase in colony losses, while farmers have seen a reduction in pollination success (personal observation).

Synthetic chemicals remain the most common method of Varroa mite treatment in Europe and the US. However, the development of resistance to these chemicals is a serious concern and has been reported worldwide (Dujin et al., 1991; Lodesani et al., 1995; Elzen et al., 2000; Pettis, 2004). Residue build up within the hive is also an issue and has been reported to cause negative effects on colony health, such as, a reduction in size and weight of emerging queen bees and an increase in worker mortality (Lodesani et al., 1992; Haarmann et al., 2002; Pettis et al., 2004). Therefore, beekeepers should consider using alternative control options, such as organic chemicals (i.e. formic acid and thymol) and biomechanical methods (i.e. drone brood removal and screen-bottom boards) in their Varroa treatment programs.

The majority of studies on drone brood removal (DBR) have been conducted in temperate regions, where brood production is highly seasonal. There is little information on the abundance and seasonality of drone production in tropical regions or on the effectiveness of DBR in environmental conditions similar to Hawaii. In this chapter, I will first discuss the worldwide spread of the Varroa mite and the ecological and behavioral parameters underlying the use of DBR for mite control. I will then present colony survey data that illustrates the seasonal trends of drone production in two apiaries on Oahu, and finally, I will describe the efficacy of the drone brood removal method as a potential control option for beekeepers in Hawaii.
3.2.1. The Varroa Mite: A Worldwide Problem

The Varroa mite was first described by Oudemans (1904) as an external parasite of the Asian honeybee, *Apis cerana* from Java, Indonesia. In 1962-63 the Varroa mite was found parasitizing the European honeybee, *A. mellifera*, in Hong Kong and the Philippines (Delfinado, 1963). Since Varroa expanded its host range to include *A. mellifera*, it has rapidly spread worldwide, owing largely to the movement of queen bees and managed hives by beekeepers from infested areas.

*Varroa destructor* was first reported in the United States in 1987 in Florida and Wisconsin. Since then, Varroa has spread throughout the United States eliminating much of the feral honeybee population and significantly impacting the honeybee industry. New Zealand and Hawaii remained Varroa free due to strict regulations prohibiting the importation of live honeybees. However, in 2000 *V. destructor* was detected in three honeybee colonies on a property in northern New Zealand (Zhang, 2000) and in 2007, Varroa was first detected in Hawaii on the island of Oahu. As of this writing Australia is the only continent that remains Varroa free.

3.2.2. Trapping Mites in Drone Brood

*V. destructor* is able to reproduce on both drone and worker brood of *A. mellifera*, contributing largely to the mite’s success as a parasite of the European honeybee. However, the reproductive potential of female Varroa mites is greatly influenced by the sex of the brood parasitized. Drone bees are larger and have a longer development time compared to worker bees, and both of these factors contribute to increased reproductive output of female mites on drone brood. Previous work has shown that foundress mites can produce an average of 2.2 – 2.6
viable female offspring on drone brood compared to 0.7 – 1.0 viable female offspring on worker brood (Martin, 1995a; Calderon et al., 2007; Mondragon et al., 2006; Medina and Martin, 1999; Ghami & Hoopinggarner, 2003). The reproductive advantage derived from drone use is reflected in the strong preference (8-11 fold higher) by *V. destructor* females to invade drone over worker cells (Fuchs, 1990; Boot et al., 1995), and this natural tendency has been used to develop the “drone comb” as a biomechanical tool for *Varroa* control. When using this control strategy beekeepers can designate a frame solely for drone production within their hives by using a Pierco plastic drone frame which contains drone cell foundation (~2000 cells). The drone frame is usually inserted near the middle of the hive next to other frames containing brood. Female *Varroa* mites will enter the open drone cells containing drone larvae just before the cell is sealed. When the majority of drone cells are sealed by the worker bees, the drone frame can be removed from the hive along with the trapped mites and cleaned for reuse. Usually a single drone frame is used in colonies consisting of one deep hive box (10 frames), however, depending on the size and strength of the colony, multiple drone frames can be used simultaneously.

