

ABSTRACT

WANTUCH, HOLLY ANNE. Modified Drone-Brood Removal to Control *Varroa destructor* in *Apis mellifera* Colonies. (Under the direction of Dr. David R. Tarpy).

The parasitic mite *Varroa destructor* Anderson and Trueman (Acari: Varroidae) has plagued European honey bees (*Apis mellifera* L.) in the Americas since its introduction to the United States in the 1980s. For many years, these mites were sufficiently controlled using synthetic acaricides. Recently, however, beekeepers have experienced increased resistance by mites to chemical pesticides, which are also known to leave residues in hive products such as wax and honey. Thus, there has been increased emphasis on non-chemical IPM control tactics for *Varroa*. Because mites preferentially reproduce in drone brood (pupal male bees), we developed a treatment strategy focusing on salvaging parasitized drones and removing mites from them.

In our initial study, we tested 10 colonies of honey bees in each of four treatment groups: 1) negative control (no treatment); 2) positive control (treatment with fluvalinate); 3) periodic drone-brood removal and freezing; and 4) periodic drone-brood removal and return of adult drones to colony after physically removing any mites. We found that there were no significant differences measured between the mean mite levels of the treatment groups ($P > 0.05$). However, there were numeric trends indicating that both drone-brood removal groups were intermediate in mite levels compared to the negative and positive controls.

In a subsequent study, we removed drone brood from colonies in which there is no acaricidal application and banking it in separate “sacrificial” colonies treated with pesticides to kill mites emerging with drones. We tested 20 colonies divided into three treatment groups: 1) negative control (no mite treatment); 2) positive control (treatment with fluvalinate and thymol); and 3) drone-brood trapping. Two colonies were selected from the drone-brood trapping group to serve as sacrificial colonies. We found that drone-brood trapping significantly lowered mite numbers during the early months of the season, eliminating the need for additional control measures in the spring. However, mite levels in the drone-brood removal group increased later in the summer, suggesting that this benefit

does not persist throughout the entire season.

We recommend that drone-brood trapping can be utilized as an element of an integrated control strategy to control varroa mites. If implemented successfully, this method of drone-brood removal and rescuing may serve to eliminate a large portion of the *Varroa* population with limited chemical treatments, while simultaneously retaining any benefits of having adult drones in the colony.

Modified Drone-Brood Removal to Control *Varroa destructor* in *Apis mellifera* Colonies

by
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DEDICATION

I dedicate this work to my mom and dad. Any success of mine is yours as well, because you have made me who I am. I think of each of you every day, and so you were there with me through all of this, even if you didn't know it. Thank you, and I love you both very much.

BIOGRAPHY

Holly Wantuch is a North Carolina Native, raised in the rural community of Dudley Shoals in Caldwell County. As a high school junior, she was accepted as a student by the North Carolina School of Science and Mathematics in Durham, NC, from which she graduated in 2002. She went on to acquire a Bachelor's degree from North Carolina State University, where she majored in Animal Science with a minor in Anthropology. Following completion of her undergraduate studies, she was accepted as a Master's student by the Entomology Department at North Carolina State University. She was offered a position in Dr. David Tarpy's lab where she researched non-chemical control methods of *Varroa destructor* in honey bee colonies. She received her Masters of Science in Entomology from NCSU in 2009.

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CHAPTER 1. LITERATURE REVIEW

Host-Parasite Ecology

Parasites and their hosts remain in a perpetual battle – the host continuously evolves defenses while the parasite evolves to evade them. Each exerts selection pressure on the other, resulting in an evolutionary arms race. The cycle of coadaptation between parasites and hosts is often a cyclical one, referred to as the “Red Queen hypothesis” (Van Valen 1973) Van after the Red Queen in Lewis Carroll’s *Through the Looking Glass* (who was constantly running but never getting anywhere, explaining that “Now, *here*, you see, it takes all the running *you* can do, to keep in the same place”). In such a situation, the parasite is strongly selected to parasitize the most common host phenotype, which constantly shifts. Therefore, the allele frequencies in both host and parasite population change in a cyclical manner, never straying too far from any previous point (Lively 1996).

Social insects offer a unique epidemiological opportunity to study host-parasite relationships. The nest environment is particularly ideal for parasites. Host insects are in very close contact with one another, facilitating movement of parasites among individuals. Also, the microenvironment of the colony remains at a relatively constant temperature and humidity, both within a single colony and between colonies, enabling parasites to adapt more easily (Morse and Nowogrodzki 1990). Social behavior also lends itself well to parasitism. For example, communal brood care provides numerous opportunities for an immature insect to encounter parasites that may be transported by its caretakers. It is typically seen that the more sophisticated a host’s social organization, the higher the specificity of its parasites. For example, an organized division of labor creates a heterogeneous environment for parasites (Schmid-Hempel 1998).

One such insect society is that of the western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) (Seeley 1985(Winston, 1999 #153, Graham 1999, Winston 1999). It evolved in Africa during the Tertiary period, later spreading throughout Asia and Europe. Its

natural habitat ranges from the southern tip of Africa into Northern Europe, though it has been introduced nearly worldwide by beekeepers. *A. mellifera* colonies nest in cavities, building several vertical wax combs consisting of numerous hexagonal cells. These cells are used to store the colony's food as well as for brood rearing.

Bees are holometabolous, having egg, larval, pupal, and adult life stages; the honey bee remains in its brood cell for all developmental stages, only emerging as an adult. Honey bees consume honey made from plant nectar, as well as pollen, which is the protein source provided to developing brood. A honey bee society consists of three castes: the queen, drones, and workers. The queen is the colony's mother, the single reproductive female laying all of the eggs, and she typically lives for several years. Drones, a colony's reproductive males, perform no work for the colony and only function to mate with queens from foreign colonies (after which they immediately die). During the mating season, there are typically several hundred drones present in an average-sized colony, and they may live for a matter of weeks, sometimes months. All colony maintenance is performed by the worker caste, including comb construction, brood care, and food collection. The social structure of this eusocial bee is very well-developed and relies upon an age-based division of labor. Workers typically live about 6 weeks, but those that overwinter with the colony experience increased longevity and live for several months. There are typically tens of thousands of workers in a colony, and therefore they comprise the majority of its population. Workers and queens are both female and diploid, arising from fertilized eggs, whereas the male drones are haploid arising from unfertilized eggs.

Honey bees are hosts to a wide range of parasites, pathogens, and pests. For example, the parasitic tracheal mite *Acarapis woodi* lives in the airway passages of adult bees. These small arachnids can kill entire colonies by themselves or by creating secondary infections after compromising the immunodefenses of workers (Bailey 1981). Another notable pest is the microsporidian, Nosema (*Nosema apis* and *N. ceranae*), which infects the gastrointestinal tract of adults (Shimanuki 1993). American foulbrood (AFB) is the most serious brood pathogen of honey bee colonies. AFB is extremely difficult to eradicate from honey, wax, and hive equipment once they have been contaminated, and is the main reason why

historically it is the most costly disease for beekeepers (Bailey 1981, Schmid-Hempel 1998). Another disease, similar in symptoms to AFB, is European foulbrood (EFB) caused by the bacterium *Melissococcus pluton* and other opportunistic organisms (Shimanuki 1993). Several viruses also infect honey bees, causing diseases such as sacbrood and acute bee paralysis, and are often associated with parasitic infestations (reviewed below). One important fungal infection known as chalkbrood, *Ascosphaera apis*, attacks developing larvae in a manner similar to AFB, although it is significantly less virulent. This abbreviated review of honey bee diseases is by no means comprehensive (Bailey 1981, Shimanuki 1993), but nonetheless illustrates that honey bees serve as hosts to a wider range of parasites and pathogens than any other social insects (Schmid-Hempel 1998).

While many of the above diseases are economically important, *Varroa destructor* Anderson and Trueman is the most serious pest of honey bees worldwide because it has been implicated in the deaths of millions of colonies (Sammataro et al. 2000, Martin 2001). *Varroa* is a large (1,100 μm long X 1,600 μm wide) ectoparasitic mite that feeds on pupal and adult honey bees (DeJong 1990), but it is most damaging when feeding on pupae (DeJong 1990, Sammataro et al. 2000). *Varroa* mites invade worker and drone cells, generally avoiding queen cells, though they are capable of feeding on queens (Santillan-Galicia et al. 2002). This parasite threatens the survival of managed and feral honey bees, as well as the continuation of the apicultural industry. Furthermore, it is a concern for the agricultural industry overall, since so many food products rely upon honey bees for pollination of crops (Robinson et al. 1989, Southwick and Southwick 1992, Morse and Calderone 2000).

Varroa Biology

The host-parasite relationship between *Varroa destructor* and the honey bee is complex. *Varroa* is fairly unusual for a mite, in that it is truly parasitic and cannot survive more than a few days away from its host (DeJong 1990, Schmid-Hempel 1998, Schmid-Hempel 1998). When mites are parasitic, they generally belong to the Mesostigmata group

(of which varroa is a part). The parasite develops different feeding strategies and locations depending on the age of the bee it is parasitizing. The mite can switch from pupal to adult host, enabling it to disperse to another colony. Horizontal transfer of parasites between colonies of social insects requires close physical contact, which is most likely to occur if common food sources are shared (such as flowers in the case of honey bees; (Schmid-Hempel 1998)). Another factor that contributes to successful transfer of parasites among colonies is the aggressive protection of stored food reserves in many social insects – this is certainly true of honey bees, especially in times during which most flowers are not producing nectar. This mite is aided in its dispersal across great distances not only by its bee hosts, but by the movement of colonies for pollination of crop fields and human commerce (Sammataro et al. 2000). Varroa is also very successful as a parasite because on many occasions it significantly debilitates but does not directly kill its host, which enables it to spread more easily than if its host dies more quickly (Schmid-Hempel 1998). .

There are three species of varroa mites: *Varroa destructor*, *Varroa jacobsoni* Oud., and *Varroa underwoodii* Delfinado-Baker; of these, *Varroa jacobsoni* is the most widely distributed (Anderson and Trueman 2000). *Varroa jacobsoni* was shown to have a high genetic variation and has been determined to be a species complex (Anderson 2000).

Varroa destructor was originally a parasite of the Asian honey bee *Apis cerana* on mainland Asia (Anderson and Trueman 2000). It was found to be a distinct species from *Varroa jacobsoni*, a species exclusively parasitizing *Apis cerana* (Anderson and Trueman 2000). There are 18 haplotypes of Varroa parasitizing the Asian honey bee, but only two of them have undergone a host switch to parasitize *Apis mellifera* (Robinson et al. 1989), specifically, the Korea and Japan-Thailand *Varroa destructor* haplotypes (Anderson and Trueman 2000). There are six haplotypes of *Varroa destructor* genetically distinct from *V. jacobsoni* that parasitizes the Asian honey bee exclusively (Anderson and Trueman, 2000). They are morphologically distinct, as *V. destructor* females are larger and less spherical than *V. jacobsoni* (Anderson and Trueman, 2000).

The host-parasite relationship between Varroa and its native host, *Apis cerana*, is evolutionarily stable. The Asian honey bee coevolved with the mite and possesses several

features that enable it to tolerate it. A study by Buchler et al. (1992) showed that, in comparison to *Apis mellifera*, the Asian honey bee is much quicker to respond to phoretic mites, and it is more successful in removing them. They found that workers of Asian honey bees begin autogrooming much more quickly and with a higher frequency than European bees when a mite was experimentally placed on the thorax of an adult bee. *A. cerana* workers also performed allogrooming, which is almost never observed in *A. mellifera* in response to a varroa mite. The behavior is initiated by a parasitized bee when it performs a shaking dance, after which neighboring worker bees come to the parasitized bee and comb its hairs with their mandibles, paying special attention to the petiolus region usually presented to them by the parasitized bee (Buchler et al. 1992).

Another characteristic that affords the Asian honey bee defense against the mite is its ability to capture mites in their mandibles. Buchler et al (1992) observed this in about one-third of their observations of *A. cerana*, but never witnessed it in *A. mellifera*. *A. cerana* also more readily detects and removes parasitized brood before it emerges, usually resulting in the death of mites and their offspring within the cell (Boot et al. 1999). It has been suggested by Boot et al. (1999) that this behavior is in response to mite activity within the cell. They noted a higher rate of detection and removal of mites originating from *A. mellifera* brood that was artificially transferred to *A. cerana* brood. This, however, occurred 2 days following the transfer, which indicates a response to the mites' activity. In addition to activity cues, it has been suggested that Asian bees also remove brood in response to the scents of foreign objects (Rosenkranz et al. 1993). That study noted another interesting behavior: when a mite originating from *A. cerana* brood was detected, the bees sometimes opened the cell and removed the mite leaving the brood intact, thus allowing its survival.

Varroa jacobsoni infesting *A. cerana* also seem to be less virulent towards their host, allowing a more stable relationship than that between *V. destructor* and *A. mellifera*. One major difference between the two mites is their reproductive strategy (Boot et al. 1999). Varroa mites in Asian honey bee colonies reproduce solely within drone cells, whereas in European honey bees it produces offspring in worker brood as well (Anderson 1994, Boot et al. 1995, Boot et al. 1999, Chandler et al. 2001). Boot et al. (1999) found that 80% of mites

removed from *A. mellifera* brood cells laid eggs in worker cells of both *A. mellifera* and *A. cerana*, compared to less than 10% of mites from *A. cerana* brood that were found to do this within worker cells of either species. Anderson (1994) observed no *V. jacobsoni* reproducing in the cells of *A. mellifera* or *A. cerana* workers. This is an important distinction, especially since mites occasionally invade worker cells in *Apis cerana* but refrain from laying eggs (Boot et al. 1999). This means that the parasite is less detrimental to *A. cerana* colonies, not only because mite populations only increase during the limited time when drone brood is present, but also because much less damage is done to the worker population.

There are several possible explanations for this reproductive behavior. One possibility is that the mites would not have sufficient time to reproduce offspring successfully within *A. cerana* worker brood, since their post-capping duration is 11 days long, whereas that of *A. mellifera* workers is 12 days long (Kapil, 1959, Tan et al. 1993, Le Conte and Curnuet 1989). A second possibility is that mites which successfully reproduce are at a higher risk of experiencing mortality, especially due to the high removal response of Asian honey bee workers, thus decreasing their individual fitness (Boot et al. 1999).

Cell Invasion

Mated female mites enter worker cells 15-20 hours prior to cell capping in workers, and approximately 40-50 hours prior to cell capping in drones (Boot et al. 1992). Mites enter larval cells when the larvae are large enough to cover the entire cell bottom; past this point, the distance between the larva and the rim of the brood cell decreases (Boot et al. 1992). They invade cells based on several different factors, especially olfactory and mechanical cues. Aumeier et al. (2002) found stage-specific odor compounds found in larval cuticles including five methyl esters and 14 hydrocarbons (kairomones) such as methyl palmitate, ethyl palmitate, and heneicosane, all of which are thought to be attractive to female mites (but see Nazzi et. al (2001), who suggested that methyl palmitate is probably not an attractant to mites). They found that the composition of larval odors changes significantly between the fourth and fifth instars, with levels of methyl alkanes significantly decreasing and levels of alkenes and esters significantly increasing. However, the total volume of volatiles also

increased during this time, presumably aiding in mite recognition of the appropriate host stage. Nazzi et al. (2001) suggest that semiochemicals in larval food also attract varroa mites prior to cell capping, since ether and acetone extracts of larval food elicited the same response from the mites as did the actual food itself. The mites' response to food samples from fifth instar bees was much stronger than that to food from sealed brood cells (Nazzi et al. 2001). The rate of cell invasion by mites is affected by the amount of brood present, such that mites enter worker brood at a higher rate when there is more brood present (Boot et al. 1992).

Sometimes several Varroa mites will be found infesting a single honey bee brood cell, which has both positive and negative implications for mites. On one hand, having more than one mite laying eggs on a single host provides an opportunity for outbreeding (as mating usually occurs among siblings). Also, having more males present to mate with in a cell increases the ease with which females, who re-mate frequently, can locate a mate (Donze and Guerin 1994). On the other hand, as the number of foundress mites per cell increases, there is a decline in the number of eggs produced per mite within a single brood cell (Martin 1995, DeGrandi-Hoffman and Curry 2004). For example, the maximum number of viable offspring found by Martin (1995) within a cell was eight and 16 for worker and drone cells, respectively. This decreased per capita reproduction, which is thought to be a result of increased mite mortality rather than decreased fecundity. In drone cells, when there may be as many as four foundress mites present, the mortality rate for offspring remains fairly constant for the first three offspring, but then increases rapidly as more offspring hatch from their eggs (Martin 1995). Death of young mites is often the result of competition among each other for access to feeding on the host, since even in the case of multiple infestations there is only one feeding site (Donze and Guerin 1994). Protonymphs are at a competitive disadvantage against deutonymphs and adults (Donze and Guerin 1994, Martin 1995), and this competition is much stronger than competition between adult female mites (Martin 1995). Younger offspring tend to take more time locating the feeding site and can lose their place in line to, or are shoved aside by, larger stronger mites searching for the feeding site (Donze and Guerin 1994). When cells are multiply infested, mites not only share the host-

feeding site, but also site of the fecal patch. Thus, they fit the definition of parasociality, in which individuals share a common nest site but lack some or all other social characteristics. Even in highly crowded situations, cannibalism amongst mites has not been observed (Donze and Guerin 1994).

Boot et al. (1995) showed that as long as waiting times between larval hosts are relatively short (7 days or less – average waiting times are about 6 days), it is beneficial for mites to reproduce only within drone brood. Indeed, mites prefer drone brood, parasitizing them between 5.5-12.1 times more often than they do workers (Boot et al. 1995, Martin 1998, Santillan-Galicia et al. 2002, Wilkinson and Smith 2002b, Coffey 2007). As a result, significantly more mites are found in drone cells (Boot et al. 1992, Calderone and Kuenen 2001). Calderone and Kuenen (2001) found that the proportion of drones having one or more mites within their brood cell was 0.763 ± 0.043 , compared to 0.253 ± 0.043 for workers. Also, drones had an average of 2.296 ± 0.239 mites per host versus an average of 0.330 ± 0.237 mites per worker, which could be because foundress mites begin entering cells earlier and over a longer period of time. After all, drone cells are attractive 2-3 times longer than worker cells, but the invasion rate is known to decrease over the last 20-25 hours prior to cell capping (Boot et al. 1992). It is also possible that Varroa mites may enter drone cells at a higher rate simply by chance, since their cells are 1.7 times larger than worker cells and they are visited more often by nurse bees (which often carry mites; (Boot et al. 1992). However, it has been shown that when drone and worker larvae are swapped into each other's respective cells, a higher proportion of worker larvae in worker cells have at least one mite and have more average mites per host compared to workers reared in drone cells (Calderone and Kuenen 2001). This seems to indicate that cell size alone may not contribute much to drone preference by mites. The maximum number of offspring found per cell by Calderone et al. (2007) was six in workers and seven in drones. However, mites in drone cells have a higher average fecundity than those in worker cells (2.9 ± 1.4 offspring per mite in worker brood 3.8 ± 1.6 offspring per mite in drone brood; (Calderone and Kuenen 2001). Also, mites in drone cells appear to be more fertile than those in workers, (76.5 % of mites in worker cells were fertile compared to 79.3% in drone cells; (Ifantidis 1984)), and a higher percentage of

mites in drone cells produced viable offspring compared to those in worker cells (38.1% and 13.8%, respectively; (Calderon et al. 2007b)

Activity within brood cells

Varroa-host interactions within the brood cell have been thoroughly documented (Ifantidis 1983, Donze and Guerin 1994, Sammataro et al. 2000, Chandler et al. 2001, Calderon et al. 2007a). Roughly 40 minutes following cell capping, the honey bee larva begins to summersault while feeding in bouts on food at the cell bottom. The foundress mite must alternate feeding positions between the larval extremities and ventral side, which contact the cell wall least often, to avoid being crushed. During this same time and subsequently while the larval bee spins its cocoon, the mite remains on the moving host feeding approximately every 1.5 hours for several minutes at a time. The bee's cocoon is completely spun roughly 33-36 hours after capping in a worker cell and about 48-52 hours after capping in a drone cell. During this time, the mite must remain on the bee to avoid being trapped in the cocoon. In both worker and drone brood, the initial egg is that of a haploid male mite, and it is laid at about 70 hours post-capping. Female eggs are diploid and are laid singly at 30-hour intervals following the initial male egg. Even though they are haploid, male mites are thought to originate from fertilized eggs; no unmated foundresses have been observed to produce male offspring, and closely related Dermanyssid mites have been shown to exhibit such "pseudo-arrhenotoky" (Helle et al. 1978, De Jong et al. 1981, Martin et al. 1997, Cruickshank and Thomas 1999).

Foundress mites can produce a maximum of six offspring within a single worker cell, and up to seven in a drone cell, but more typically 2-3 and 3-4 eggs are laid per cell in worker and drone cells, respectively (Calderon et al. 2007b). Varroa mites go through several phases during their maturation within the sealed cell: pharate larvae, mobile protonymph, pharate deutonymph, mobile deutonymph, pharate adult, and adult. Mites in mobile stages are feeding and growing, while pharates are quiescent. It takes a male mite about 5.5 days to undergo this development, while it takes female mites roughly 7.5 days to

do so. During the prepupal stage of bee development, the foundress mite feeds hourly on the abdominal pleural pads for about 2-3 minutes, however once the bee has pupated, she feeds about half as often but for about 6 minutes at a time. Feeding by the mother mite decreases following oviposition of the first egg in accordance with decreased nutrient demand. The preferred feeding site on the pupal bee is the fifth abdominal segment, which can be difficult for mites to reach, and therefore necessitates rearrangement of the exarate bee's legs by the mite. Commonly, if the fifth abdominal segment cannot be reached, mites will instead choose a feeding site located on one of abdominal segments six through eight, although feeding at any one of these carries the risk of hemorrhage of the pupa, which results in drowning of all resident mites. Mites do not feed on the head or thorax of the bee, which minimizes the potential damage to its appendages.

Donze and Guerin (1994) have shown that the maternal mite participates in parental care of her nymphal offspring after they hatch from the egg. Her first action is to prepare a single feeding site that will be used commonly by all individuals within the cell, which takes considerable effort and requires an investment of energy by the foundress. This is important for the survival of her male offspring, as his chelicerae are modified into spermadactyls, and he would otherwise be unable to feed. Male feeding enables him to produce sperm during the five-day span during which adult females are available to mate, which is the time between a male's imaginal ecdysis and the emergence of the adult bee from its cell. The foundress also prepares a fecal accumulation on the roof of the cell near the anus of the honey bee, which serves as a rendezvous point near the feeding site for her offspring, and facilitates mating.

All mating takes place within the sealed brood cell of the parasitized bee, which typically occurs between sibling mites. The fecal accumulation, prepared by the maternal mite within a cell, serves as a meeting place for her offspring and is conveniently placed near the feeding site (Donze and Guerin 1994). Here, the male mite spends most of his time awaiting the arrival of his adult female siblings. This eliminates excess time spent searching for a mate within the cell, thus 90% of matings occur at the fecal accumulation (Donze and Guerin 1994). Male mites have been observed to begin the actions of the mating sequence

with their maternal mites but always cease very quickly and without exception (Donze and Guerin 1994).

Non-reproduction of varroa mites within brood cells is a limiting parameter to their population growth, and there are several reasons that a female foundress mite may not produce viable offspring. She may fail to lay eggs, her eggs may not be viable, she may produce only male offspring, or her single male offspring may die prematurely before mating with his sister mites (Martin et al. 1997). Since mites can only mate within their natal brood cell, premature death of the male mite leads to the inability for his sisters to reproduce following their emergence (Martin et al. 1997). According to Donze and Guerin (1994), the incidence of premature male death is higher for mites in drone cells because of the drone's longer development time; mites have sufficient time to lay two eggs during the prepupal stage of the drone, meaning that the male protonymph is already mobile at pupation, thus exposing it to the moving drone and increasing its chances of being crushed. Calderone et al. (2007) also found that a larger proportion of female mites produce only females when in drone cells than when in worker cells. However, Martin et al. (1997) found that 8-20% of male offspring die in worker cells before mating, as compared to a rate of 10% premature male death in drone cells. Male death, however, cannot account for all forms of non-reproduction in Varroa as it does not occur frequently enough to explain all instances of unfertilized females (though it does correlate to higher female infertility; Martin et al. 1997). This is especially obvious in the case of foundresses producing only male offspring since, despite the haploid nature of male mites, they originate from fertilized eggs and cannot be produced by unmated females (Martin et al. 1997). Martin et al. (1997) found that mite reproduction, or lack thereof, was generally uniform between brood cycles regardless of the whether they were infesting drone or worker brood (meaning that if adult female mites reproduced normally once, they would continue to do so in the future, and the same for those not reproducing normally). However, it has been shown that nearly a quarter of mites that produce immature or otherwise nonviable offspring in worker brood will produce viable offspring when placed into drone cells, and almost three quarters of mites that do not reproduce at all when in worker cells produced offspring in drone cells (Calderon et al.

2007b). Results found by Ifantidis (1984) also show that only 4% of mites in drone cells were non-reproductive compared to 19% in worker cells. Interestingly, in mite-tolerant bees like Asian honey bees and Africanized bees (see below), mites are usually non-reproductive in worker brood, slowing their population growth. This has been determined to be a trait of the mite rather than an effect caused by the bee stock (Boot et al. 1995).

When the adult bee emerges from its cell, all surviving adult female mites enter a phoretic phase, during which they hitchhike on adult bees feeding on hemolymph until they are ready to enter a brood cell and lay eggs. *V. destructor* prefers younger “in hive” nurse bees to older foragers (Sammataro et al. 2000). Phoretic periods can last about 4-11 days, the duration of which largely depends upon brood availability (Martin 1998, Sammataro et al. 2000).

Harris et al. (2003) found two factors to be linear predictors of Varroa population growth: percentage of reproducing female mites and proportion of total mites in capped brood. Conversely, mortality of mites in cells, growth of the honey bee population, area of capped brood present at the end of the test, and duration of the test were all factors not found to be predictors of population growth. According to Wilkinson and Smith (2002), the most important factors to mite population growth are the timing of the beginning and end of foraging season and brood rearing. This is because of the associated increased mite mortality and the length of time brood is available (not, however, the amount of brood available). Likewise, according to Calis et al. (1999), a longer brood rearing period will drastically increase the mite population, as will increased drone brood area. Although mite population growth is exponential at the peak of brood rearing (Calis et al. 1999a), once mite populations reach a high level, their rate of increase declines because mites in multiply infested cells do not reach their reproductive potential (DeGrandi-Hoffman and Curry 2004).

Parasitic Mite Syndrome

Parasitism by varroa mites is considered a pathological condition referred to by several names: varroatosis, varroosis, varrosis, and parasitic mite syndrome or PMS

(Shimanuki et al. 1994, Hung et al. 1996, Sammataro et al. 2000). Symptoms are seen year-round, but especially in mid-summer and fall, when varroa populations are at their height (Shimanuki et al. 1994). Symptoms of PMS in adult bees include the visible presence of *V. destructor*, reduced colony population, evacuation of the hive by crawling morbid bees, and increased queen supercedure (Shimanuki et al. 1994, Hung et al. 1996). Symptoms of PMS in developing bees include an inconsistent or “spotty” brood pattern, formation of hardened scales from dead pupal remains (distinguishable from those formed from American foulbrood infections), and other symptoms resembling brood diseases but no odor or larval ropiness (Shimanuki et al. 1994). Not all symptoms are present within a given colony or at any one time (Shimanuki et al. 1994). While no specific microbes have consistently been associated with this PMS, numerous viruses are often present within parasitized colonies and transmitted by the mites (Shimanuki et al. 1994, Hung et al. 1995, Hung et al. 1996).

Deformed Wing Virus

Perhaps the most prevalent and harmful virus to invade honey bee colonies, deformed wing virus (DWV) is often found in colonies that appear healthy (Allen and Ball 1996, Martin 2001, Chen et al. 2005b, Yue and Genersch 2005, Tentcheva et al. 2006). Nonetheless, DWV is closely associated with colony collapse in honey bee colonies infested with *Varroa destructor* (Nordstrom et al. 1999, Yue and Genersch 2005). Like many viruses that infect the honey bee, DWV is a single-stranded RNA picorna-like virus (Chen et al. 2006a) that can be transmitted both vertically and horizontally and may cause deformity and premature death in adults and brood (Allen and Ball 1996, Bowen-Walker et al. 1999, Chen et al. 2005b, Yue and Genersch 2005, Yue et al. 2006, de Miranda and Fries 2008). Many or all of the unparasitized bees in a colony often carry DWV without showing symptoms and have low virus titers (Yang and Cox-Foster 2005, Yue and Genersch 2005, Yang and Cox-Foster 2007). Moreover, viral titers in adult and pupal bees have been shown to be higher towards the later end of the season when mite densities are highest (Gauthier et al. 2007). Though it may or may not be found present in honey bee colonies with low or no Varroa

populations, it is generally ubiquitous in colonies with sizeable mite numbers (Nordstrom et al. 1999, Martin 2001, Yue and Genersch 2005). Even a relatively small mite population of 2000-3600 present in late summer can kill a strong colony of 30,000-40,000 workers because DWV damages the wings of workers that are required for the colony to survive over the winter (Martin 2001).

Varroa mites are effective vectors of DWV, spreading it from infected to uninfected hosts (Bowen-Walker et al. 1999, Martin 2001, Nordstrom 2003, Chen et al. 2005b, Yue and Genersch 2005, Tentcheva et al. 2006). If a bee is parasitized by a mite that has previously fed on a bee with DWV, the present host faces a higher risk of deformity or death (Bowen-Walker et al. 1999). However, DWV is not transmitted between host and parasite at all perceived opportunities (Nordstrom 2003). For example, a foundress mite carrying DWV does not necessarily infect her host bee and vice versa. Likewise, immature mites do not necessarily become infected by an infected host, although they are typically infected from their host rather than the maternal mite (Nordstrom 2003). It is unclear if host morbidity or mortality from DWV increases with the number of mites feeding on a given bee; Bowen-Walker et al. (1999) found a significant relationship, but Nordstrom (2003) found that multiple infestation of a brood cell by mites did not significantly increase the chance of the host bee developing DWV. Bees that are fed upon by mites during development more often emerge with some level of deformity, and DWV titers generally correlate positively with mite numbers within the cell, and increasing levels of deformity (Bowen-Walker et al. 1999, Chen et al. 2005b, Shen et al. 2005b, Yang and Cox-Foster 2005, Tentcheva et al. 2006, Yang and Cox-Foster 2007). Though nearly all deformed bees are found to have mites, there are cases of mite infestation without deformity, indicating that it is not parasitism alone that causes the presentation of DWV symptoms (Yang and Cox-Foster 2005). Also, viral titers have been shown to be increased by parasitism, even by mites known to be free of DWV (Allen and Ball 1996, Shen et al. 2005b). It has been determined that Varroa parasitism activates and amplifies DWV within the pupal honey bee (Allen and Ball 1996, Shen et al. 2005b, Yang and Cox-Foster 2005) and that the combination of parasitism and microbial challenge often results in deformity or mortality of bees (Yang and Cox-Foster

2005, Yang and Cox-Foster 2007). In a study by Yang and Cox-Foster, (2007), when parasitized and mite-free bees were both infected with *E. coli*, non-parasitized bees recovered and survived just as long as control bees that were exposed to non-infectious materials, whereas those that were parasitized died significantly earlier. This suggests that mite infestations hinders the immune response of honey bees, and indeed it has been shown that Varroa parasitism immunosuppresses bees (Shen et al. 2005b, Yang and Cox-Foster 2005, Yang and Cox-Foster 2007). Parasitism suppresses the expression of genes important to immune function, such as those that encode for enzymes such as phenol oxidase (Yang and Cox-Foster 2007), glucose oxidase, glucose dehydrogenase, and lysozyme (Yang and Cox-Foster 2005). Thus, DWV, typically present as a latent infection, is transformed in the presence of mites into a crippling or fatal disease that attacks immunosuppressed bees; not surprisingly, DWV titers are negatively correlated with expression of immune-related enzymes (Yang and Cox-Foster 2005).

Proportional to their weight, varroa mites often have virus titers much higher than that of their hosts (Bowen-Walker et al. 1999, Gauthier et al. 2007), and the virus has been shown to be present in and capable of replicating within some (but not all) adult female mites (Chen et al. 2005b, Yue and Genersch 2005). Viral replication in mites correlates with host deformity but not with infestation level; mites sampled from crippled bees display viral replication whereas those from asymptomatic bees display none (Yue and Genersch 2005). Indeed, mites emerging from cells of deformed honey bee hosts generally have DWV titers significantly higher than those coming from cells of asymptomatic bees (Bowen-Walker et al. 1999, Nordstrom 2003).

Mites, however, are not always necessary in the transmission of DWV (Bowen-Walker et al. 1999, Chen et al. 2005b, Yue and Genersch 2005, Tentcheva et al. 2006, Yue et al. 2006, de Miranda and Fries 2008). Yue et al. (2006) was the first to show that DWV RNA sequences were present in drone sperm. In fact, all samples positive for DWV also contained sequences for acute paralytic bee virus (APBV), showing that multiple viruses could potentially be transmitted via sperm. Not only is the virus present in drone sperm, venereal transmission of DWV has been documented (de Miranda and Fries 2008). When

semen from DWV-infected drones was used to artificially inseminate DWV-free queens, in multiple instances the virus was later found not only in the queens' spermathecae (which could be because of passive transfer), but also in the ovaries proving venereal transmission (de Miranda and Fries 2008). In that study, venereally infected queens passed the viral infection to their offspring – even to drones, which were not inseminated by the infected sperm. Moreover, Chen et al. (2006b) found queen spermathecae to be positive for DWV, as well as drone and worker offspring at all life stages. However, neither venereal transmission nor vertical transmission to offspring was 100% effective, and since in these instances 100% of individuals sampled were DWV positive, mating cannot be the sole means of transmission of DWV (Yue et al. 2006, de Miranda and Fries 2008). DWV has also been detected in stored pollen, as well as brood food, indicating that it may be secreted in salivary glands, hypopharyngeal glands, or both (Yue and Genersch 2005, Tentcheva et al. 2006). It has been suggested that DWV may be spread from nurse bee to developing larvae through feeding (Yue and Genersch 2005, Tentcheva et al. 2006), and the presence of DWV in the feces and digestive tracts of queen honey bees also suggests horizontal transmission through feeding (Chen et al. 2006b).

Acute Paralysis Virus

In overt cases of apparent acute paralysis virus (APV) infection, honey bees are observed to be unable to fly and begin to noticeably tremble (Bailey 1968). APV is associated with colony mortality when mites are present, and overt infections are rarely found in their absence (Nordstrom et al. 1999, Martin 2001). Indeed, APV was found to be the main cause of colony mortality in German colonies with high mite infestations, though colonies with low infestations had low virus prevalence as well (Ball and Allen 1988). Mites have been found to be competent vectors of APV, and it has been suggested that they can also activate the virus (Ball and Allen 1988, Brodsgaard et al. 2000, Martin 2001). Though APV infections in the absence of mites are asymptomatic, mortality frequently occurs in developing bees when varroa parasitism is combined with APV (Brodsgaard et al. 2000,

Martin 2001). This may be because fewer virus particles are necessary to cause morbidity and mortality when they are injected directly into the hemolymph (as occurs during parasitism) than when they are introduced orally (as during feeding of the larvae by nurse bees (Brodsgaard et al. 2000, Martin 2001). It is obvious that APV is transferred to young larvae during feeding aside from varroa parasitism because the virus has been isolated in young larvae from unsealed cells as well as from older capped brood (Ball and Allen 1988). However, the disease kills its host very rapidly and so is self limiting; it is generally only capable of causing the collapse of a colony if there is a large mite population (10,000+) present at the time of infection (Martin 2001). APV is one of only a few viruses tested for, but not found in, queen honey bees (Chen et al. 2005b, Chen et al. 2005a, Chen et al. 2006b).

Kashmir Bee Virus

Parasitism by varroa mites is also associated with an increased infection of bees with Kashmir bee virus (KBV; (Shen et al. 2005b, Todd et al. 2007), as well as increased KBV RNA titers compared to non-infested bees (Shen et al. 2005b). KBV has been found in *V. destructor* mites themselves as well as their saliva, implicating them as vectors of the disease (Shen et al. 2005a, Todd et al. 2007). It also seems that parasitism activates KBV infection in bees (Shen et al. 2005b). Another means of horizontal transmission may be from adults to larvae through food, as KBV RNA has been detected in honey, pollen, royal jelly, and brood food (Allen and Ball 1996, Shen et al. 2005a). The virus, however, is thought to be transmitted vertically through the ovaries. For example, Shen et al. (2005b) found KBV to be present in queens, as well as eggs and all other developmental stages of the bee. KBV is detected more often in the autumn, when mite populations are highest, and it has been connected to the collapse of infested colonies (Todd et al. 2007). KBV, though, has also been known to cause sporadic disease outbreaks independent of varroa mites (Allen and Ball 1996).

Cloudy Wing Virus

Cloudy wing virus (CWV) has been found to be common in honey bee colonies (Nordstrom et al. 1999, Todd et al. 2007), and is present throughout the season (Todd et al. 2007). The presence of CWV is not dependent upon the presence of *Varroa destructor*, nor the size the colony population (Nordstrom et al. 1999, Todd et al. 2007). As such, it has not been found to have any association with Parasitic Mite Syndrome (Nordstrom et al. 1999).

Sacbrood Virus

Sacbrood virus (SBV) mainly acts on the honey bee brain and corpora allata, which control molting through the production and regulation of hormones (Bailey 1968). In prepupal bees infected with SBV, the final larval skin separates from the pupal integument (as expected), but rather than being shed, it forms a tough sac in which ecdysial fluid accumulates, and the affected bee dies soon thereafter (Bailey 1968). SBV accumulates in the heads of adult worker bees, especially within the hypopharyngeal glands. The virus is found to multiply within adult bees when injected or fed to young bees (adults older than 4-8 days cannot be infected orally; Bailey 1969). Infection cannot be spread between adults, but is most likely passed between larvae and adults through feeding and cannibalization of diseased brood (Bailey 1968). RNA titers of SBV are not significantly increased by Varroa infestation (Shen et al. 2005b). However the virus has been found in adult mites as well as mite saliva, implicating *V. destructor* as a vector of the virus. SBV has been found to co-infect both honey bees and varroa mites (Shen et al. 2005a). Varroa, however, seems to have limited influence on the occurrence of this disease, as colonies tend to have highest SBV titers in the spring when mite populations are low, whereas other viral infections are often at their peak in the late summer and fall (Bailey 1968, 1969, Ball and Allen 1988, Shen et al. 2005b, Gauthier et al. 2007). This may be because, besides transfer of the virus by mites, SBV appears to be capable of transovarial transmission, since infected queen bees have been shown to pass the virus to their eggs (Shen et al. 2005a).

Black Queen Cell Virus

Black queen cell virus (BQCV) has not been shown to correlate with presence of *Varroa destructor* (Nordstrom et al. 1999, Todd et al. 2007). Instead, it is only infective to adults, is found most often during the summer, and is associated with the presence of *Nosema apis* (Ball and Allen 1988, Nordstrom et al. 1999, Gauthier et al. 2007, Todd et al. 2007). It has been found to be present in the feces of queen bees, suggesting feeding as a means of transmission (Chen et al. 2006b).