There is very little information regarding the natural levels of drone production of managed hives, however, it is well known that in temperate regions where temperature and climate greatly influence brood production, drones are seasonal, and consequently DBR is restricted to the warmer summer months when colonies are most active reproductively. In principle, the introduction of a Pierco drone frame into a colony that is in reproductive mode, facilitates drone cell construction and also, encourages the bees to concentrate the drones in a single area, making it much easier to remove drones from the hive. Rearing large numbers of drones is energetically costly for honeybee colonies due to the production of drone cells and larval food. Nevertheless, researchers evaluating the efficacy of DBR on the mainland USA,
kept colonies consisting of two deep hive boxes (20 frames) and used as many as three drone frames without adversely affecting colony growth (Calderone, 2005; Wantuch & Tarpy, 2009). These studies also found that mite levels could be suppressed to tolerable levels throughout summer until early fall, which eliminated the need for additional control measures in the spring. Santas and Lazarkis (1984) reported foregoing the need of chemical treatments for two and a half years in colonies under DBR, whereas control colonies (receiving no Varroa treatment) required chemical treatment after only one year.

Hawaii’s tropical climate presents several interesting questions regarding the reproductive strategies of the colony and the associated control options for Varroa control. Given that local colonies can produce brood, including drones, year-round, the implementation of a DBR component as an alternative for Varroa control seems appropriate. However, the efficacy of a drone-base control method for Varroa will depend, in part, on the number of drone cells that are produced at any one time in a colony, and the effort required from the beekeeper to monitor, remove, and replace the drone comb frames at an apiary level. In this chapter, I will examine the seasonal trends of drone production in two Hawaii apiaries, and discuss how microclimates may affect drone levels at each site. The data collected will help determine whether seasonal trends in drone production can be identified and exploited to maximize the efficacy of DBR in Hawaii.

3.3. Material and Methods

3.3.1. Study Sites and Hive Setup

The study was conducted on Oahu, Hawaii, from November 2008 to October 2009. A total of 22 colonies were utilized in this experiment from two different apiaries (~26 km apart).
Sixteen of the study colonies were kept at an apiary in a macadamia nut grove at the University of Hawaii Waimanalo Research Station. The second apiary, where six colonies were monitored, was located at the University of Hawaii Bekesy Laboratory in Manoa. Each colony was kept in a standard Langstroth hive, consisting of a single full-depth hive body with 10 full-depth worker combs. All the colonies used in this study were European honeybees of Italian stock. The study colonies were managed using standard apiculture techniques, including swarm management by adding and removing honey supers, and the feeding of sugar syrup and pollen patties during early stages of colony growth.

Sixteen colonies from the Waimanalo apiary were randomly chosen to be in the study group and as such the only Varroa control method used in these colonies was drone brood removal. The study group initially started with five colonies in November 2008 and was increased to sixteen colonies by June 2009 as our apiary grew in size due to colony splits and catching honeybee swarms. Study colonies were equipped with either a Pierco plastic drone frame or a handmade half-frame, which consisted of a Langstroth frame divided horizontally by a thin wooden bar with the top half of the frame containing worker wax foundation and the bottom half without foundation (Figure 3.1). The empty space provided was used by worker bees to draw comb for rearing done brood. All six colonies at the Manoa apiary were included in the study group and each received a Pierco plastic drone frame. At both apiaries, the drone frames were placed in the 3rd position within the colonies (third frame from the right of the hive).

3.3.2. **Drone Production and Drone Comb Utilization Monitoring**

The drone comb use and drone production (total number of capped drone cells/ drone frame) for each colony was monitored throughout the experimental period. At the Waimanalo
apiary, drone combs were removed and replaced each month for one year (except twice in August), totaling 13 drone comb removal dates and a total of 80 drone frames sampled. At the Manoa apiary, drone combs were removed and replaced approximately every three weeks, totaling 17 removal dates during the study period with 66 total drone frames sampled. Removed frames were taken to the laboratory where the total number of capped drone cells on each frame was recorded. Occasionally a study colony failed to produce drones during a sampling period, and in these cases the frames were simply left in place in the hive until the next removal date.