Effects of Varroa Parasitism on Drones

Parasitism by varroa mites has been found to have significantly detrimental effects on drones, which can reduce the available population of drones for mating with local queens. Parasitized drones experience increased mortality as compared to their non-infested counterparts (Rinderer et al. 1999, Collins and Pettis 2001). Collins and Pettis (2001) have shown that parasitized drones are more often deformed following pupation, making them immediately unfit because they cannot fly. Of those that were functional upon emergence, 1/3 fewer parasitized drones lived to sexual maturity compared to non-infested drones. And, although they did not go on fewer flights per drone, parasitized individuals died more frequently after reaching flight age. Besides having higher mortality, infested drones have lower sperm viability as well as decreased sperm counts, seminal gland weights, mucous gland weights, and overall body weights (Rinderer et al. 1999, Zoltowska et al. 2007). They also suffer from decreased sugar concentration, especially trehalose and glycogen, indicating increased sugar turnover resulting from parasitism, which could be detrimental (Zoltowska et al. 2007). In the end, Varroa infestation of colonies in an apiary will most likely lead to fewer drones available to mate, but those that do mate will probably be of similar quality to those in non-infested colonies (Collins and Pettis 2001).

Effects of Parasitism on Workers and Implications for Overwintering

Parasitism by *Varroa destructor* has been shown to lower worker weight upon emergence (DeJong et al. 1982, Janmaat and Winston 2000b). Parasitized workers weigh 6-25% less than do their non-infested counterparts, with weight loss directly correlating with mite number within the brood cell (DeJong et al. 1982). *Varroa* mites have also been shown to decrease overall longevity of workers, which has serious implications for colony survival (Janmaat and Winston 2000b).

Varroa infestation can be fatal to a honey bee colony preparing to overwinter in temperate climates, since overwintering requires brood production to cease and workers to extend their longevity. By weakening individual worker bees, parasitic mites hinder their ability to live through the winter, thus decreasing the entire colony's chances of surviving until spring (Amdam et al. 2004, Rice et al. 2004). As winter progresses and the honey bee population dwindles without new brood to bolster the worker population, the mite-to-bee ratio increases (Bowen-Walker et al. 1997, Rice et al. 2004). For example, Bowen-Walker et al. (1997) showed that most bees had one phoretic *varroa* mite at the outset of winter, but by the end of the season the majority had two or more. This is because mites have lower winter mortality than honey bees, and, should their host die, they are capable of transferring to another host before the dead bee falls from the winter cluster to the hive floor. In agreement with this, mites have shown to be capable of switching between live hosts under winter cluster conditions, as well as from dead or dying bees to live ones 75% of the time (Bowen-Walker and Gunn 1998). However, Fries and Perez-Escala (2001) disagree and do not differentiate between mortality of bees and mites, finding that mites do not concentrate themselves on overwintering bees. During the winter, mites most often feed in a preferred location between the third and fourth ventro-lateral tergites on the abdomen of the honey bee, and generally they are found on the bee's left side (Bowen-Walker et al. 1997). Mites probably prefer this feeding site because of its proximity to the ventriculus, which is distended during the winter because of the bees' decreased opportunities to take cleansing flights. Thus, it would stand to reason that hemolymph taken from such a location would be higher in nutrient concentration as a result of diffusion from the ventriculus (Bowen-Walker

et al. 1997).

Amdam et al. (2004) suggest that parasitism on the developing pupae that of workers weakens them and alters their physiological “programming” to the extent that they may not be capable of maintaining the longevity needed for overwintering. Workers that had been parasitized as pupae showed a rapid drop in vitellogenin following emergence. This has very negative implications for winter bees whose vitellogenin levels should remain high throughout the winter, significantly more so than typical summer workers (Fluri et al. 1982). Vitellogenin is required for winter bees to have the extended lifespan necessary for successful overwintering (Amdam et al. 2004), and it is also important in the production of brood food that will be needed to feed the new work force once the queen resumes egg laying in the spring (Amdam et al. 2003). In agreement with lowered vitellogenin titers, parasitized workers tend to have elevated ecdysteroid levels, which is negatively correlated with overall protein synthesis (Amdam et al. 2004).

Parasitized workers also seem to have a hampered immune function. Yang and Cox-Foster (2005) found that when mites fed on developing bees, expression of certain genes important to immune responses was suppressed. In parasitized bees, genes encoding for antimicrobial peptides such as abaecin, defensin, and hymenoptaecin were suppressed, as were genes encoding for immunity-related enzymes including phenol oxidase, glucose dehydrogenase, glucose oxidase, and lysozyme. They are also known to have lower mean proportions of normal hematocytes than mite-free bees upon emergence and in the days following thereafter, which indicates injury to the cellular immune system resulting from parasitism (Amdam et al. 2004).

Parasitism by *Varroa* actually alters the survivorship curve for honey bees; mite-free bees generally die of old age (Type I curve), parasitized but asymptomatic bees have a constant death rate (Type II curve), and physically deformed bees die very quickly (Type III curve; (Yang and Cox-Foster 2007). This influence by mites on survivorship has serious implications for overwintering abilities of parasitized honey bee colonies.

Mite Tolerance in Honey Bees

Characteristics of Tolerant Bees

Grooming behavior – the removal of phoretic mites from adult bees by nest mates – is a behavioral trait that retards the growth of Varroa populations, affording them increased tolerance to the mites (Buchler et al. 1992, Arechavaleta-Velasco and Guzman-Novoa 2001). Estimates of mite populations from colonies that express grooming behavior may be misleading, however, because the bees are actively removing and injuring mites at a higher rate end (Arechavaleta-Velasco and Guzman-Novoa 2001).

Though it may not be as effective as grooming behavior in maintaining low mite populations, hygienic behavior is beneficial as a means of lowering the rate of Varroa population growth. Colonies can be selected for their workers to remove diseased or parasitized brood from the hive before they emerge, which has been shown to lower Varroa infestations compared to unselected bees (Arechavaleta-Velasco and Guzman-Novoa 2001, Spivak and Reuter 2001, Rice et al. 2004, Delaplane et al. 2005, Ibrahim et al. 2007). Spivak and Reuter (2001) found that hygienic bees were quite tolerant of varroa mites when mite levels were fairly low, but additional control measures were necessary when infestations exceeded 15% on worker brood and adult bees. It is unclear what triggers hygienic bees to remove brood. Aumeier and Rosenkranz (2001) found that brood cells containing dead mites were removed as often as those with live mites, which excludes mite activity as the cue for brood removal (but see Boot et al. 1999). In the same colony, brood with mites originating in Carniolan and Africanized colonies was removed at similar rates, so it appears that a hive-specific scent may impact hygienic behavior (Aumeier and Rosenkranz 2001). The role of scent seems to play some role, but mites whose odor volatiles had been removed triggered a similar hygienic response as untreated mites, so hygienic bees are not responding solely to olfactory cues (Aumeier and Rosenkranz 2001). Aumeier and Rosenkranz (2001) found Africanized bees to be more sensitive to scent as a trigger for hygienic removal of parasitized brood. Hygienic behavior is a trait that can be maintained in honey bee populations even

when allowing queens to mate naturally, which allows it to be maintained easily in commercial beekeeping operations (Spivak and Reuter 2001, Ibrahim et al. 2007). Stocks of hygienic bees, compared to other stocks, have been shown to have similar adult and brood populations, similar honey production, and fewer signs of disease, especially chalkbrood (Spivak and Reuter 2001).

Some honey bees have been selectively bred with the goal of expressing suppression of mite reproduction (SMR), traits that reduce the fecundity of mites invading their brood cells. According to Harris and Harbo (2000), this effect is attained by increasing the proportion of mites that die in the cocoon of the developing bee and by decreasing the proportion of mother mites that produce offspring. They found that 65% of the mites in SMR colonies were non-reproductive, whereas only 10-15% of those mites in non-SMR colonies were non-reproductive. When non-SMR queens were swapped with SMR queens, mite reproduction was shown to decrease (as measured by inspecting brood cells to observe the percentage of foundress mites having no offspring); the reverse is true of colonies in which SMR queens were exchanged with non-SMR queens. This SMR trait has been determined to be more Varroa Sensitive Hygiene, or VSH (Harris 2007, Danka et al. 2008)) More recently, Ibrahim and Spivak (2006) have shown that honey bees expressing SMR traits show hygienic behavior selectively towards brood infested with varroa mites and that they do so at even higher frequencies than do standard hygienic stocks. Additionally, they showed that varroa mites placed on SMR brood, regardless of what bee stock they originated from, produced significantly fewer viable female offspring than those placed on hygienic bees. This indicates that SMR bees do in fact express some form of mite suppression by decreasing reproductive success of mites. This stock, however, has been shown to have somewhat lowered brood production (Delaplane et al. 2005). Harris (2007) found that VSH honey bees removed significantly more parasitized brood than did non-VSH control bees. VSH bees seem to be especially active in removing parasitized brood 3-5 days after the cell is capped, and so it is likely that they are responding to some stimulus during this time span (Harris 2007). These bees control mites so efficiently that acaricide application (see below) can often be forgone completely. Though stocks of pure VSH honey bees perform better,

hybrids and colonies founded by free-mated VSH bees have Varroa resistance that is on par with other well-known mite tolerant stocks (Danka et al. 2008).

According to DeGrandi-Hoffman et al. (2002), it may not be possible to select for traits that decrease Varroa fecundity directly. They suggest that larval and pupal characteristics most likely do not impact mite reproduction and so selection focusing on these traits will not reduce Varroa population growth. However, according to Wilkinson and Smith (2002), it may be possible to select for other traits such as post-capping time (defined as the time between when a cell is sealed and adult emergence; post-capping time for workers is typically 12 days and for drones is 14 days). On one hand, they show that shortening the post-capping time of developing brood by 10% would reduce the mite population by 60% in workers. On the other hand, a reduction in post-capping time of only 4-5% would actually result in an increase in the mite population, as it would not prevent the development of younger mites to adulthood, but would allow for earlier emergence resulting in a faster generation time (Wilkinson and Smith 2002a).

Russian Bees

Stocks of bees from the Primorsky region of Russia have also been shown to be tolerant of varroa mites (Rinderer et al. 2001). These *A. mellifera* populations were introduced to the area in the 1800s, which was adjacent to the natural range of *A. cerana*. As such, these European bees have long been exposed to parasitism by the mite, and have presumably evolved defenses through natural selection (Rinderer et al. 2001), allowing them to coexist with Varroa without experiencing drastic mortalities seen in other European honey bee populations. The USDA-ARS Honey Bee Breeding, Genetics, and Physiology laboratory developed a breeding program incorporating these resistant bees, and the Russian honey bees are proving to hold much potential (Wood 1999). De Guzman et al. (2007) found that, compared to Italian honey bees (a typical commercial stock), Russian bees have lower mite numbers, lower proportions of infested brood, and fewer multiply-infested brood cells (both workers and drones). This mite tolerance could be the result of several characteristics.

For example, brood from Russian colonies has been shown to be less attractive to mites, and when mites do enter Russian brood cells, they tend to have lower reproduction rates. Also, mites on Russian bees also have extended phoretic periods, which decreases the opportunity for mites to reproduce while leaving them more exposed to autogrooming, a behavior frequently expressed by these bees (Rinderer et al. 2001, De Guzman et al. 2007). All of these factors together lead to varroa mite populations in Russian colonies having significantly lower growth rates (Rinderer et al. 2001, De Guzman et al. 2007). Importantly, these traits are genetically based and have been shown to be heritable (Rinderer et al. 2001). Russian bees maintain significant levels of tolerance even when hybridized with other genetic lines of bees, though the effect is somewhat muted in comparison to pure Russian stock, with effects observed mostly at the beginning and end of the bee season (Tarpy et al. 2007).

Africanized Bees

Africanized honey bees (AHBs) are also known to be tolerant of varroa mites (Medina and Martin 1999, Mondragon et al. 2006, Carneiro et al. 2007). This is largely a consequence of higher mite mortality rates developing in AHB cells compared to mortality rates in European honey bee (EHB), especially those of the male and second female offspring (Medina and Martin 1999, Mondragon et al. 2006). According to Medina and Martin (1999), only about 40% of foundress mites in AHB cells produced viable female offspring, compared to approximately 75% of mites in EHB colonies. Also, Mondragon et al. (2006) showed that fecundity of mites decreased significantly in multiply infested AHB cells compared to singly infested ones, especially in drone cells. In fact, mites in drone brood often became non-reproductive when two or more foundress mites were present, and while multiple infestation is rare in AHB worker brood, it is quite common in drone brood. Mites infesting Africanized bees specialize on drone brood, in which female mites produce an average of 2.6 ± 0.01 offspring per drone (Calderon et al. 2007a). In worker brood, however, mites have high percentages of non-reproducing foundresses (Boot et al. 1995). Varroa was also found to produce only immature offspring 8% of the time, only female offspring 26.5 % of the time,

and only males 7.6% of the time (Calderon et al. 2007a). These factors may help to limit population growth of *V. destructor* in AHB.

In Brazil, it had been thought that AHB tolerance to Varroa was due in large part to the less virulent Japan-Thailand haplotype abundant in that area. However, it has recently been found that the Korean haplotype is by far the most common, and accordingly, mites in Brazil are increasingly fertile, now approaching the levels of mites in Europe long known to be of the Korea haplotype (Garrido et al. 2003). Carneiro et al. (2007) report an increase in the percentage of fertile female mites in Brazil from 56% to 86% over the past three decades and an increase in the percentage of female deutonymphs produced from 35% to 72% during roughly the same period. The AHB/varroa mite relationship remains stable with infestation levels remaining low, however, suggesting that tolerance to the mite does not depend solely on its haplotype (Garrido et al. 2003, Carneiro et al. 2007).

Other Factors

Colonies headed by younger queens seem to maintain lower mite levels than do those with queens aged 2 years or older (Akyol et al. 2007). Also, colonies with higher pollen stores have been shown by Janmaat and Winston (2000a) to remove mite-infested brood at a higher rate than did colonies with lower pollen stores. Additionally, they have shown that having high pollen stores in a colony has been shown to offset the negative effect that *Varroa destructor* parasitism has on worker longevity. It is suggested that this is because both parasitism and low pollen availability will result in a worker bee beginning foraging at an earlier age, which results in decreased longevity. Thus, a colony's mite tolerance may be bolstered by administering supplemental pollen (Janmaat and Winston 2000a, b).

In some situations, it is possible that unmanaged honey bee colonies live for many years while infested with varroa mites (Fries et al. 2006, Fries and Bommarco 2007, Le Conte et al. 2007, Seeley 2007). For example, Fries et al. (2006) have shown that honey bee colonies existing in an isolated environment to have co-adapted with their resident mite populations when the colonies were left totally unmanaged. Though the majority of these

colonies died within the first three years without treatment to control mites, those that survived eventually displayed decreased mite infestation, decreased winter colony mortality, and increased swarming frequency (Fries et al. 2006). Contrary to this, Le Conte et al. (2007) found no significant difference in swarming frequencies between colonies treated for mites and those surviving untreated for many years, and they suggest that swarming does not influence tolerance to mites. Meanwhile, colonies surviving with varroa mites have been shown to produce less honey than treated colonies (Le Conte et al. 2007).

It is thought that in some instances, it is not the bees which develop resistance to the mites, but rather the mites that become less virulent (Seeley 2007). For such adaptation to occur, colonies must be isolated from managed colonies chemically treated for mites. Other research has shown that bees may develop resistance to mite populations (Garrido et al. 2003), surviving for many years without mite treatment, regardless of whether the mites originated in treated or untreated colonies (Fries and Bommarco 2007). It was observed that such resistant bees produced less total brood (both workers and drones) compared to treated colonies, although average colony size did not differ. They also maintained a lower proportion of mites in sealed brood than did treated colonies with a higher proportion remaining on adult bees (Fries and Bommarco 2007). Perhaps because of such adaptations, Varroa population growth rates in untreated colonies were reduced by 82% (Fries and Bommarco 2007), and infestation rates in such colonies have been shown to be reduced to levels three times lower than those of treated colonies (Le Conte et al. 2007). After living with Varroa for some time, resistant colonies seem to reach a new host-parasite equilibrium, and they show mortality rates no higher than those to which mite treatments are applied (Le Conte et al. 2007).

Varroa Management and Integrated Pest Management Strategies

Integrated Pest Management (IPM)

IPM strategies are designed to control pest populations before they reach levels at which they cause damage while simultaneously applying varied yet minimal control measures (especially chemical pesticides) (Koul and Cuperus 2007, Horne and Page 2008). There are numerous non-chemical approaches in managing pests, such as biological control, mechanical control, and genetic host resistance. IPM strategies are beneficial for several reasons. First, such strategies are generally more cost-effective to the producer, as chemical pesticides can be expensive to purchase and apply. Second, multifaceted approaches provide fewer opportunities for pests to develop resistance mechanisms. Third, they reduce chemical applications and therefore decrease pesticide residues, which is generally more environmentally safe and sustainable.

There is often an acceptable population size that a pest may reach before it warrants any control actions (known as the ‘treatment;’ or ‘economic threshold’) (Koul and Cuperus 2007, Horne and Page 2008). Below this level, the producer does not experience any losses due to pest presence. Above this level, however, treatment measures must be taken to prevent economic losses. It is therefore critical to regularly monitor pest levels when implementing a proper IPM strategy. This, along with knowledge of the pest’s life history and biology, will enable the producer to gain a clear picture of the population dynamics and take action accordingly.

Varroa Monitoring

In a well-formed strategy for controlling any pest, the single most important element is that of monitoring pest populations. Sampling tools are intended to be used by the beekeeper in order to identify the time when mite populations reach their economic threshold, at which time treatment is necessary to reduce mite populations in order to avoid

injury to the colony. It is suggested to monitor populations on at least a monthly basis normally and on a bi-weekly basis if mite populations seem to be increasing rapidly (Sammataro et al. 2002). One common method for sampling mites within a colony is to place an adhesive *sticky board* on the bottom board of a hive to catch all mites falling to the hive bottom over a given period (usually 24, 48, or 72 hours). Sticky boards are thought by some to be more efficient and accurate than other invasive measures that monitor mites from adults, brood, or both (Sammataro et al. 2000, Sammataro et al. 2002, Branco et al. 2006), but they are best used only under certain circumstances. For example, colonies should contain brood, and not be experiencing collapse from varroosis (Branco et al. 2006). Delaplane and Hood (1999) calculated the season economic threshold for varroa mites in the southeastern US. They found that early-season colonies (with smaller adult populations of about 12,000 to 13,500) require treatment if sticky board counts are 1-10 in a 24 hours, but late season colonies (with larger populations of 24,808 – 33,699 bees) require treatment when sticky board counts exceed 59-187 mites in 24 hours. Similarly, Martin (1998) suggests economic thresholds of 250-500 in winter and 20-40 in the summer to, which accounts for changes in the ratio of live to dead mites, depending upon the amount of sealed brood. Though generally 24-hour mite counts are highly correlated with total mite populations, there are some inconsistencies since colony population size is not the only factor of mite mortality (Ostiguy and Sammataro 2000, Branco et al. 2006). For example, sticky board counts are known to correlate negatively with mite infestations in colonies that express high levels of grooming behavior; mite populations are typically lower, but only because more mites are being actively removed from adult bees and fall to the hive bottom (Arechavaleta-Velasco and Guzman-Novoa 2001). One important distinction in interpreting total mite count obtained by sticky board measurements is that they provide estimates of the existing mite population, but they should not be used as accurate predictors of future mite infestation levels (Branco et al. 2006).

Ether rolls are another tool for monitoring varroa mite populations. About 100-200 bees are caught in a jar to which a few ml of ether is applied to cover the inner surface. The jar is then rolled to dislodge and count the mites. Similar to ether rolls, powdered *sugar rolls*

remove mites from 100-200 adult bees captured in a container, preferably with a solid bottom (Aliano and Ellis 2005b). This method, however, is non-lethal to the bees and has proven harmless to brood within the colony except in cases in which large amounts of powdered sugar are applied directly to the cells (Aliano and Ellis 2005a). The mites are removed from the bees when the small particles of powdered sugar interfere with their grip on the exoskeleton. Loose mites are then shaken through a mesh lid to be counted. This method, though easy and inexpensive, may also prove inaccurate in determining colony-level mite populations (Sammataro et al. 2002). Alternatively, *alcohol washes* utilize ethanol (usually 70% EtOH) added to a sample of adult bees, which is subsequently strained so that mites can be counted (Sammataro et al. 2000). Each of these monitoring techniques provides an estimate of the mite-to-adult-bee ratio, because they allow the beekeeper to know exactly how many mites were present in a given number of bees sampled. The threshold for treatment according to an ether roll is a late-season count of 15-38 mites per sample and an early-season count of about 0.5 – 3 mites in an overwintered colony (Delaplane and Hood 1999). The location within the nest from which samples are taken should be kept consistent, because mites have been shown to be present in higher proportions in the brood areas versus honey storage areas (Calderone and Turcotte 1998). These techniques are relatively easy to perform, but their accuracy is questionable in determining the actual number of mites within the colony (Sammataro et al. 2002).

Combined with measuring the percentage of adult bees infested with mites, examining brood samples can be a useful method of monitoring mite populations as well (Branco et al. 2006). Uncapping pupal cells using a capping scratcher is a common technique used to achieve this measurement (Sammataro et al. 2000). If uncapping 100 drone brood cells, a 15% infestation rate by mites is a conservative treatment threshold (Wilkinson and Smith 2002b). This method, though, is labor intensive and its accuracy is questionable (Sammataro et al. 2002).

To build an effective plan to control varroa mite populations, especially when combining multiple forms of control in an IPM strategy, it is critical to closely monitor the pest population. In doing so, it is advisable to use two or more monitoring techniques (e.g.

sticky boards and sugar shakes) because it provides a more complete picture of mite population within the colony and enables a beekeeper to choose a more effective control strategy.

Mite Control

Without treatment to control *Varroa destructor*, the vast majority of honey bee colonies will succumb to mite infestations within 1-3 years (Nordstrom et al. 1999, Fries et al. 2006). Whatever mite control method is used, it is very important to have Varroa populations under control by late summer or early fall. The infestation level at this time has direct implications for the winter survival of colonies, especially due to the detrimental effects of parasitism on the worker population that will overwinter and be needed to care for brood in the spring (Martin 2001, Amdam et al. 2004, DeGrandi-Hoffman and Curry 2004, Amrine and Noel 2006, Fries et al. 2006, Akyol et al. 2007, Todd et al. 2007).

Chemical Acaricides

For many years, the sole chemical treatment of *V. destructor* was the pyrethroid fluvalinate (Apistan[®]) applied as plastic chemically impregnated strips placed between brood frames (Sammataro et al. 2000). Apistan[®] is a contact poison that acts as an axonic nerve poison against varroa mites (Stanghellini and Raybold 2004). Pyrethroids show more rapid and higher toxicity to mites than organophosphates (Elzen et al. 2000). The current formulation of this acaricide, however, is very toxic to honey bees as well, especially in the presence of synergists (such as piperonyl butoxide, a pesticide adjuvant often used in the vicinity of urban apiaries; (Frazier et al. 2008). Fluvalinate has been shown to have sublethal effects on drones, such as decreased longevity, seminal gland weight, mucus gland weight, sperm count, and body weight. Thus, queen breeders are advised to treat for mites just prior to drone rearing to prevent increased drone mortality (Rinderer et al. 1999). Treatments should be applied in spring and fall as needed, but applications should be avoided during the

nectar flow (Sammataro et al. 2000). It is suggested that fluvalinate be used in rotation with an acaricide from a different chemical class to avoid selecting for mites resistant to this pesticide (Elzen et al. 1998).

Coumaphos (Checkmite +[®]) is an organophosphate acaricide. It is also applied in the form of plastic chemically impregnated strips hung between brood frames. Like fluvalinate, the labeled recommendation is for applications to be made in spring and fall as required, but not during the nectar flow (Sammataro et al. 2000). It has proven useful in the past, especially against mite populations that have become resistant to fluvalinate.

Mite Resistance to Pesticides

Mites in many geographic locations have become resistant to fluvalinate (Elzen et al. 1998, Rice et al. 2004, Sammataro et al. 2005, Frazier et al. 2008). Resistance of mites to fluvalinate is an unstable trait, and as such Apistan[®] can be reintroduced only after waiting sufficiently long after previous use (Elzen et al. 2000). Resistance may be due in part to the persistent residual presence of the chemical in wax combs; such exposure to sublethal amounts of acaricide can encourage resistance to it (Korta et al. 2001).

Spraefico et al. (2001) was the first to document Varroa resistance to coumaphos (Checkmite[®]) at label-recommended doses, and increasing resistance has been shown subsequently, even in colonies not treated with coumaphos (Elzen and Westervelt 2002, Rice et al. 2004, Sammataro et al. 2005). As is the case with fluvalinate, residues in wax combs encourage development of resistance to the chemical (Korta et al. 2001).

Though it has not been found to leave behind chemical residues (Korta et al. 2001), resistance to the formamidine Amitraz[®] has been reported (Elzen et al. 2000, Sammataro et al. 2005). Amitraz[®] was known to be effective in the past, but it is no longer a registered product in the US (Elzen et al. 2000). This is clear evidence of off-label pesticide application by some beekeepers.

Acaricidal Residues

Coumaphos is lipophylic and accumulates in wax over time and has a half-life of 115 days in that medium (Thrasyvoulou and Pappas 1988, Kochansky et al. 2001, Korta et al. 2001, Martel et al. 2007). Following application, coumaphos residues are four to fifteen times higher in wax than honey samples, and it is found throughout the colony in both brood and honey combs (Martel et al. 2007). It can, however, be transferred from wax to syrup and honey, in which it has a half-life of 69 days (Kochansky et al. 2001, Martel et al. 2007). Levels of coumaphos in wax, syrup, and honey begin at low concentrations and increase over the months; after 26 weeks of contact, wax with 1000 ppm of coumaphos will transfer 430 ppb of coumaphos to honey or syrup (Kochansky et al. 2001). The pesticide has been shown to persist in honey for 30 days by Martel et al. (2007), but Korta et al. (2001) has shown it to be stable in honey for over 9 months following application; its levels in honey, though, decrease drastically after 3 months of storage, and after that time have been shown to be safe for human consumption (Thrasyvoulou and Pappas 1988). It can persist in wax for over a year following treatment (Martel et al. 2007). Coumaphos remains present in beeswax during industrial recycling processes, even after heating (Martel et al. 2007). In a recent study, 100% of wax samples analyzed for chemical residues contained coumaphos (Frazier et al. 2008).

Like coumaphos, fluvalinate is also lyphophylic and persists in wax throughout and following industrial recycling, even if it is boiled at higher temperatures or for longer than is typical (Bogdanov et al. 1998, Korta et al. 2001, Martel et al. 2007). In fact, in a recent study by Frazier et al. (2008), every sample of wax taken from 88 colonies in the US contained fluvalinate residues. Levels of up to 204 ppm have been found present in brood wax and pollen (Frazier et al. 2008). It is stable in honey for over 9 months after treatment (Korta et al. 2001). Fluvalinate residues have been found in brood combs after only a single treatment, and residues were 5-10 times higher in the brood comb than in honeycombs (Bogdanov et al. 1998). Residues increase with increasing duration of treatment and persist at higher levels in new wax than in old (Bogdanov et al. 1998).

Amitraz residues are not detected in wax one day after treatment with Apivar® (Korta

et al. 2001, Martel et al. 2007). Amitraz has a half-life of only 6.3 hours in wax, and it is the only chemical acaricide known that is not stable in that medium (Korta et al. 2001). It is only briefly detectable in honey, and is almost totally degraded 10 days following treatment (Korta et al. 2001).

Plant-Derived and Non-Synthetic Acaricides

Essential oils are highly volatile liquid compounds from the flowers, roots, wood, fruit, and seeds of plants, and they are characterized by a strong odor. To be classified as an essential oil, a plant must consist of at least 0.1% oil (Imdorf et al. 1999). Most essential oils or components of such have been found to be ineffective in *Varroa* control in field trials, however, thymol (an essential oil component) is the exception to the rule and has consistently been used in apiculture with good results against the mite (Imdorf et al. 1999, Floris et al. 2004). In fact, thymol has been shown to produce 90-100% mite mortality, without any relevant side effects to colony health (Imdorf et al. 1999, Baggio et al. 2004). However, Floris et al. (2004) reported significant reduction in brood area during treatment with thymol products, suggesting that the product should not be used during typical times of colony growth. Calderone et al. (1997) found that thymol products used as fumigants were much less effective when applied in the presence of brood, and so this is another reason to wait for brood production to be low before applying this treatment. Judging by mite fall, thymol-based products have lasting effects on mite populations extending beyond actual treatment time (Baggio et al. 2004). Apilife VAR® is a varroacide that has thymol as its active ingredient and also contains menthol, eucalyptol, and camphor (other essential oils) in smaller amounts. Api Life VAR® tablets are placed upon brood frames and its oils are passively evaporated into the colony to kill varroa mites (Imdorf et al. 1999). Because it relies on passive evaporation, however, effectiveness is variable depending on factors such as external temperature (20-25° C is optimum) and colony condition (strong colonies distribute the substance better than weak ones) (Baggio et al. 2004, Floris et al. 2004). It is useful as an element of an IPM strategy, especially when applied in late fall, but probably is not effective

as a stand-alone treatment (Imdorf et al. 1999, Pettis and Shimanuki 1999, Rice et al. 2004, Stanghellini and Raybold 2004). Api Life VAR leaves very low residues behind in honey, even after long-term treatment (Imdorf et al. 1999). However, it should not be applied during the nectar flow to avoid affecting the taste of honey (Imdorf et al. 1999). In wax, residues drop to near the detectable threshold after 1 year following treatment (Imdorf et al. 1999). Apiguard® is another thymol-based product that is available in gel form and has proven effective against varroa mites. This formulation has been observed to release more thymol into the colony than does Apilife VAR® during the first week of treatment (Floris et al. 2004). Apiguard has also shown variable mite control depending on temperature and other biological factors (Floris et al. 2004).

Oxalic acid, a non-synthetic pesticide consumed by honey bees, is a protoplasmic poison active against Varroa that acidifies the bees' hemolymph (Stanghellini and Raybold 2004) and is known by many to have good mite control when the colony is broodless, but not when brood is present (Gregorc and Planinc 2001, Stanghellini and Raybold 2004). However, Sammataro et al. (2008) found oxalic acid to significantly reduce mite levels, even when there was brood present. Similar mite mortality was observed in colonies treated with oxalic acid as in those treated with Apistan®, and Apilife VAR® (Stanghellini and Raybold 2004). There have been no side effects concerning colony health observed resulting from the use of oxalic acid, and it has been shown to leave behind no residues in beeswax and propolis because of its hydrophilic properties (Gregorc and Planinc 2001, Sammataro et al. 2008).

Sucrose octanoate (Sucroside®) is a non-synthetic acaricide deriving from the tobacco plant, shows good mite control, is safe to use, and can be used throughout the season (Stanghellini and Raybold 2004). It was shown by Stanghellini and Raybold (2004) to have similar efficacy against mites as did oxalic acid, Apilife VAR®, or Apistan®. However, they did caution that its application may prove overly labor intensive for beekeepers with many colonies to maintain. In other studies, Sucroside® was found to lack ability to control varroa mites over a long period of time (Sammataro et al. 2008).

Entomopathogenic Fungi

Entomopathogenic fungi have proven to be prime candidates for biological control of *Varroa destructor* in honey bee colonies even though the environment within a colony is not one typically suitable for fungal growth, which generally requires high humidity for sporulation and moderate temperatures of about 15-27° C (Chandler et al. 2001, Shaw et al. 2002). However, there are many fungal strains and certain isolates have been shown to be capable of growth at the low humidity in the hive (up to 70% relative humidity (RH), but often in the range of 40-50% RH) and high summer temperatures found within the colony that are often at 32-37° C (Chandler et al. 2001, Shaw et al. 2002). Also, mitosporic fungi can be mass produced and are fairly easily applied to the colony. The contact activity of entomopathogenic fungi allows for easier infection of the mite than would an agent that was transmitted orally, as they would need to be transmitted during feeding, which could be problematic in application (Chandler et al. 2001). The fungi infect their hosts through conidial attachment to the host cuticle, where the fungus germinates (Chandler et al. 2001, Roy et al. 2006). An appressorium then forms, penetrating the integument, allowing the fungus to multiply within the hemocoel and soft tissues of the host, generally causing mortality within 3-10 days. Mortality is caused by several factors: water loss, gross mechanical damage, nutrient deprivation, and toxic action. Fungal applications are most effective during times when there is little or no brood present in the colony, such as in early spring or late fall (Kanga et al. 2006). Entomopathogenic fungi have added potential as agents of *Varroa* control since it is unlikely that the mites will develop resistance to them; fungal action involves multiple target receptors, compared to a single mode of action of a chemical acaricide (Kanga et al. 2006). Encouragingly, should a suitable fungal pathogen be identified for use against varroa mites, it could potentially offer global control of the pest since honey bee colonies across the world all share a consistent internal environment (Chandler et al. 2001, Shaw et al. 2002, Kanga et al. 2006).

The fungal pathogen, *Metarhizium anisopliae* has shown significant success in controlling mites when applied as both a dust and on strips coated with conidia (Shaw et al. 2002, Kanga et al. 2003, Kanga et al. 2006). Strip application of *M. anisopliae* was most

effective at reducing mite numbers (Kanga et al. 2003). Numbers of mites sealed in brood cells of colonies treated with *M. anisopliae* were comparable to those of colonies treated with Apistan®, and effects were seen 42 days after treatment (Kanga et al. 2003, 2006). The fungal pathogen has also been shown to have a wide temperature range, and some isolates active against Varroa are capable of growth at the high temperatures found within the colony during the summer (Chandler et al. 2001). Kanga et al. (2003) have shown *M. anisopliae* to have no negative effects on adult or brood population levels or to colony development; however, Shaw et al. (2002) found that it did in fact have the potential to induce honey bee mortality at maximum challenge doses. The fungus has been shown to successfully spread amongst colonies in an apiary through drifting of drones and workers which gives it potential to be sustainable in the field and a useful component in an IPM program (Chandler et al. 2001, Kanga et al. 2003, 2006).

Hirsutella thompsonii is a fungus that has been shown to be fatally infectious to varroa mites under laboratory conditions, and shows some promise as a biological control tool (Peng et al. 2002, Shaw et al. 2002). The mite is infected by *H. thompsonii* when conidia adhere to its aurolia (the smooth membranous structures at the end of each leg which can be retracted into the leg stalk) (Peng et al. 2002). The mite retracts its aurolia in an attempt to remove the conidia through grooming, but the conidia stick to the membrane and are not removed. The fungus germinates 12 hours after initial contact within the humid environment of the leg stalk and eventually forms a fungal germ tube that penetrates the body cavity and kills the mite. *H. thompsonii* has not been observed to affect honey bees of any age, under laboratory conditions; it is has thus far been known to be active only against Acari (Chandler et al. 2001, Peng et al. 2002). Also, it is capable of growth at the high temperatures found within the bee colony (Chandler et al. 2001). There are some potential obstacles to overcome before the fungus could be of use to the apiculture industry however: mass production of the culture would have to be made possible; a formulation would have to be developed that could maintain infectivity in colonies in the field; this formulation would have to be one that did not contaminate hive products (Peng et al. 2002).

Treatment with *Beauveria bassiana* conidial isolates collected from *Varroa*

destructor have been shown to be fatal to the mite (Shaw et al. 2002) and lead to significant knockdown, peaking between 5-8 days after treatment, in honey bee colonies when applied in a wax powder mix (Meikle et al. 2007, 2008). However, such treatment is only effective to those mites exposed to the fungus at the time of application; those that emerge from sealed brood cells later will remain untreated (Meikle et al. 2008). Varroacidal effects of the fungus have been shown after more than a month has passed since treatment, and *B. bassiana* seems to be spread between colonies through drifting or robbing by bees from treated colonies (Meikle et al. 2007). It does not have a negative impact on parameters indicative of colony health such as total adult bee weight, area of capped brood or honey, or colony survival (Meikle et al. 2008). *B. bassiana* is a promising biopesticide agent for use in controlling *Varroa destructor* (Shaw et al. 2002).

Mechanical Control

Dusting with powdered sugar is a mechanical means of mite control; the fine sugar particles interfere with the suction mechanism of the mite's ambulacrum, causing it to lose its grip on the bee's exoskeleton and fall off. Fakhimzadeh (2001) shows 91% effectiveness in mite knock-down when the sugar is applied directly to bees that are then shaken and rolled in a container and 62% effectiveness with air-assisted dusting inside a colony. The particle size of the sugar powder is comparable to that of pollen, and it was found not to obstruct the perpetually open T2 spiracle of honey bees when examined with a scanning electron microscope, nor were they in the respiratory ducts (Fakhimzadeh 2001). Also, application of powdered sugar has been shown to be harmless to immature bees unless large amounts of sugar are applied directly to open brood cells (Aliano and Ellis 2005b). It is suggested that this technique be performed during broodless periods for maximum efficiency, since otherwise some amount of the Varroa population will emerge with new bees and will thereafter remain in the colony. This method of mite control is fairly disruptive to colony function, however, and is only of limited effectiveness when applied to an entire colony, since one cannot shake or roll an entire colony (and this is the most effective method of sugar

application) (Fakhimzadeh 2001). Also, dusting with powdered sugar may prove time consuming and labor intensive (Aliano and Ellis 2005b).

Screen bottom boards allow mites to fall out of the colony when they fall to the bottom of the hive, whereas with regular bottom boards, they may be able to hitch a ride back in on another passing worker. They have been shown to exhibit mite control, sometimes significantly (Pettis and Shimanuki 1999, Delaplane et al. 2005, Coffey 2007). As an additional benefit, colonies with screen bottom boards have been shown to have significantly more sealed brood than those with normal bottom boards (Pettis and Shimanuki 1999). They can have variable results and should not be used as the only measure of mite control, but screen bottom boards may be a good addition to an integrated management system, especially considering the limited cost and effort required (Pettis and Shimanuki 1999, Rice et al. 2004, Coffey 2007).

Drone Brood Manipulation

Because mites spend significant time sealed within brood cells, trapping them there is an attractive method of mechanical control (Boot et al. 1995). This technique has been explored using worker brood, but was found to be too labor intensive and time consuming (Boot et al. 1995). Much brood was needed (mites are not very concentrated within worker comb), and after removal Varroa trapped within had to be selectively killed so as to preserve the valuable worker brood (Boot et al. 1995). These obstacles are avoided, however, if mites are trapped in drone comb. Less comb is necessary since mites seek it out preferentially, and it can be disposed of along with the mites, as it is not critical to colony survival (Boot et al. 1995). Conveniently, such treatment can easily be accomplished with little extra time spent if incorporated into swarm prevention management activities already in place (Boot et al. 1995, Wilkinson and Smith 2002b).