To describe trends of drone comb utilization at the Waimanalo apiary, I compared the tendency of individual colonies to produce drone brood during different months of the year. Colonies were scored as “active” if they used the drone frame at least once during a given period, otherwise the colonies were considered as “inactive”. The active versus inactive score was a binomial response, independent of the total number of drones produced. To further quantify the degree of variability in drone production, I also scored colonies based on their reproductive output. Colonies were considered having high drone investment if they produced ≥ 1,000 drones/frame during consecutive drone comb removal dates.

3.3.3. Drone Brood Sampling

Upon removal, the drone frames were frozen to kill the developing bees and mites trapped within the cells. Fifty capped drone cells were randomly sampled from each drone frame (25 cells per side). As of January 2009 at the Waimanalo apiary, “mite families” from each infested cell were “reconstructed” by counting the total number of foundress mites, male mites, mature daughter mites, deutonymphs, protonymphs, and eggs. The complete procedure of sampling drone cells and reconstructing mite families is described in Chapter Two. The drone
frames collected from the Manoa apiary were only sampled for infestation rate and no mite family data were recorded. When combs could not be examined immediately, they were stored in a freezer at -20°C for later examination.

DBR traps and removes foundress mites as well as the daughter mites developing within the brood cells. It is important however, to distinguish between viable and non-viable daughter mites since only those daughters that are fertilized and complete development before the bee emerges will contribute to mite reproduction. To determine if a daughter is likely to reach maturity before the bee emerges from the cell we used the development chart produced by Medina and Martin (1994), which compares mite nymph stages to drone pupal development and allows us to determine whether a particular Varroa nymph will have enough time to reach the adult stage. In addition, because fertilization takes place in the cell, daughters need a mature male in the cell to be considered viable.

3.3.4. Measuring the Efficacy of DBR

The efficacy of drone removal as a mite control method will depend on: 1) the frequency of utilization of the drone comb by each colony, 2) the number of drones removed per frame, and 3) the percent infestation of the drone cells. Data regarding the frequency of drone comb use and drone production by individual colonies were obtained during the regular drone comb removal dates. The percentage infestation of each comb and data on mite families were derived from the drone comb sampling as described previously.
**Estimating adult mites removed via drone comb**

The efficacy of DBR in this study was estimated by comparing the total number of adult mites removed in a drone frame with a four-week cumulative mite drop following an application of Mite Away Quick Strips (MAQS), a commercial mite treatment.

The total number of mites trapped and removed in each drone frame was estimated as:

\[ T = \frac{A \times B}{C} \]

where \( T \) is the total number of mites removed, \( A \) is the number of mites found in the cells sampled, \( B \) is the total number of capped drone cells on the frame, and \( C \) is the number of cells sampled. This formula was used to estimate the total foundresses removed/frame and the total adult mites (foundresses and mature daughter mites) removed/frame.

**Estimating total colony mite level**

As a way to measure the relative impact of DBR, the total number of adult mites removed/frame (described above) was compared with the total colony mite level. To estimate the total number of mites within a colony, I used the average mite fall after MAQS treatment, a number that includes both phoretic mites (mites not in brood cells) and mites found in worker and drone cells.

MAQS function as a fumigant, formulated as gel pads placed on the top of the brood chamber. The data used derive from five trials conducted from July 2009 to July 2011 on the islands of Oahu and the Big Island. The study colonies were assigned to either a treated group which received the recommended dose of MAQS (\( n = 32 \) colonies), and a control group which did not receive any treatment (\( n = 19 \) colonies). The mite drop was monitored by outfitting the hives with bottom boards covered with a 3 mm mesh screen. “Sticky boards” were placed under
the mesh screen to collect the fallen mites. The “sticky boards” were removed and replaced at weekly intervals over four weeks, and the collected mites were counted and recorded. The total mite fall from the first day of MAQS application to the end of the fourth week was considered the total mite kill from the product. Based on the field data collected from treated and control colonies, the MAQS efficacy was 83% (74% - 94%) (Villalobos, In preparation). To obtain an estimate of colony mite levels, we used the total mite drop of MAQS and corrected the count using the reported 83% efficacy in Hawaii:

\[ C = T \times 0.83 \]

where C is the estimated colony mite level and T is the total mite drop (includes foundress and mature daughter mites) under MAQS treatment.