Calderone (2005) evaluated the effect of supplying two drone comb frames to colonies and periodically removing them, trapping the brood within along with any mites infesting it; frames were then replaced with either empty drone comb or drone comb that had

been previously frozen. Providing bees with entire frames of drone comb equaling about 20% of the total comb area within the hive (a natural percentage of drone comb for honey bees) has been found to limit the amount of worker comb converted into drone comb, leaving “cleaner” frames for beekeepers (Seeley 2002). Meanwhile, supplying the colonies with drone comb consistently but removing it periodically such that no drones emerged in the colony was found to have no detrimental effects on colony health (Charriere et al. 2003, Calderone 2005). In fact colonies whose drone brood was trapped and removed in drone comb produced significantly more honey than did control colonies in which drone brood was allowed to emerge within the colony, while worker populations were similar for the two groups (Calderone 2005). Likewise, Seeley (2002) found that colonies supplied with drone comb that was left in place throughout the season produced less honey than those colonies without drone comb at all, or with small amounts of it. Perhaps more importantly, colonies that underwent periodic removal and replacement of drone brood had significantly lower mite-to-bee ratios than did control colonies which were provided with drone comb that was left in place (Wilkinson and Smith 2002b, Calderone 2005, Coffey 2007). Seeley (2002) found that if drone comb was provided to a colony but left in place, mite counts were elevated above those colonies that had little or no drone comb, so it is important for beekeepers to remove any drone comb provided prior to emergence. If drone trapping is to be practiced, though, beekeepers should not wait for mite levels to reach treatment thresholds to begin trapping – instead the combs should be put in place in March or April (Wilkinson and Smith 2002b). Besides being environmentally safe and seemingly impervious to development of resistance by mites, this technique has been proven to successfully suppress mite populations through summer and early fall (though it is not suggested to use it as the only control), and is a valuable addition to an integrated approach to managing varroa mites (Boot et al. 1995, Wilkinson and Smith 2002b, Charriere et al. 2003, Calderone 2005, Coffey 2007).

The drawback to methods of drone trapping currently practiced to control varroa mites is the negative impact on the drone population. Though the absence of drones is not harmful to individual colonies (Seeley 2002), they are essential to colony reproduction. I

will employ a drone trapping technique that controls varroa mites while preserving the drone population. A healthy breeding population is vital to honey bee population health, and having low drone numbers or drones of poor quality could have negative effects on queen mating (Sammataro et al. 2000, Coffey 2007, Kraus et al. 2007). Having drones present in an apiary may have the added benefit of increasing the frequency of mite-resistant alleles in the overall population; this is feasible since mites exert direct selection pressure on drones, and so natural selection should select mite-resistant genotypes (Jandricic and Otis 2003). A method of drone trapping that does not destroy drones may be an excellent addition to an IPM program to control varroa mites.

CHAPTER 2. DRONE BROOD REMOVAL FROM *APIS MELLIFERA* COLONIES TO CONTROL *VARROA DESTRUCTOR* AND SALVAGING OF ADULT DRONES

Introduction

Varroa destructor Anderson and Trueman is an ectoparasitic mite of the European honey bee (*Apis mellifera* L.). Since its introduction to the United States in the 1980s, it has been the most serious problem facing the US apiculture industry, and it is found nearly everywhere honey bees are present. The Varroa mite was originally a parasite of the Asian honey bee (*A. cerana*), but it has recently undergone a host shift to parasitize the European honey bee as well (Oldroyd 1999, Anderson and Trueman 2000). Of the Varroa species complex that includes *Varroa jacobsoni* Oud. and *V. underwoodii* Delfinado-Baker, only *V. destructor* is capable of reproduction within *A. mellifera* colonies (Anderson and Trueman 2000). The mite causes relatively little injury to its native host, because it generally reproduces only within the cells of developing males (drone brood), leaving the colonies' workers unharmed (Boot et al. 1995, Boot et al. 1999, Chandler et al. 2001). Moreover, Asian honey bee adults readily recognize the presence of phoretic mites and remove them with their mandibles, a grooming behavior that is elicited by a dance to recruit neighboring workers (Buchler et al. 1992). *A. cerana* nurse bees are more adept than European honey bees at recognizing and removing parasitized and diseased brood before it emerges, thus preventing mite spread (Boot et al. 1999). These factors all help to stabilize the host-parasite relationship between Varroa and *A. cerana*. Similar behaviors are lacking in *A. mellifera*, leaving European honey bees vulnerable to parasite induced morbidity and mortality (Kraus and Page 1995, Sammartaro et al. 2000).

To reproduce, a female mite "foundress" enters the brood cell of a late instar bee and begins laying eggs about 60 hours after the cell is capped. Eggs are laid singly at 30-hour intervals; the first egg produced is unfertilized resulting in a haploid male offspring and subsequent offspring are diploid females (Donze and Guerin 1994, Chandler et al. 2001, Sammartaro et al. 2002). Mature males mate with their female sibling(s) as they mature within the cell (Donze and Guerin 1994, Sammartaro et al. 2000, Chandler et al. 2001, Calderon et al. 2007b). Only adult female mites exit the cell when the host bee emerges from

its pupal confinement. The male mite and any immature offspring will not survive outside of a capped brood cell (Sammataro et al. 2000).

Varroa mites feed directly on the hemolymph of late instar larvae, pupae, and adult honey bees. Mites are most damaging to pupal bees. Loss of nutrients and hemolymph is especially harmful at this critical stage of development for the honey bee. Parasitism results in decreased adult body weight and longevity, and reduced sperm count, mucous gland weight, and seminal vesicle weight in drones (DeJong et al. 1982, Rinderer et al. 1999, Janmaat and Winston 2000a, Zoltowska et al. 2007). Indirectly, varroa mites may sufficiently compromise immune defenses of the honey bees, enabling facultative viruses to become pathogenic. They can also vector—and in some cases amplify—viral diseases within and among colonies. This combination of parasitism and viral infection is often referred to as parasitic mite syndrome (PMS) or varroosis. *V. destructor* is a biological vector of deformed wing virus (DWV), perhaps the most prevalent and harmful of the known bee viruses (Yue and Genersch 2005, Tentcheva et al. 2006). Indeed, DWV is a major factor in the collapse of colonies infested with *V. destructor* (Bowen-Walker et al. 1999, Nordstrom et al. 1999, Martin 2001, Nordstrom 2003, Chen et al. 2005a, Yue and Genersch 2005, Tentcheva et al. 2006). In the absence of mites, DWV is often not problematic for honey bees, which remain asymptomatic (Allen and Ball 1996, Martin 2001, Chen et al. 2005b, Yue and Genersch 2005, Tentcheva et al. 2006). Likewise, varroosis exacerbates acute paralysis bee virus (APBV) infections within bee colonies. When Varroa is present to vector and activate this virus, it can be fatal to a colony, but it is otherwise a latent infection causing no obvious harm (Ball and Allen 1988, Nordstrom et al. 1999, Brodsgaard et al. 2000, Martin 2001). The mite is also implicated as a vector of Kashmir bee virus (KBV) (Shen et al. 2005a, Todd et al. 2007) and sac brood virus (SBV) (Shen et al. 2005a), although the mites play a smaller role in the pathogenicity of the viruses, which are more often spread by other means.

Ever since their introduction, synthetic acaricides have been used to combat Varroa mite infestations. Chemical agents used include the pyrethroid fluvalinate (Apistan®), the organophosphate coumaphos (Checkmite+®) and the formamidine amitraz (Apivar®), which

is no longer registered). These highly effective pesticides also have significant drawbacks, especially acaricide resistance of and chemical residues left behind in wax and honey. Though susceptible mite populations exist, widespread resistance has developed to each of these acaricides (Elzen et al. 1998, Elzen et al. 2000, Spreafico et al. 2001, Rice et al. 2004, Sammataro et al. 2005). Resistance often develops due to overuse and abuse of chemical pesticides, as well as consistent exposure of mites to sub-lethal doses that persist within the hive in honey and especially wax (Korta et al. 2001). In particular, lipophilic fluvalinate and coumaphos accumulate in beeswax (Thrasyvoulou and Pappas 1988, Kochansky et al. 2001), residues remain through the processing of industrial wax recycling (Martel et al. 2007). These chemicals, though less stable in honey, can be detected for months following chemical application. Alternative pesticides (e.g. oxalic and formic acid) and botanical pesticides (such as sucrose octanoate, a tobacco derivative, and essential oils) have gained attention, but often their use is labor-intensive and may not be as effective as stand-alone treatments (Imdorf et al. 1999, Gregorc and Planinc 2001, Rice et al. 2004, Stanghellini and Raybold 2004). Other potential control measures include entomopathogens, inert dusts, and screen bottom boards; all lack efficacy and may be labor intensive or disruptive to the colony (Pettis and Shimanuki 1999, Chandler et al. 2001, Fakhimzadeh 2001, Kanga et al. 2003, Aliano and Ellis 2005b, Delaplane et al. 2005, Kanga et al. 2006, Coffey 2007, Meikle et al. 2007, 2008). These methods, however, may be useful if incorporated into an integrated mite control plan.

One form of cultural control for *V. destructor* is drone-brood removal (Boot et al. 1995, Wilkinson and Smith 2002b, Charriere et al. 2003, Calderone 2005). This technique takes advantage of the mite's natural preference for parasitizing drone brood. Mites selectively prefer drone brood because it increases their reproductive output; because drones have a longer post-capping duration than workers, drone brood enables mites to produce more offspring (Calderone and Kuenen 2001). When practicing drone-brood removal, beekeepers provide entire frames of drone comb allowing queens to concentrate their drone-laying to isolated frames. Mite infested frames are later removed from the hive after the cells are capped. This allows the frames to act as a sink for the mite population, minimizing the

loss of valuable worker brood. Most of the previous forms of this cultural practice involve the drone frames being destroyed or frozen, killing all mites present as well as drones (Huang 2001, Calderone 2005). Drone-brood removal has achieved low mite levels through summer and early fall without the use of synthetic acaricides, and it is a form of control to which mites will not likely become resistant (Boot et al. 1995, Wilkinson and Smith 2002b, Charriere et al. 2003, Calderone 2005, Coffey 2007).

It is critical to the health of a colony that the queen mates with many drones to assure sufficient genetic diversity within the colony, subsequently reducing susceptibility to disease and parasitism (Seeley and Tarpay 2007). Traditional drone-brood removal techniques, in which drone brood is destroyed, result in a decrease in the number of drones available to mate with queens. In contrast, allowing mites to actively exert selection pressure on drones may result in increased survival of individuals that have increased mite tolerance. Since drones are haploid, any alleles responsible for such tolerance would be more readily expressed and passed on to offspring. Although mites increase drone mortality, surviving parasitized drones remain reproductively competitive (Rinderer et al. 1999). Thus, the concept of removing mites from drones, then returning the previously infested drones to their colonies is attractive because it does not decrease the available mating pool, and it may also result in the natural selection of individuals that are more tolerant of varroa mites.

In the present study, we investigate a variation of drone-brood removal that would allow the survival of the drone population, thus leaving them to bolster the mating population and potentially act as vehicles to disperse any mite resistant alleles they may possess, while simultaneously decreasing the number of mites within a colony without the use of synthetic acaricides. This approach, which we term “drone-brood rescuing”, may therefore have benefits in both the short term (e.g., decreased mite loads) as well as the long term (e.g., increased mite-tolerant alleles in the population).

Materials and Methods

We began the study in mid-April, 2007, with 40 five-frame nucleus colonies made up

of Italian stock honey bees (*Apis mellifera*). All colonies were split from others sharing a common apiary. The colonies were placed in an apiary adjacent to a corn field on the Lake Wheeler Research Station in Raleigh, NC. The location was shaded for most of the length of a day, and there was a water source nearby. Within several days, we increased the hives' size to 10-frames in standard Langstroth hives. The added frames consisted of two frames of drone comb (one drawn and one foundation only) placed in positions 2 and 9 within the hive, two standard foundation frames placed in positions 1 and 10, and another frame of drawn comb placed next to a drone frame. To boost colony growth and development, we provided all colonies with supplemental sugar syrup and pollen. When colony populations were sufficiently large, we added second hive bodies on a colony-by-colony basis. These second brood chambers consisted of three foundation frames placed in the outmost positions, one honey frame, and six frames of drawn, empty comb. Over the course of the season, we managed colonies using standard apicultural techniques, (i.e., swarm control, adding and removing supernumerary hive boxes, feeding of sugar syrup, application of robbing screens, etc.). The exception to this was Varroa mite control, which was dictated by treatment group.

Colony strength was evaluated before treatment assignments. We evaluated adult worker population by estimating the percentage of each frame covered with bees and then summing all percentages to get total number of frames covered per colony. Similarly the, brood populations in were estimated as the percentage of each comb that was occupied by developing bees in all stages of development and summing percentages. Also, we counted the numbers of capped drone brood cells outside of the provided drone frames for each colony.

Prior to treatment assignments, mite populations within each colony were evaluated using white adhesive sticky boards (Calderone and Turcotte 1998, Ostiguy and Sammartaro 2000). Sticky boards were covered with the rubber screen to prevent bees from adhering to the adhesive but allowing sufficient contact to capture dislodged mites. Sticky boards were placed on the bottom board of each hive for 24 hr. Adhering mites were counted with the aid of a grid placed over the sticky board. Increased activity within the colony could potentially lead to increased mite drop, and so to ensure that mite counts were not elevated from colony

disturbance, we did not open or manipulate the hives during the time the sticky boards were in the hives.

We then assigned four treatment groups to the colonies. To correct for potential position effects, we divided the colonies into 10 blocks of four adjacent colonies and randomly assigned treatments within each block. We analyzed pre-treatment measurements of the four groups so all groups were statistically equivalent with respect to adult population, brood population, number of capped drone cells outside of drone frames, and 24-hour mite drop prior to the start of treatment. Treatment groups were as follows: Group 1 – Negative control, no preventative varroa-mite treatment; Group 2 – Positive control, Apistan[®] strips (plastic impregnated with the acaricide fluvalinate) placed between frames towards the periphery of the cluster with two strips per brood box, which were removed and replaced every 6 weeks; Group 3 – drone-brood removal and freezing, where every month the drone frames were removed, shaken to remove adult bees, placed into a freezer, and replaced with empty drone frames; and Group 4 – drone-brood removal and rescuing, where frames of drone comb were removed monthly and replaced with empty frames, placed in individual cages within an incubator set at brood-nest temperature (37°C), and checked daily for emerged adult drones. For all emerged drones, we then dusted them with powdered sugar, placed them into a plastic jar with a wire-mesh lid, shook to remove mites (Fakhimzadeh 2001), and placed the drones back into their natal colonies. We provided a small number of worker bees in each incubated box to help the drones emerge from their cells and feed. We recorded the number of mites removed each day from emerging drones in each colony in Group 4, as well as the number of drones returned to their colony of origin. We began treatments in mid-late May, including the application of Apistan[®] strips and the removal and replacement of drone frames. We continued treatments over the course of the summer, which we terminated in August when drone production had halted for the remainder of the season.

We performed Pettis tests (Pettis and Jadczyk 2005) on a representative sub-sample of five colonies within the apiary in an attempt to identify any fluvalinate resistance present in the mite population. We collected a sample of 150 bees from each colony and put into a 500 ml wide-mouth canning jar with a mesh cover (coarse enough to keep bees in, but fine

enough to allow mites to fall through). We cut and stapled a 3/8" X 1" piece of a fresh Apistan[®] strip to the center of an index card. The treated card was placed in the jar of bees and was incubated for 6 hours at 37° C. After this time, we removed the jars from the incubator and inverted them to shake out all dead mites. Jars were shaken over a white surface to facilitate counting and the number of mites was recorded. We then poured sufficient ethanol into the glass jars submerge and kill all bees within them. The contents of the jars were poured through a mesh filter to allow mites, but not bees, to pass through and into a white bucket. Mites washed from the bees were counted and the numbers recorded. Mites washed from the bees were considered not susceptible to the pesticide. We calculated the proportion of susceptible mites by dividing the number of mites killed initially with the total number collected. If more than 50% of mites present were killed by Apistan[®] we considered them susceptible.

During the course of the study, we measured the mite levels within each colony every month using sticky board counts (see above) and sugar shakes (Fakhimzadeh 2001, Aliano and Ellis 2005b). To perform sugar shakes, we collected approximately 200 bees from brood frames into a plastic jar with a mesh-screen lid. We applied approximately ¼ cup of powdered sugar to the bees in the jar and let stand for 1-2 minutes to ensure that the sugar was sufficiently distributed. We inverted the jar and gently shook it over a light-colored surface to allow dislodged mites to fall through the mesh lid and be counted. When no more mites could be shaken from the jar, we returned the bees to their respective colonies.

Periodically, we removed individual drones from incubated frames (from colonies in Group 4) as they emerged from brood cells. We recorded the number of mites within their cells and on their bodies and physically removed the mites from each using forceps. We then weighed the drones using a digital scale, and recorded body weights. We later returned the drones to their original colonies.

During the study, one colony was diagnosed with American Foulbrood (AFB), and was removed from the study and destroyed. We treated remaining colonies with the antibiotic terramycin as a prophylactic measure. We removed another colony from the study after it became queenless and developed laying workers. A third colony died during the

study as a result of poor general health.

After the final course of treatments, we assessed colony strength by estimated adult bee and brood populations as we did prior to the start of the study.

Statistical Analysis

Means of pre-treatment measurements of strength were compared between treatment groups using an analysis of variance (ANOVA) performed using SAS software (SAS Institute, Cary, NC). These measurements included total frames of adult bees, frames of brood, 24-hour mite drop using sticky boards, and area of drone comb outside of provided frames. Mean 24-hour mite counts from sticky boards for treatment groups were compared by ANOVA to determine whether the mite counts between treatment groups differed significantly between groups in any month during the study. The mean mite counts as measured by sugar shakes were compared among treatment groups to determine significant differences between groups during any month of the study. Pair-wise comparisons of means between treatment groups were performed using Tukey tests. Means of post-treatment measurements of strength were compared between treatment groups using ANOVA. These measurements included total frames of adult bees, frames of brood, 24-hour mite drop using sticky boards, and area of drone comb outside of provided frames.

Results

There were no significant differences between any of the pretreatment groups at the start of the study (Fig. 2.1); measures of colony strength were statistically similar with regard to adult bee population ($F = 0.70$, $df = 3, 36$, $P = 0.5629$) and mean brood population ($F = 0.83$, $df = 3, 36$, $P = 0.4479$; Fig. 2.2). Likewise, the colonies did not differ significantly with regard to mean 24-hour mite drop on sticky boards ($F = 0.86$, $df = 3, 36$, $P = 0.5975$; Figure 2.3) or the levels of drone brood external to drone frames provided ($F = 0.17$, $df = 3, 36$, $P = 0.3314$).

The mean 24-hour mite drops were highly variable among groups regardless of

treatment. The means did not differ significantly between treatment groups during any month of the study ($P > 0.05$; Fig. 2.3). This variability may be due in part to differences in adult bee populations between colonies, which would affect the mite to bee ratio, not measured using this assay.

Mean mite counts from monthly sugar shakes were compared between treatment groups. In June, mean mite count in the positive control group was significantly lower than in the negative control group ($F = 2.76$, $df = 3, 35$, $P = 0.0089$; Fig. 2.4), and the drone-brood freezing and drone-brood rescuing groups were numerically intermediate but not significantly different from either control group. In other months, all treatment groups were statistically similar (all $P > 0.05$; Fig. 2.4), though in all groups mite count increased greatly as the season progressed. All treatment groups were similar with respect to post-treatment measurements of adult bee populations ($F = 1.44$, $df = 3, 33$, $P = 0.2676$; Fig. 2.5) and brood populations ($F = 0.36$, $df = 3, 33$, $P = 0.7135$; Fig. 2.6). At the end of colony manipulations, no colonies were producing drone brood, and so comparisons among treatment groups for capped drone brood outside drone frames were not warranted.