### 3.3.5. Statistical Analysis

A mixed model Analysis of Variance was conducted using PROC MIXED (SAS Institute, 2003) to detect significant differences in the means of drone numbers during the peak and low months at both apiaries and to detect significant differences in the means of foundress mites removed/frame at different drone levels and at different periods of the year. A Wilcoxon Signed Rank Test was conducted to detect significant differences between passive mite fall levels one week prior to removing the drone comb and one week post drone comb removal. The relationship between the number of mites removed/frame and the number of drones/frame was measured using a regression analysis (SigmaStat, 2006). Kruskal-Wallis One Way Analysis of Variance on Ranks was performed to detect significant differences in the means of total adult mites removed per drone frame at different drone levels. Mann-Whitney Rank Sum Test was
conducted to detect significant differences in the means of total adult mites removed per drone frame at different periods of the year.

3.4. Results

3.4.1. Drone Production

Drones were produced year round at both study sites, however the individual colonies at each apiary varied in their frequency of drone comb use (see below) and drone production levels (capped drone cells/frame). At the Waimanalo site, individual colonies were highly variable in the number of drones produced, with the average drone frame having 567.18 ± 54.88 (mean ± SE) capped drone cells (Table 3.1). Drone production appeared to be seasonal in the Waimanalo apiary, and the peak of drone production occurred between June and September, when the average drone frame contained 787.64 ± 78.09 (mean ± SE), compared to 323.50 ± 54.89 drones/frame between October and May ($F_{1;78} = 22.74, P < 0.0001$) (Figure 3.2). The period of high drone production (June to September) will hereafter be referred to as the peak drone months, while the period of low drone production (October to May) will be referred to as the low drone months.

At the Manoa apiary, colonies averaged 550.47 ± 50.75 (mean ± SE) capped drone cells/frame. Although variation in drone production among colonies was observed, there was no seasonal difference during the peak and low drone months as observed at the Waimanalo apiary. From June to September, an average of 533.10 ± 71.83 (mean ± SE) drones per colony were produced at the Manoa apiary compared to 560.88 ± 69.24 from October to May ($P = 0.988$) (Figure 3.3).
3.4.2. Drone Comb Utilization

At the Waimanalo apiary, where drone production exhibited a seasonal trend, drone comb utilization seemed to be influenced by the time of year as well. All the study colonies (16/16) were scored as “active” (as defined above) during the peak drone season compared to 81% (13/16) during the low drone season. In addition, one-fourth of the Waimanalo study colonies had high drone investment during the peak drone period compared to no colonies with high done investment during the low drone period. At the Manoa apiary, there was no seasonal difference in drone comb use; all the study colonies (6/6) were scored as “active” during both peak and low drone seasons. Additionally, there were two colonies with high drone investment during the peak months and three colonies during the low months, which again show no seasonal trend.

3.4.3. Passive Mite Fall

The number of drones being produced and removed from study colonies was found to have an impact on mite drop levels throughout the year. During peak drone months at the Waimanalo apiary, there was a significant difference in weekly passive mite falls before and after the removal of the drone comb. Study colonies had significantly fewer mites the week following drone brood removal (mean ± SE = 160.35 ± 19.13) compared to the week prior to drone brood removal ([242.43 ± 35.78]; z = -3.657; P < 0.001, Wilcoxon signed rank test). However, during the low drone months, there was no significant difference between weekly mite falls before and after (285.66 ± 35.26 and 287.16 ± 36.89) the removal of the drone comb (t = -0.0516; df = 35; P = 0.959). However, mite fall numbers two weeks after drone comb removal during both peak and low drone months were not significantly different from mite fall numbers one week prior, suggesting that the impact of DBR is relatively short (Figure 3.4).
At the Manoa apiary, differences in weekly mite falls before and after drone comb removal were also found throughout the year. During the months of June to September, passive mite fall numbers were significantly less one week after removing the drone combs (mean ± SE = 90.61 ± 13.72) compared to the week prior to drone comb removal (143.11 ± 23.96; z = -2.76; P = 0.006). During the months of October to May, a significant difference in mite falls before and after drone comb removal was also found. Colonies averaged 204.79 ± 24.83 (mean ± SE) mites one week before removing the drone comb, compared to 151.68 ± 17.76 mites one week after (z = -3.88; P < 0.001). Mite fall numbers two weeks after drone comb removal during both peak and low drone months were not significantly different from mite fall numbers one week prior (Figure 3.5).

3.4.4. Measuring the Efficacy of DBR

Estimating foundress mites removed

The “mite family” data collected from the drone combs at the Waimanalo apiary were used to estimate the number of foundress mites trapped and removed in each drone comb (Table 3.2). The number of capped drone cells and the number of foundress mites removed per drone frame were positively related (F1,55 = 28.169; R² = 0.339; P < 0.001). For instance, colonies producing more than 1000 drones per drone frame trapped 891.25 ± 192.31 (mean ± SE) foundress mites, compared to 264.21 ± 40.39 (mean ± SE) foundress mites when less than 500 drones were produced (P < 0.0001) (Figure 3.7).

During the peak drone months at the Waimanalo apiary, drone combs trapped and removed significantly more foundress mites than during the low drone months (F1,55 = 7.44; P <
Drone combs removed 597.43 ± 87.93 (mean ± SE) foundress mites during peak drone months compared to 296.32 ± 59.38 foundress mites during the low drone months.

*Estimating total adult mites removed*

When taking into account all adult mites (foundress mites and mature daughter mites) trapped within the drone cells, the total number of individuals removed per drone frame increased as expected (Table 3.2). In colonies producing over 1,000 drones, drone combs trapped and removed a large number of adult mites (mean ± SE = 1262.25 ± 789.33). Colonies producing 500 – 1,000 drones and those with fewer than 500 drones removed 836.45 ± 455.56 and 370.45 ± 276.31 adult mites respectively (H = 24.85; df = 2; P < 0.001). During the peak drone months, drone brood in colonies was trapping and removing 819.21 ± 101.91 (mean ± SE) adult mites per frame compared to 495.33 ± 91.04 adult mites during the low drone months (Mann-Whitney U-test, P = 0.002). Similar to the number of foundress mites removed, there was a positive relationship between total adult mites removed and the number of capped drone cells (F₁,₆₃ = 36.277; R² = 0.365; P < 0.001, Figure 3.9).

*Estimating total colony mite level*

The average mite level within a hive was estimated using colonies that reached the passive mite fall threshold of 50 mites/day before being treated with MAQS. These colonies (n = 32 colonies) under MAQS treatment dropped an average of 2,200 adult mites during a four-week period. Taking into consideration the product’s reported efficacy in Hawaii (83%), the estimated colony mite level was approximately 2,650 mites. This estimation was used to measure the relative proportion of mites DBR can potentially remove from a colony. During the
periods of high drone investment when colonies produced over 1,000 drones, large numbers of mites were being trapped (as reported above), which resulted in DBR removing approximately 48% of the total mite population. Colonies producing 500 – 1,000 drones and those with fewer than 500 drones removed 32% and 14% of the total mite population respectively.