Discussion

Though no statistically significant differences were detected in this study, numeric trends in mite counts were seen among treatment groups, with both drone-brood removal groups being intermediate between the negative control group (which had the highest mite counts) and the positive control group (which had the lowest mite counts). These trends match the hypothesized outcomes, and the differences seen may have been greater if manipulations began earlier or were performed as needed in each colony.

The large variance observed in the 24-hour sticky board mite counts was likely due to the lack of data concerning the adult bee population within each colony at the time of the measurements. It is important to take into account the bee population when conducting sticky board counts because the total mite drop is a positive function of the size of the colony. Additionally, in some instances, the consistency of mite counts using the boards was

questionable because some mites did not adhere to the board. These inconsistencies have been noted in previous studies as well (Ostiguy and Sammataro 2000).

The lack of significant differences between treatment groups in measures of colony strength suggest that drone brood removal is not detrimental to colony health and development. This finding is consistent with those of other studies (Charriere et al. 2003, Calderone 2005), which suggests that there are minimal costs to colony productivity by employing a drone-brood removal strategy for mite control.

There was no significant difference between the mite counts of either drone brood removal group and the negative or positive control groups during any month of the study. It is possible that greater treatment effects might have been seen if they were initiated earlier in the season when the majority of drones are produced by colonies. Therefore, drone brood removal might be more effective when a larger proportion of the mite population would be attracted to drone brood. It is our recommendation that in the southern states, drone-brood removal should begin no later than April, with monitoring for drone production beginning in early March.

It is also likely that removing drone frames on a monthly basis was not sufficient to prevent significant numbers of drones from emerging within the colony. This would make the practice counter productive, in fact allowing mites an opportunity to locate drone brood easily and multiply quickly. It is imperative that drone frames be removed before any emerge in a colony, with the benefit of increased mite control outweighing the additional labor involved in monitoring the colonies at least weekly to catch drone frames at the appropriate stage for removal. It would benefit a beekeeper to take detailed notes of the ages of brood to form a removal schedule for each colony individually, which could be combined with conventional swarm-control practices.

In conclusion, drone-brood “rescuing” may be a potentially useful method for controlling *V. destructor* in honey bee colonies. Moreover, it is possible to trap mites while preserving the drone population without risking colony health. This has encouraging implications for both the possibility of instilling mite-tolerant alleles in the population, using drones as a vehicle for their distribution, and bolstering the pool of drones available for

mating. These possibilities are feasible, since parasitized drones that live to sexual maturity are known to remain reproductively capable and competitive (Rinderer et al. 1999). When using this technique, however, it is important that treatment begin as soon as drone production begins within the colony; in the southeastern region of the US, this would most likely be mid-April, with monitoring for drone brood beginning in early March. Also, drone frames should be removed on a colony-specific schedule that maximizes the amount of capped brood removed while preventing any from emerging within the colony. It is best if examination of drone brood frames is performed weekly in order to maintain an effective schedule, which should be easily combined with swarm-control methods.

CHAPTER 3. REMOVAL OF DRONE BROOD FROM *APIS MELLIFERA* COLONIES TO CONTROL *VARROA DESTRUCTOR* AND DRONE CONSOLIDATION INTO COLONIES TREATED WITH ACARICIDE

Introduction

Varroa destructor Anderson and Trueman is an ectoparasite of the European honey bee, *Apis mellifera* L. It was introduced to the United States from mainland Asia in the 1980s and has since been the largest parasitic disease facing the US apiculture industry. Its range is very widespread, and it is now found nearly worldwide. The varroa mite's native host is the Asian honey bee, *Apis cerana*, but it has shifted hosts to parasitize European honey bees also (Oldroyd 1999, Anderson and Trueman 2000). *V. destructor* is the only species of the Varroa species complex that reproduces within *A. mellifera* colonies; this complex also includes *V. jacobsoni* Oud. and *V. underwoodi* Delfinado-Baker (Anderson 2000). The mite is fairly innocuous to its native host because it reproduces only within the cells of developing males (drone brood), leaving developing workers unparasitized and restricting the mite's population growth to those times of the season during which drone brood is present (Boot et al. 1995, Boot et al. 1999, Chandler et al. 2001). When mites are present in *A. cerana* brood cells, nurse bees often detect it and remove the parasitized pupa from the colony. This behavior limits the mites' spread within a colony (Boot et al. 1999). Also, adult Asian honey bees have well-developed behavioral defenses against the mite. They quickly detect the presence of phoretic mites and commonly remove them from their own bodies (autogrooming), or they perform a dance eliciting the assistance of neighboring bees to remove the mites for them (allogrooming). *A. mellifera* does not possess such defenses, and it is much slower to recognize phoretic mites. They do not remove the mites as efficiently through autogrooming as Asian bees, and allogrooming is rarely observed in European bees (Buchler et al. 1992). Thus *V. destructor* has proven to be far more injurious to *A. mellifera* than it is to *A. cerana* (with which it shares a relatively stable host-parasite relationship), and both managed and feral European colonies experience high rates of mortality and morbidity as a result of parasitism.

To reproduce, a gravid female mite enters the brood cell of a fifth instar bee and begins laying eggs approximately 60 hours after the cell is capped. Thereafter, eggs are laid

singly at 30-hour intervals; the first is a male, which is haploid, and subsequent offspring are females, which are diploid (Donze and Guerin 1994, Chandler et al. 2001, Sammartaro et al. 2002). Mature males then mate with their sibling(s) as they reach maturity within the sealed cell (Donze and Guerin 1994, Sammartaro et al. 2000, Chandler et al. 2001, Calderon et al. 2007b). Only adult female mites leave the cell when the host bee emerges after its own development is complete. The male mite and immature individuals cannot not survive outside of a capped cell (Sammartaro et al. 2000).

Parasitism by *V. destructor* is directly damaging to late instar larvae, pupae, and adult honey bees because vital nutrients are lost when the mite consumes the bees' hemolymph. Most of this injury is incurred during the bees' pupal development, during which time the invading female (foundress) mite and her developing offspring are feeding. This is a critical stage of development for the honey bee, and parasitism results in decreased adult body weight and longevity, as well as sperm count, mucous gland weight, and seminal vesicle weight in drones (DeJong et al. 1982, Rinderer et al. 1999, Janmaat and Winston 2000a, Zoltowska et al. 2007). Indirectly, however, Varroa is even more detrimental due to its competency as a vector for numerous viral diseases, as well as its ability to activate otherwise latent infections. This has been termed parasitic mite syndrome (PMS), or varroosis (Shimanuki et al. 1994). This mite has been shown to transmit and amplify deformed wing virus (DWV), which is perhaps the most prevalent and harmful of the known bee viruses (Yue and Genersch 2005, Tentcheva et al. 2006). Its presence is a major factor in the collapse of colonies infested with this parasite (Bowen-Walker et al. 1999, Nordstrom et al. 1999, Martin 2001, Nordstrom 2003, Chen et al. 2005a, Yue and Genersch 2005, Tentcheva et al. 2006). DWV is highly prevalent in honey bee populations, but infected individuals remain largely asymptomatic in the absence of varroa mites (Allen and Ball 1996, Martin 2001, Chen et al. 2005b, Yue and Genersch 2005, Tentcheva et al. 2006). Similarly, varroosis intensifies acute paralysis bee virus (APBV) infections within bee colonies. In the presence of the varroa mite, the virus is activated and spread rapidly, in some cases leading to colony fatality. Alone, however, APBV causes no obvious harm (Ball and Allen 1988, Nordstrom et al. 1999, Brodsgaard et al. 2000, Martin 2001). *V. destructor* has also been proven to vector

Kashmir bee virus (KBV) (Shen et al. 2005a, Todd et al. 2007) and sacbrood virus (SBV) (Shen et al. 2005a). These diseases, however, are generally spread by other means, and mites contribute much less to their pathogenicity than in the cases of DWV and APBV.

All forms of injury resulting from Varroa parasitism take their highest toll on honey bees during the overwintering phase of colony life. The health of the overwintering worker population, as well as that of the generation of workers that care for them, is critical to colony survival through the winter and into the following spring. As such, autumn Varroa populations are particularly harmful to colonies (Martin 2001, DeGrandi-Hoffman and Curry 2004, Amrine and Noel 2006, Fries et al. 2006, Akyol et al. 2007, Todd et al. 2007). Winter workers need to be long-lived, at least in temperate climates, surviving to produce the new generation of brood in the spring. Besides introducing pathogens that will weaken them, Varroa parasitism actually changes their physiology in ways that hinder increased longevity. For example, parasitism has been shown to decrease vitellogenin, which is crucial for winter survival in long-lived workers as well as the production of food for spring brood (Fluri et al. 1982, Amdam et al. 2003, Amdam et al. 2004). In these ways, *V. destructor* contributes significantly to the frequency of collapse in feral and managed colonies.

Synthetic acaricides have long been the most commonly used tool to control varroa mites. Among them is the pyrethroid fluvalinate (Apistan[®]), the organophosphate coumaphos (Checkmite+[®]), and the formamidine amitraz (Apivar[®], which is no longer registered for use). Though they were once highly effective, varroa mites are developing increased resistance to all of these chemicals, in many cases making them insufficient for controlling infestations (Elzen et al. 1998, Elzen et al. 2000, Spreafico et al. 2001, Elzen and Westervelt 2002, Sammataro et al. 2005). Resistance has been propagated by overuse and misuse of pesticides, as well as exposure of mites to sublethal residues left behind in honey and wax (Korta et al. 2001). Because of their lipophilic properties, fluvalinate and coumaphos accumulate in beeswax over time and persist even after the industrial processing that occurs before it is recycled and reused as wax foundation (Martel et al. 2007). Though the half-lives of these chemicals are considerably shorter in honey, they can be detected in honey for months following application (Kochansky et al. 2001, Korta et al. 2001, Martel et

al. 2007). Moreover, pesticide residues pose potential health hazards for human consumers of honey and beeswax products. Alternative pesticides are available (such as oxalic and formic acids), as well as plant-derived pesticides (such as the tobacco derivative sucrose octanoate and essential oils such as thymol). Though these acaricides may be appealing as alternatives to synthetic substances, they are often more labor-intensive to apply and may not offer sufficient mite control when used alone (Imdorf et al. 1999, Gregorc and Planinc 2001, Rice et al. 2004, Stanghellini and Raybold 2004). Non-chemical control measures, such as application inert dusts, fungal pathogens, and use of screen bottom boards are sometimes used, but these measures do not offer consistently high efficacy when used alone and may be disruptive to the colony (Pettis and Shimanuki 1999, Chandler et al. 2001, Fakhimzadeh 2001, Kanga et al. 2003, Aliano and Ellis 2005b, Delaplane et al. 2005, Kanga et al. 2006, Coffey 2007, Meikle et al. 2007, 2008). Used in conjunction, however, a combination of these treatments can be used to formulate an effective integrated management plan to control varroa mite populations.

Another method of non-chemical control of varroa mites is drone-brood trapping, which is designed to take advantage of the mite's natural preference for parasitizing drone brood (Boot et al. 1995, Wilkinson and Smith 2002b, Charriere et al. 2003, Calderone 2005). Reproducing in drone cells increases the reproductive potential of a female mite. The drone pupal stage is longer than workers, within the cells to produce more mature offspring before the bee emerges (Calderone and Kuenen 2001). Traditionally, to perform this control technique, frames of drone comb are placed into a hive, left there until there is capped drone brood in the cells, and then removed from the colony to be frozen. The drone brood acts as a sink for varroa mites, which are removed from the colony in a way that has minimal impact on worker brood. Drone-brood trapping has been shown to maintain low mite populations through the late summer when used without any other mite treatment, and it is unlikely that varroa mites will develop any behavioral resistance to this form of treatment (Boot et al. 1995, Wilkinson and Smith 2002b, Charriere et al. 2003, Calderone 2005, Coffey 2007).

This application of drone-brood trapping and subsequent freezing effectively kills the mites trapped within it (Calderone 2005), but it also kills all of the developing drones.

Genetic diversity is vital to the health of a colony, and this is accomplished when the queen honey bee mates with many drones. When the queen does not mate with a sufficient number of males, her colonies are often weaker and more susceptible to parasitism and disease (e.g., Seeley and Tarpy, 2007). Current methods of drone-brood trapping result in a decrease in the drone population available for mating with local queens. Also, exertion of selection pressure from mites on drones may result in increased survival of individuals that have increased mite tolerance. Drones are haploid, and so any alleles responsible for such tolerance would be passed on to offspring. Thus, it would seem beneficial if drones could be salvaged after their removal from colonies while killing the mites that are present with them, particularly since parasitism does not seem to unduly affect their ability to mate (Rinderer et al. 1999). A method of treatment that accomplished this would not decrease the mating pool, and it may allow the natural selection of those individuals who are most fit under the conditions of parasitism. In the present study, we investigated a variation of drone-brood removal that permits the survival of the drone population, thus leaving them to bolster the mating population and potentially act as vehicles to disperse any mite resistant alleles they may possess. We hypothesize that this method will decrease the number of mites within a colony with very limited use of synthetic acaricides, which will be applied only to a small number of colonies in the apiary.

Materials and Methods

We began the study in early March with 20 ten-frame colonies in standard Langstroth hives. The colonies were European honey bees of Italian stock. The hives were placed in a row parallel to a corn field on one side and a tree line on the other on the Lake Wheeler Research Station in Raleigh, NC. The tree line provided shade for much of the day, and a nearby stream provided a source of water. Each hive included two frames of drawn drone comb placed in positions two and nine, approximately one-and-a-half frames of pollen, two frames of honey, and four frames of brood. To supplement the colonies' nutrition and encourage rapid colony growth and development, all colonies were fed supplemental sugar

syrup (one part water to one part granulated sucrose) and pollen patties to support brood production. As colony populations increased, second hive bodies were added to existing hives on a colony-by-colony basis. These additional brood boxes each consisted of two foundation frames placed in the outmost positions and eight frames of drawn, empty comb. Colonies were thereafter managed using standard apicultural techniques, (i.e., swarm management, adding and removing supernumerary hive boxes such as brood boxes and medium-sized honey supers, feeding sugar syrup to supplement nectar flow, and installation of robbing screens). Varroa mites, however, were managed according to treatment group assigned. At outset of the study, all colonies were treated with the antibiotic Fumagillin to protect against *Nosema*, a protozoan disease of adult honey bees.

Before we assigned treatment groups, we evaluated various aspects of colony strength. We measured adult worker population by estimating the proportion of each frame's surface area that was covered by adult bees. We then summed these proportions to find the total number of frames of adult bees per colony. Similarly, we estimated the percentage of each comb occupied by immature bees to find the number of frames of brood in each colony, which included all stages of development. Colony weights were measured in the field using a digital scale. During the study, before any equipment was added or removed from a colony, it was weighed to ensure accurate measurement of changes in colony weight.

Initial measurements of mite populations in each colony were also made by taking 24-hour mite drop counts using adhesive sticky boards (Delaplane and Hood 1999, Ostiguy and Sammataro 2000, Sammataro et al. 2002). Sticky boards (Mann Lake Ltd.) consisted of adhesive-coated cardboard, which were placed on the bottom boards of hives for 24 hours. Any varroa mites that fell to the bottom of the hive during that time adhered to the boards and were counted after they were removed. A rubber screen was placed over the board to prevent bees from sticking to it as well. Because mites are more likely to become dislodged during colony disturbance, we did not manipulate the colonies in any way during and for at least 12 hours before sticky boards were inserted to ensure that mite counts were not biased by human activity. For a more accurate evaluation of proportion of bees infested with mites, these counts were also corrected for the total number of adult bees in each colony.

We then assigned three treatment groups to the colonies. To control for position effects within the apiary, the colonies were divided into four blocks of five adjacent colonies, and treatments were randomly assigned within each block. Treatment groups were as follows: Group 1 – Negative control, no preventative treatment for varroa mites; Group 2 – Positive control, Apistan[®] strips (plastic impregnated with fluvalinate) hung between outer frames such that the cluster of bees was located between apistan strips; two strips were applied per brood box, according to label instructions; these were removed and replaced with new strips every 6 weeks; colonies were also treated with Apilife VAR[®] (thymol granules) according to label instructions in late summer; Groups 3 and 4 – drone-brood removal, drone comb frames removed, shaken to remove adult bees, put into “sacrificial” colonies (when the majority of drone brood present was capped), and replaced. To be sure that no group was significantly different from the others at the start of the experiment with respect to pre-treatment measurements made, we performed ANOVAs on the frames of adult bees, frames of brood, colony weight, and 24-hour mite count per frame of bees in each treatment group, and all statistical tests were not significant.

The two colonies in the drone-brood removal group located furthest down the tree line were designated as sacrificial colonies. These colonies were treated with Apistan[®] in the same manner as were those colonies in the positive control group. When drone brood was gathered from other colonies, it was added to these colonies in deep hive body supers added to existing boxes. Drone frames were dated and left for two weeks to ensure that all brood present had emerged. Drone frames were then removed from sacrificial colonies, placed in a freezer at -20°C, and redistributed as replacement frames in other drone-brood removal colonies when needed. All treatments were started in mid-late March, including the application of Apistan[®] strips in the negative control colonies, as well as the removal and replacement of drone frames. Treatments were continued over the course of the spring and summer, and they ended when colonies ceased drone production in early September. We recorded monthly mite counts from each colony during the study using sticky boards (see above) (Delaplane and Hood 1999, Ostiguy and Sammartaro 2000, Sammartaro et al. 2002) and sugar shakes (Fakhimzadeh 2001). Sugar shakes were performed by scraping

approximately 200 bees (presumably nurse bees) from brood frames into a jar fitted with a mesh-screen lid. Powdered sugar was applied to the bees in the jar until they were sufficiently coated with it. The bees were then left for several minutes so that they would distribute the sugar among themselves through their movements. The jar was then inverted and gently shaken over a light-colored surface. Mites dislodged by the powdered sugar then fell through the mesh lid and onto a light-colored surface to be counted. Shaking of the jar continued until no more mites fell from it, after which we returned the bees to the colonies from which they were collected. Monthly estimates of adult bee population were also taken in the manner previously described, which were performed shortly before sticky boards were installed.

Three colonies died during the study. Both sacrificial colonies were gradually weakened throughout the study and ultimately died, as did one colony in the negative control group. Another colony in the drone-brood removal group was removed from the study early on after being diagnosed with European foulbrood. After the cessation of treatments, colony strength was assessed by estimating adult bee and brood populations as previously described. Also, final colony weights were measured and the change in weight was determined for each colony.

Statistical Analysis

Means of pre-treatment measurements of strength were compared among treatment groups using ANOVA to ensure that there were no differences in starting conditions. These measurements included total frames of adult bees, frames of brood, and change in colony weight. Mean 24-hour mite counts from sticky boards were divided by total number of frames covered by adult bees to control for differences in colony strength, then treatment groups were compared by ANOVA to determine whether the mite counts among treatment groups differed significantly in each month of the study. The mean mite counts of treatment groups as measured by sugar shakes were compared to determine whether there was significant difference between groups during any month of the study. Similarly, the mean number of frames covered by adult bees was compared using ANOVA to determine whether

groups differed significantly during any month of the study. Finally, means of post-treatment measurements of colony strength were compared among treatment groups using ANOVA, including total frames of adult bees, frames of brood, and change in colony weight. All post-hoc comparisons were made with Tukey tests, and all means are reported as ± 1 SEM with $\alpha = 0.05$.