3.5. Discussion

Honeybee drone production in temperate regions is strictly regulated by ambient temperatures and food availability; colonies synchronously produce drones and queens during the most favorable times of the year. The short reproductive period of temperate climates is highly predictable, and colonies produce large numbers of drones in a few months or even weeks. It seems that the subtropical climate of Hawaii, with relatively small temperature variations and overall mild climate, contributes to a decrease in the degree of synchronicity in drone production among colonies. In this study, a strong seasonal trend in drone production was evident only at the Waimanalo site, but not in the Manoa apiary. At Waimanalo, drones were most abundant throughout the summer and early fall (June – September) and during that time drone production was nearly 2.5 folds higher than the rest of the year (October – May). Drone production at the Manoa apiary remained relatively constant throughout the year. The lack of seasonality in drone abundance at the one site cannot be explained by differences in ambient temperature between the two locations but may have been influenced by differences in rainfall patterns and blooming peaks. Although the two apiaries were only 25.7 km apart, the annual rainfall is dramatically different between the two sites; for example in 2009, Manoa’s annual rainfall was 308.2 cm/year compared to 111.5 cm/year at Waimanalo (Climatological Data Annual Summary Hawaii and Pacific, 2009). In addition to the large differences in rainfall, the
Waimanalo site exhibited a seasonal pattern in precipitation, including a prolonged dry period (June-September) with an average rainfall of 3.9 cm/month. Interestingly, the months with high levels of drone production at that apiary corresponded with periods of low precipitation. In contrast the Manoa apiary is located in a valley with high and constant rainfall, and their drone production levels, although variable between colonies, did not exhibit marked seasonality. These data highlight the need for further research to fully understand the interactions between honeybee colony dynamics, Hawaii’s unique topography and associated microclimates (Giambelluca & Schroeder, 1998), and variation in local floral resources.

The study colonies displayed great variability in their energetic investment in drone production. In fact, the number of males per drone frame varied greatly among colonies in the same apiary, suggesting that drone production is influenced not only by environmental factors but also by colony characteristics, possibly such as colony strength, resident queen age, and/or colony age. While seasonality in drone production was evident at Waimanalo, there were individual colonies are not synchronous in their use of the drone comb nor the energetic investment devoted to drone production. For instance, even during the peak drone months at the Waimanalo apiary, not all the colonies invested equally in drone production. In fact during those months, fourteen drone frames had over 1,000 males, while six drone frames contained less than 200 males, including two cases where the colonies produced less than 70 drones/frame. At the Manoa apiary, where the average number of drones remained relatively constant throughout the year, there were also large inter-colony variations in drone comb use, and it was at the Manoa apiary where the largest deviations in drone production between hives were recorded. Based on the results it seems that although environmental conditions may favor drone production at certain times of the year, there is still large individual variability in the timing of the reproductive
investment by each colony. It is conceivable that seasonal rainfall patterns and blooming peaks may encourage colonies to synchronously produce reproductives (as at Waimanalo), much like cold winters influences the bees in temperate climates. However, the fact that individual colonies can produce large numbers of drones all throughout the year indicates that there is no great “penalty” for producing drones “out of season”. More research needs to be conducted to dissect the relative importance of climate versus colony characteristics in the production of drones by Hawaiian colonies.

The efficacy of the DBR can be described in terms of the number of adult mites (foundress mites and mature daughter mites) removed per drone frame. The number of adult mites removed was positively related to the number of drones produced, and, as such, the efficacy of drone removal will increase in times of high drone availability. Because drone comb removal is time consuming, it is important to consider what time of the year would maximize the effectiveness of this method. Obviously, the “pay offs” for beekeepers using this kind of control will depend on the total numbers of mites eliminated at each removal date. Sites, such as the Waimanalo apiary, which exhibited a seasonal trend in drone production, would allow a beekeeper to concentrate their drone removal efforts to a shorter, more productive peak season. Concentrating on the peak season would ensure that their management is more cost effective. During Waimanalo’s four-month peak drone season, the number of mites trapped and removed from each colony was 2 fold higher compared to the rest of the months. In addition, individual colonies invested more heavily in drone production during the peak season; 64% of the colonies sampled produced at least the “peak drone season average” of 787.64 drones/frame during June-September, compared to only 19 % of colonies during the low drone season. Consequently, the likelihood that a colony would utilize a drone comb increased in the peak season and the total
number of drones produced was also greater, making this summer peak the recommended time for inclusion of DBR in an Integrated Pest Management strategy.

Because, mite reproduction in European honeybees is not restricted to drone brood alone, and the overall impact of DBR will depend on the proportion of mites reproducing on drone versus worker brood. Measuring the precise impact of DBR would require comparing the total number of mites trapped and removed with the total mite population within a colony. These types of data are not presently available, but an estimate of average adult mite population/colony can be obtained using the total number of mites killed when a Varroa treatment is applied to a colony. Mite treatments, whether based on synthetic or organic compounds, are not 100% effective, so the total number of mites in a colony is the number of mites killed by the treatment plus, the number of mites that survive the treatment (based on the average efficacy of the control product). In this study, I used the total mite kill of the product MAQS in colonies with a passive mite fall of at least 50 mites/day before treatment and estimated that colonies contained on average 2,650 adult mites.

Although DBR is only able to target mites in drone cells, the drone comb was able to trap and remove 48% of the colony’s total mite population (1262.25 adult mites/frame). However, this efficacy was only achieved during optimum drone conditions in colonies that were producing over 1,000 drones/frame. Maintaining high drone levels is a costly investment for colonies, thus fewer than 500 drones/frame were produced during two-thirds of the year, resulting in an average DBR efficacy of 25% for the entire year (649.79 adult mites/frame). In comparison with MAQS, which targets phoretic mites and mites in drone and worker cells, treated colonies achieved an average mite kill of 2,200 adult mites (83% efficacy) (Villalobos, In preparation). Although MAQS have a higher efficacy, DBR can be a useful component in an
IPM program during the periods of peak drone production, reducing reliance on a single mite suppression technique. Because weather conditions vary greatly over short distances in Hawaii, temporal patterns in drone production can vary considerably between apiaries. Therefore, it is important that beekeepers become familiar with their apiary’s colony dynamics and seasonal drone pattern in order to maximize the efficiency of DBR.
Figure 3.1. Types of frames used to stimulate drone production. The top picture is of a plastic Pierco drone frame. The bottom picture is of a modified frame divided in half by a wooden bar. The top half of the frame is used for worker brood production, while the bottom half is used for drone brood production.
Table 3.1. Average number of capped drone cells removed from treatment colonies

<table>
<thead>
<tr>
<th>Month</th>
<th>Waimanalo apiary (n=16)</th>
<th>Manoa apiary (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  ±SE</td>
<td>Mean  ±SE</td>
</tr>
<tr>
<td>Nov. 2008</td>
<td>157.5  20.8</td>
<td>894.4  146.0</td>
</tr>
<tr>
<td>Dec. 2008</td>
<td>448.6  75.7</td>
<td>709.7  253.1</td>
</tr>
<tr>
<td>Jan. 2009</td>
<td>161.0  87.9</td>
<td>568.3  106.5</td>
</tr>
<tr>
<td>Feb. 2009</td>
<td>420.4  232.6</td>
<td>na na</td>
</tr>
<tr>
<td>Mar. 2009</td>
<td>440.5  237.6</td>
<td>374.3  161.1</td>
</tr>
<tr>
<td>Apr. 2009</td>
<td>367.8  163.2</td>
<td>233.3  233.3</td>
</tr>
<tr>
<td>May 2009</td>
<td>212.2  50.0</td>
<td>466.7  295.1</td>
</tr>
<tr>
<td>Jun. 2009</td>
<td>605.5  94.3</td>
<td>410.8  127.8</td>
</tr>
<tr>
<td>Jul. 2009</td>
<td>720.7  207.7</td>
<td>291.7  189.9</td>
</tr>
<tr>
<td>Aug. 2009</td>
<td>1045.4 142.4</td>
<td>393.8  151.3</td>
</tr>
<tr>
<td>Sept. 2009</td>
<td>577.6  142.0</td>
<td>845.8  75.8</td>
</tr>
<tr>
<td>Oct. 2009</td>
<td>299.8  138.5</td>
<td>525.0  126.0</td>
</tr>
<tr>
<td>Average</td>
<td>567.18 54.88</td>
<td>550.47 50.75</td>
</tr>
</tbody>
</table>
Figure 3.2. Average (± SE) number of capped drone cells produced per frame in colonies at the Waimanalo apiary. Between the summer months of June to September (peak drone months) colonies significantly produced more drones than the months of October to May (low drone months) ($F_{1;78} = 22.74$, $P < 0.0001$).
Figure 3.3. Average (± SE) number of capped drone cells produced per frame in colonies at the Manoa apiary. There was no significant difference in drone production between the months of October to May and June to September (P = 0.988).
Figure 3.4. Average (± SE) passive mite fall one week prior and one week after the removal of the drone comb at the Waimanalo apiary. During the peak drone months (Jun-Sept), passive mite fall numbers one week after drone comb removal was significantly less than the week prior ($z = -3.657; P < 0.001$, Wilcoxon signed rank test). During the low drone months (Oct-May), passive mite fall numbers did not significantly differ one week prior and one week after drone comb removal ($P = 0.959$).
Figure 3.5. Average (± SE) passive mite fall one week prior and one week after the removal of the drone comb at the Manoa apiary. During June to September, passive mite fall numbers one week after drone comb removal was significantly less than the week prior ($z = -2.76; P = 0.006$, Wilcoxon signed rank test). During October to May, passive mite fall numbers one week prior and one week after drone comb removal was also found to be significantly different ($z = -3.88; P < 0.001$).
Table 3.2. Average number of foundress mites and adult mites removed per drone frame from the Waimanalo apiary