Results

There were no significant differences between any of the pretreatment groups at the outset of the study concerning measures of colony strength; they were statistically similar with regard to mean adult bee population ($F = 1.83$, $df = 2, 15$, $P = 0.1942$; Figure 3.1), mean brood population ($F = 0.03$, $df = 2, 15$, $P = 0.9716$; Figure 3.2), and mean colony weight ($F = 0.99$, $df = 2, 15$, $P = 0.3952$). Likewise, they did not differ significantly with regard to mean 24-hour mite drop on sticky boards per frame of adult bees ($F = 0.46$, $df = 2, 15$, $P = 0.6390$; Figure 3.3).

Monthly 24-hour sticky board mite drop counts were adjusted by the number of frames covered by adult bees for each colony to quantify the number of mites per frame of bees (Figure 3.3). During March, April, May, and June, there were no significant differences in the number of mites counted per frame of bees among the different treatment groups (all $P > 0.05$). In July and August, however, drone brood removal (2.82 ± 2.167 ; 8.10 ± 6.940 for July and August respectively) and positive control colonies (1.02 ± 2.564 ; 4.36 ± 8.211 for July and August respectively) had significantly fewer mites per frame of bees than did the negative control group to which no mite treatment was applied (14.90 ± 2.564 ; $F = 8.98$, $df = 2, 14$, $P < 0.01$ for July; 38.69 ± 8.211 ; $F = 5.47$, $df = 2, 14$, $P < 0.05$ for August). September measurements indicated that mean number of mites per frame of bees was significantly higher in both negative control (22.07 ± 5.224) and drone brood removal colonies (17.81 ± 3.949) compared to the positive control group (1.41 ± 4.672 ; $F = 5.28$, $df = 2, 13$, $P < 0.05$).

Mite counts from monthly sugar shake measurements (Figure 3.4) differed significantly between treatment groups in June, July, August, and September. In June, the

negative control group had significantly higher mean mite counts (20.20 ± 2.940) than did the drone brood removal (6.14 ± 2.484) and positive control groups (2.40 ± 2.940 ; $F = 10.43$, $df = 2, 14$, $P < 0.005$). In July, mean mite counts from the negative control group (31.00 ± 5.620) were significantly higher than were those of the positive control group (4.20 ± 5.620), and the mean counts from the drone brood removal colonies were intermediate between the two (14.00 ± 4.750 ; $F = 5.85$ $df = 2, 14$, $P < 0.05$). Mean sugar shake counts in August were significantly higher in the negative control colonies (51.00 ± 8.803) than in the drone brood removal colonies (17.57 ± 7.440), and mite counts in the positive control group were intermediate between the two (31.80 ± 8.803 ; $F = 4.21$ $df = 2, 14$, $P < 0.05$). In September, the negative control group had a significantly higher mean mite count (232.50 ± 54.830) than did the positive control group (4.80 ± 49.041), and mean drone brood removal sugar shake counts were intermediate between the other two groups (128.29 ± 41.448 ; $F = 4.87$, $df = 2, 13$, $P < 0.05$).

Treatment groups did not differ significantly in measures of colony strength at the conclusion of the study in regards to mean frames of brood ($F = 0.29$, $df = 2, 13$, $P = 0.7520$; Figure 3.2) or mean change in colony weight ($F = 0.14$, $df = 2, 13$, $P = 0.8712$; Figure 3.5), nor were they differ significantly at any point during the study in mean frames of adult bees (all $P > 0.05$; Figure 3.1).

Discussion

Results of the study support our hypothesis that this method of drone brood removal provides sufficient varroa mite control to forgo at least one seasonal application of acaricides. During much of the study, colonies undergoing drone brood removal had significantly fewer mites than those without mite treatment (negative control group). This method of drone brood removal maintained varroa populations below the treatment threshold of 10 mites in a sugar shake sample (D. Hopkins, personal communication) until mid-late summer without the application of synthetic acaricides in the spring. Furthermore, the drone population was

preserved and developed in the absence of acaricides.

During the summer months, when varroa mite populations naturally increase (DeGrandi-Hoffman and Curry 2004), those undergoing drone brood removal (with no other mite control) maintained mite populations that were either equivalent to those of colonies treated with synthetic acaricides (positive control group) or were intermediate between the positive and negative control groups. In late summer and early fall, however, the rate of growth of mite populations increased in drone brood removal colonies, leading to mite numbers similar to those of the negative control group. This agrees with previous findings concerning drone brood removal to control mites (Charriere et al. 2003). It is important, therefore, that when this technique is used by beekeepers, they may need to apply a late summer varroa treatment in addition to drone-brood trapping. It is very detrimental to the health of overwintering colonies to have high mite levels during the time that the overwintering worker population is developing (DeGrandi-Hoffman and Curry 2004). For this reason, we do not suggest drone-brood trapping as a stand-alone treatment, and this agrees with the findings of other studies done on drone-brood trapping techniques (Charriere et al. 2003). Monitoring for drone brood can be easily incorporated into existing swarm management monitoring schedules (Calis et al. 1999b).

Results from this study suggest that drone brood removal does not interfere with colony development and has no adverse effect on colony strength. At the conclusion of the study, there were no significant differences between any of the treatment groups with regard to change in colony weight, adult bee population, or brood population. This is also in agreement with previous work (Coffey 2007).

A critical aspect of this drone-brood trapping strategy was that drone frames were removed from their natal colonies when the majority of the brood present was capped. Colonies were monitored and the decision to remove drone frames was made on a colony-by-colony basis. This was done in such a way as to ensure that no drones emerged from the frames within their original colonies, which is important because if they were allowed to emerge, mite numbers would likely increase significantly in those colonies since there would be no pesticides to kill the large numbers of mites emerging with the drones.

The two colonies in this study designated as “sacrificial” colonies, in which drone frames removed from other colonies were banked until all drones had emerged, died during the course of the study. Observations suggested that these colonies were weakened as a result of supporting such a large drone population; they had very low honey stores and relatively small worker populations. They seemed less able to defend against otherwise innocuous pests, such as small hive beetles, which did significant damage to both colonies at various times. Thus, if beekeepers choose to implement this method of drone-brood trapping, they should be prepared for the possibility that the colonies used to bank drones will not produce much, if any, honey and are more likely to die before the end of the season.

In conclusion, drone-brood trapping and banking in sacrificial colonies has much potential for being an effective element of an integrated pest management plan to control varroa mites. It was effective in maintaining low mite levels while preserving the drone population. It has been shown by Sylvester et al. (1999) that drones parasitized by mites do remain reproductively competitive, which indicates that this strategy could bolster mating populations, especially in comparison to drone-brood trapping techniques that destroy all drone brood. This method of mite control would allow beekeepers to manage varroa mite populations in a way that requires only very limited pesticide application, and which allows the potential natural selection of those individuals who are most fit after experiencing parasitism.

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Appendices

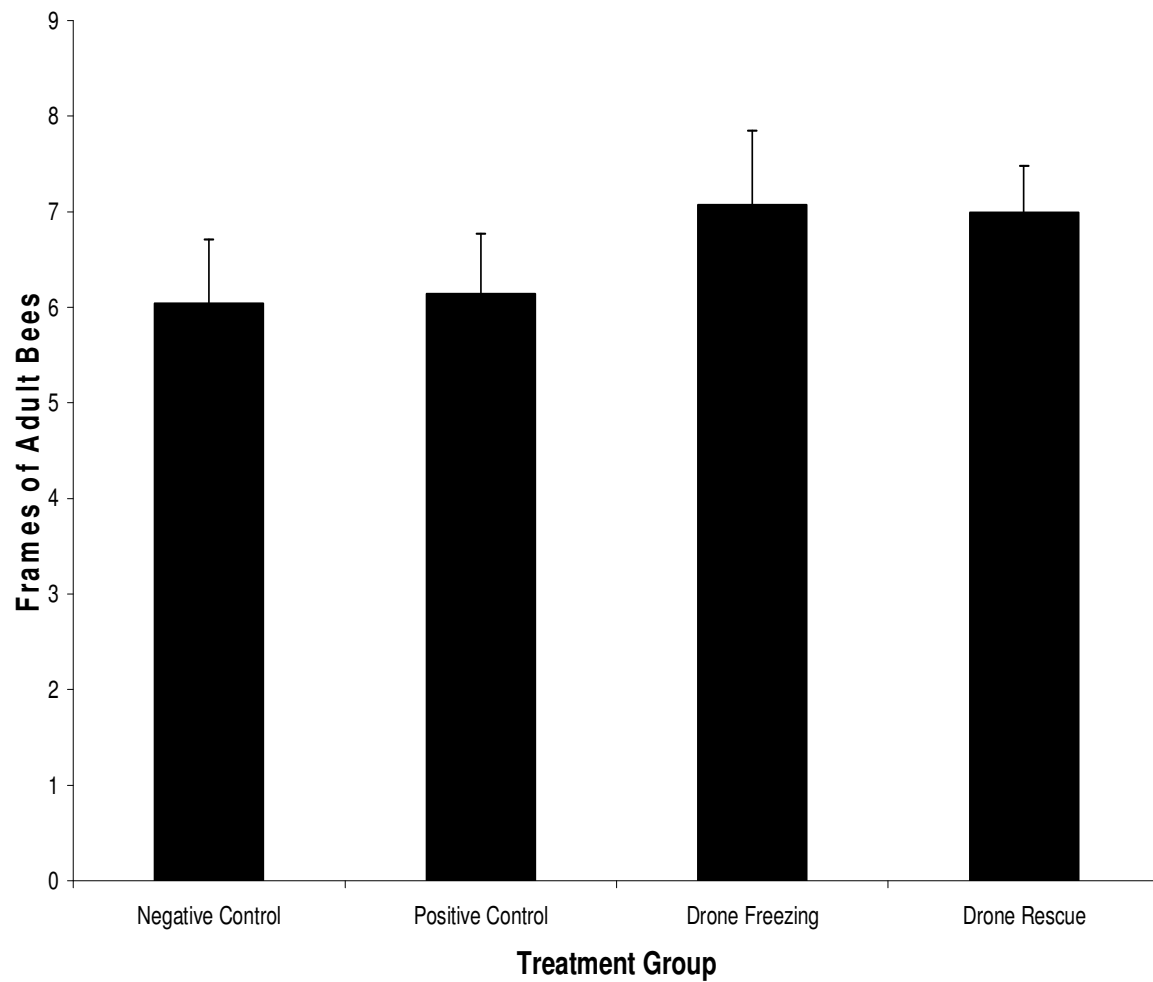


Figure 2.1

Comparisons of frames covered by adult honey bees within treatment groups prior to the start of treatments (mean \pm SE). There were no significant differences among groups. Thus, any effects observed during the study were not a result of any group being biased with regard to adult population at the outset of the study.

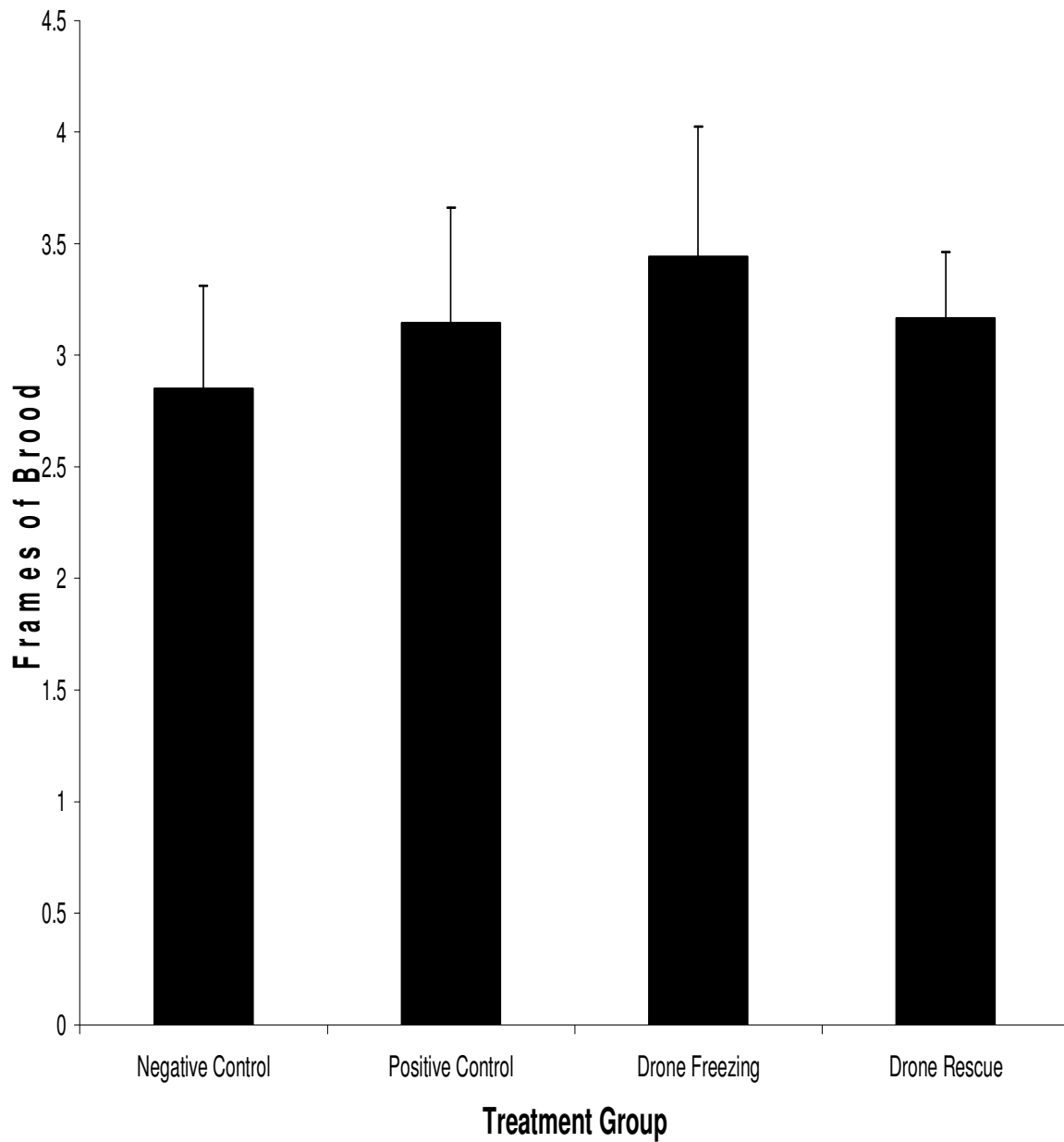


Figure 2.2

Comparisons of frames covered by brood within treatment groups prior to the start of treatments (mean \pm SE). There were no significant differences among groups. Thus, any effects observed during the study were not a result of any group being biased with regard to brood population at the outset of the experiment.

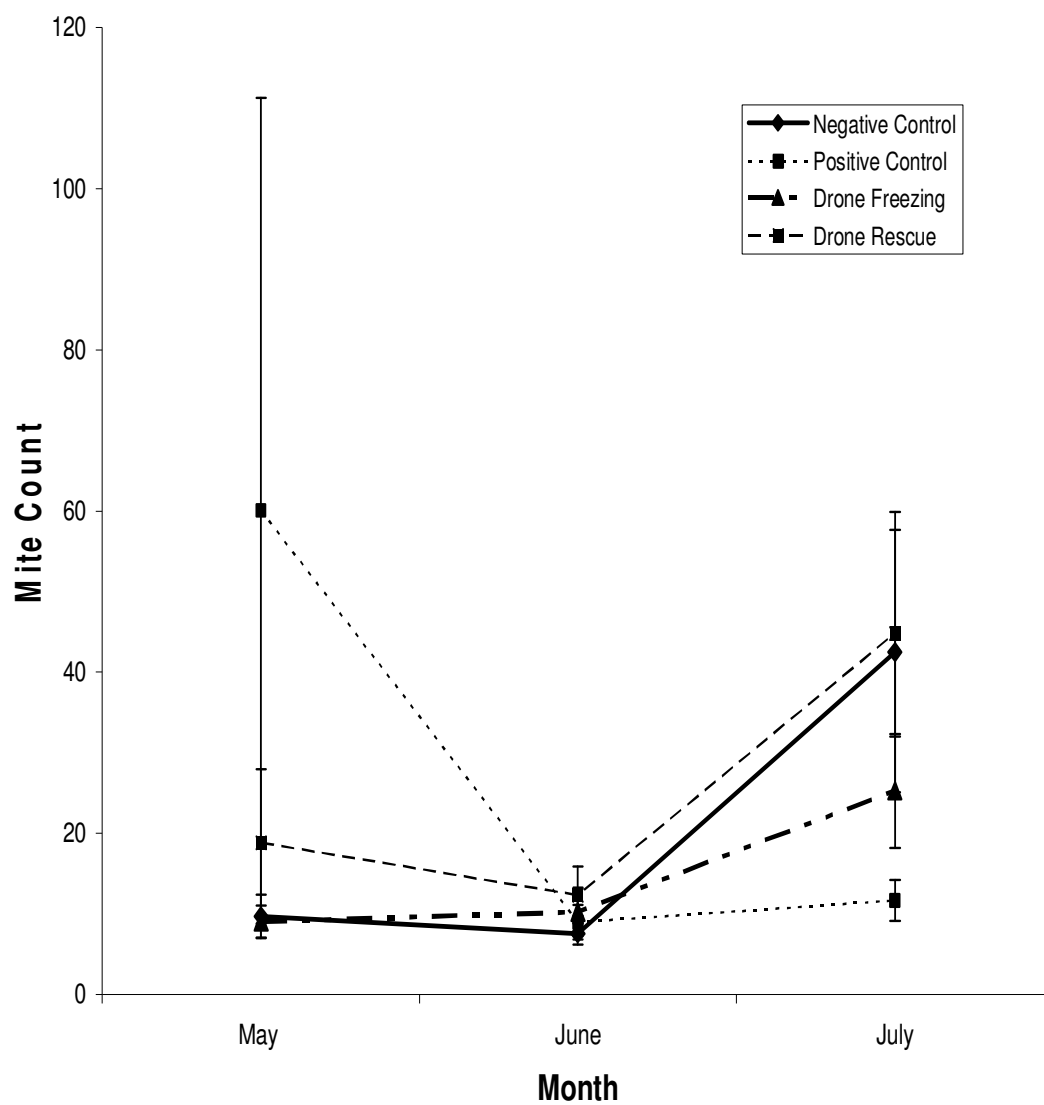


Figure 2.3

Comparisons of 24-hour sticky board mite counts within treatment groups prior to start of treatments and throughout study (mean \pm SE). There were no significant differences between groups.