<table>
<thead>
<tr>
<th>Month</th>
<th>Foundress mites (Mean ±SE)</th>
<th>Adult mites (Mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Waimanalo apiary (n=16)</td>
<td></td>
</tr>
<tr>
<td>Jan. 2009</td>
<td>126.0 ±110.6</td>
<td>264.0 ±225.6</td>
</tr>
<tr>
<td>Feb. 2009</td>
<td>420.0 ±228.8</td>
<td>784.6 ±449.5</td>
</tr>
<tr>
<td>Mar. 2009</td>
<td>411.7 ±110.1</td>
<td>542.8 ±145.7</td>
</tr>
<tr>
<td>Apr. 2009</td>
<td>284.7 ±106.4</td>
<td>388.1 ±154.2</td>
</tr>
<tr>
<td>May 2009</td>
<td>248.9 ±132.1</td>
<td>304.3 ±126.7</td>
</tr>
<tr>
<td>Jun. 2009</td>
<td>501.8 ±90.4</td>
<td>859.8 ±163.9</td>
</tr>
<tr>
<td>Jul. 2009</td>
<td>574.0 ±125.5</td>
<td>609.7 ±128.6</td>
</tr>
<tr>
<td>Aug. 2009</td>
<td>762.3 ±234.6</td>
<td>1020.5 ±229.7</td>
</tr>
<tr>
<td>Sept. 2009</td>
<td>452.6 ±197.2</td>
<td>488.0 ±181.4</td>
</tr>
<tr>
<td>Oct. 2009</td>
<td>181.8 ±103.4</td>
<td>182.5 ±104.0</td>
</tr>
</tbody>
</table>
Figure 3.6. The relationship between the number of capped drone cells and the number of foundress mites removed per drone frame. The regression line is foundress mites removed = $0.496(\text{capped drone cells}) + 161.971$ and the relationship is significant ($F_{1,55} = 28.169$; $R^2 = 0.339$; $P < 0.001$).
Figure 3.7. Average (± SE) number of foundress mites removed per drone frame at different drone levels. The number of foundress mites removed at the three drone levels (<500, 500-1000, and >1000) were all significantly different from each other (all drone levels $P < 0.05$).
Figure 3.8. Average (± SE) number of foundress mites removed per drone frame from colonies at the Waimanalo apiary. During the peak drone months (Jun-Sept), significantly more foundress mites were removed in each drone comb compared to the low drone months (Oct-May) ($F_{1,55} = 7.44, P < 0.0086$).
Figure 3.9. The relationship between the number of capped drone cells and the number of adult mites removed per drone frame. The regression line is foundress mites removed = 0.524(capped drone cells) + 229.239 and the relationship is significant ($F_{1,63} = 36.277; R^2 = 0.365; P < 0.001$).
3.6. References