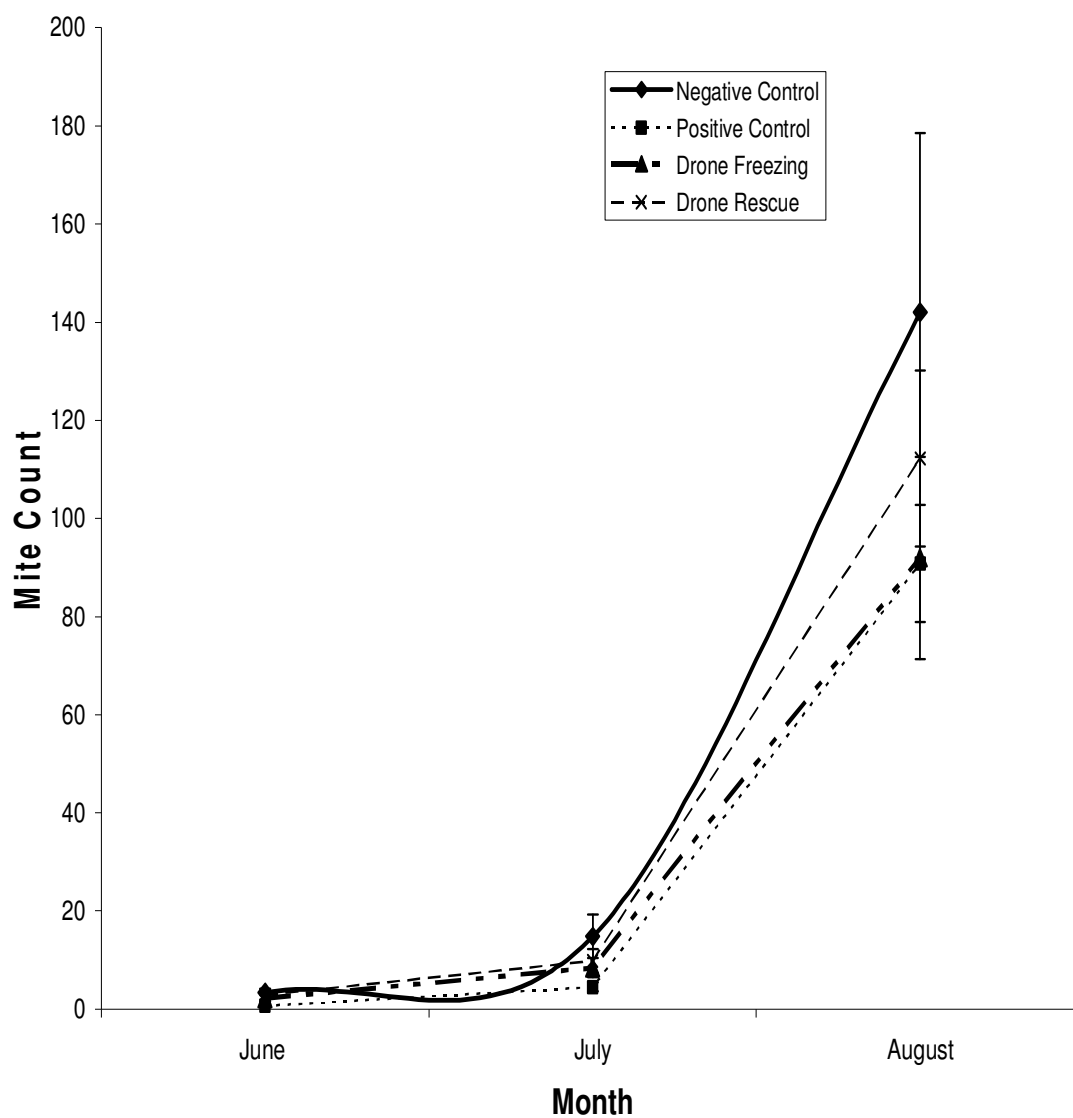


Figure 2.4

Comparisons of sugar shake mite counts within treatment groups during the study (mean \pm SE). There were no significant differences between treatment groups, however the drone-brood rescue group was numerically intermediate between the positive and negative control groups. The drone brood removal and freezing group was numerically similar to the positive control group.

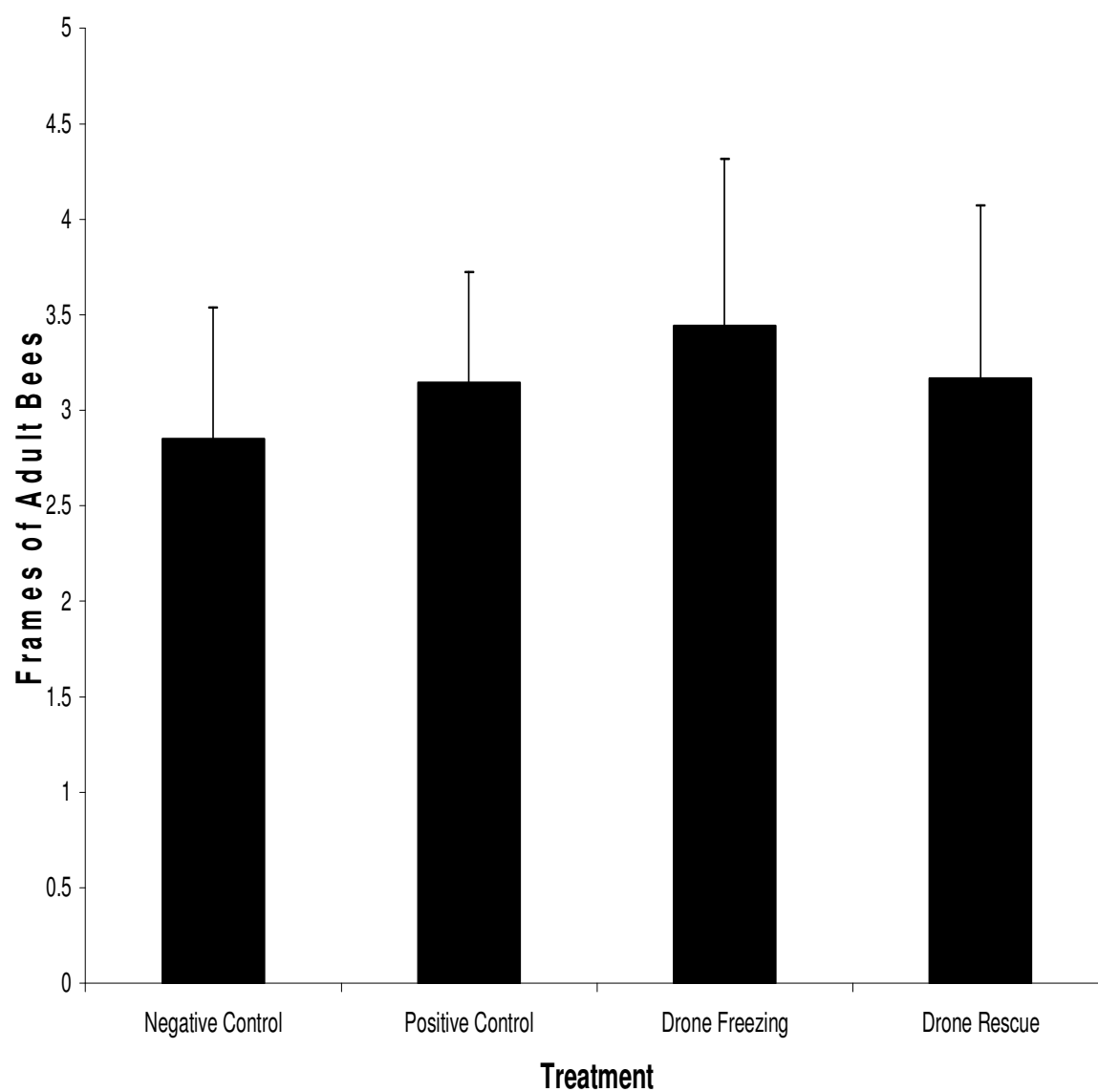


Figure 2.5

Comparisons of frames covered by adult bees within treatment groups at conclusion of study (mean \pm SE). There were no significant differences between groups, indicating that treatments applied did not compromise colony strength as relates to adult bee population.

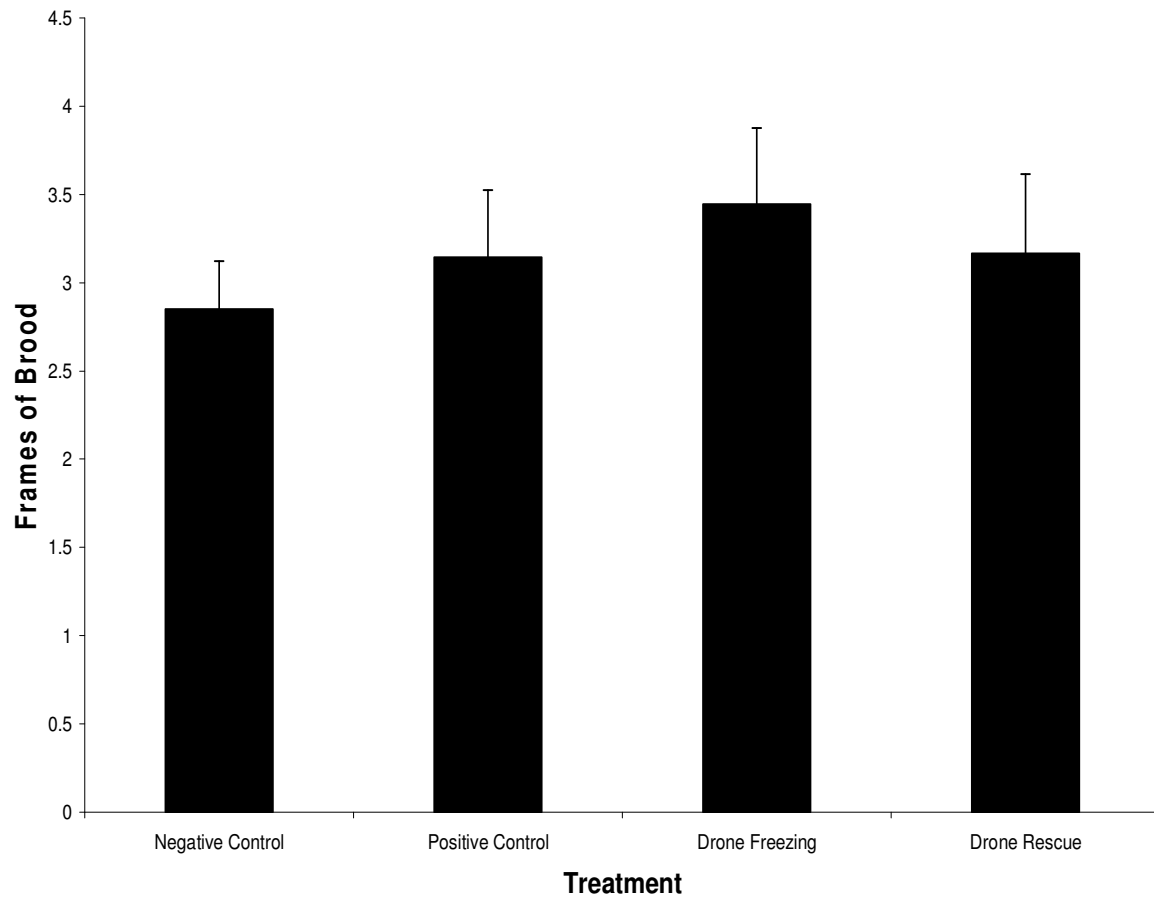


Figure 2.6

Comparisons of frames covered by brood within treatment groups at conclusion of study (mean \pm SE). There were no significant differences between groups, indicating that treatments applied did not affect colony strength as relates to brood production.

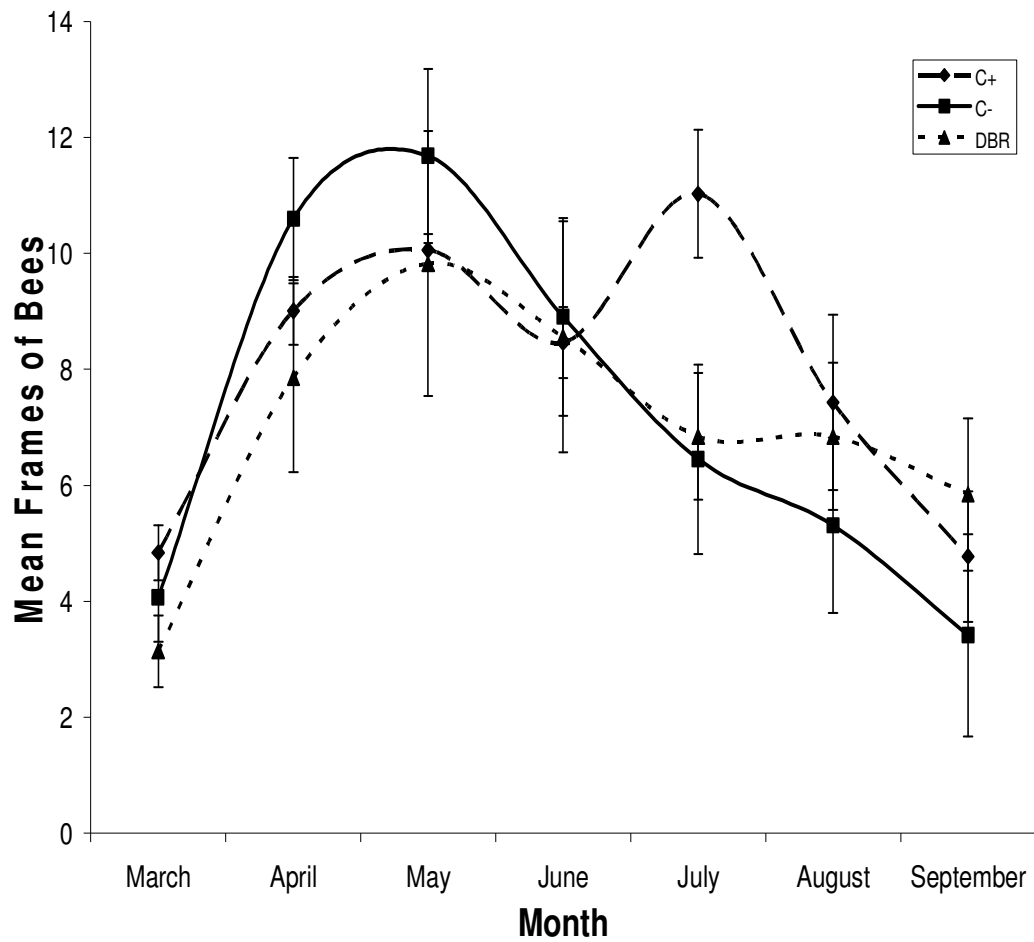


Figure 3.1

Comparisons of adult bee population in positive control, negative control, and drone brood removal groups (C+, C-, and DBR, respectively) measured by visually estimating total frames covered by adult bees in each colony (mean \pm SE). No groups differed significantly at the outset, during, or at the conclusion of the study. This indicates 1) that any effects seen during the experiment were not a result of unequal adult populations before treatment was applied, and 2) that no treatment applied was detrimental to the adult population of colonies.

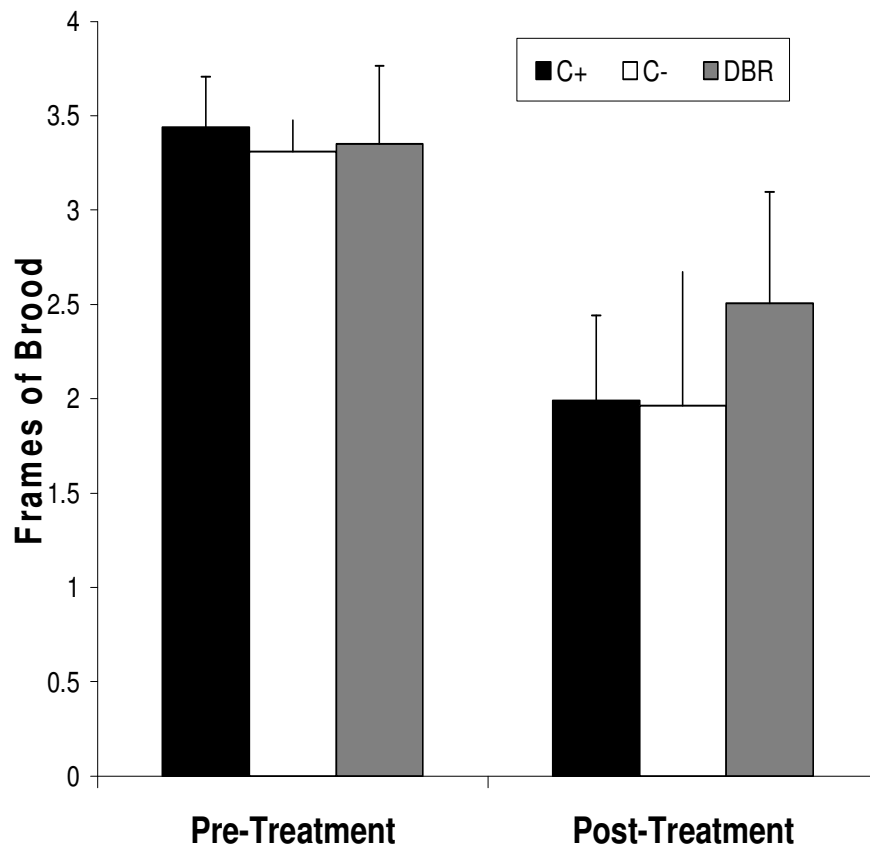


Figure 3.2

Comparisons of frames covered by brood in positive control, negative control, and drone brood removal colonies (C+, C-, and DBR, respectively) prior to the application of treatments and at the conclusion of the study (mean \pm SE). No significant differences were observed on either occasion, indicating 1) that effects observed during the experiment were not affected by unequal brood populations between treatment groups at the outset, and 2) that none of the treatments applied had detrimental effects on brood population.

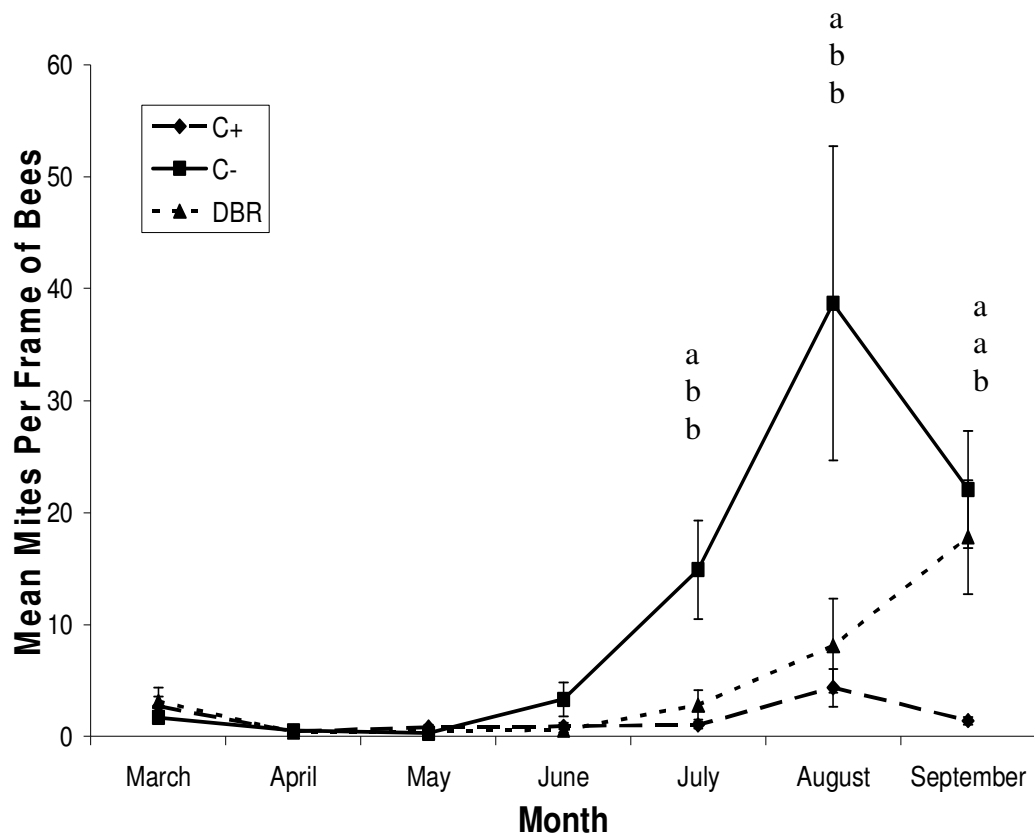


Figure 3.3

Comparisons of sticky board mite counts measuring 24-hour mite counts per frame of bees in each treatment group (positive control (C+), negative control (C-), and drone brood removal (DBR)) during each month of the study (mean \pm SE). During the months of July and August, mite counts in the C- group were significantly higher than were those of the C+ or DBR groups. In September, C- and DBR groups had significantly higher counts than did the C+ group.

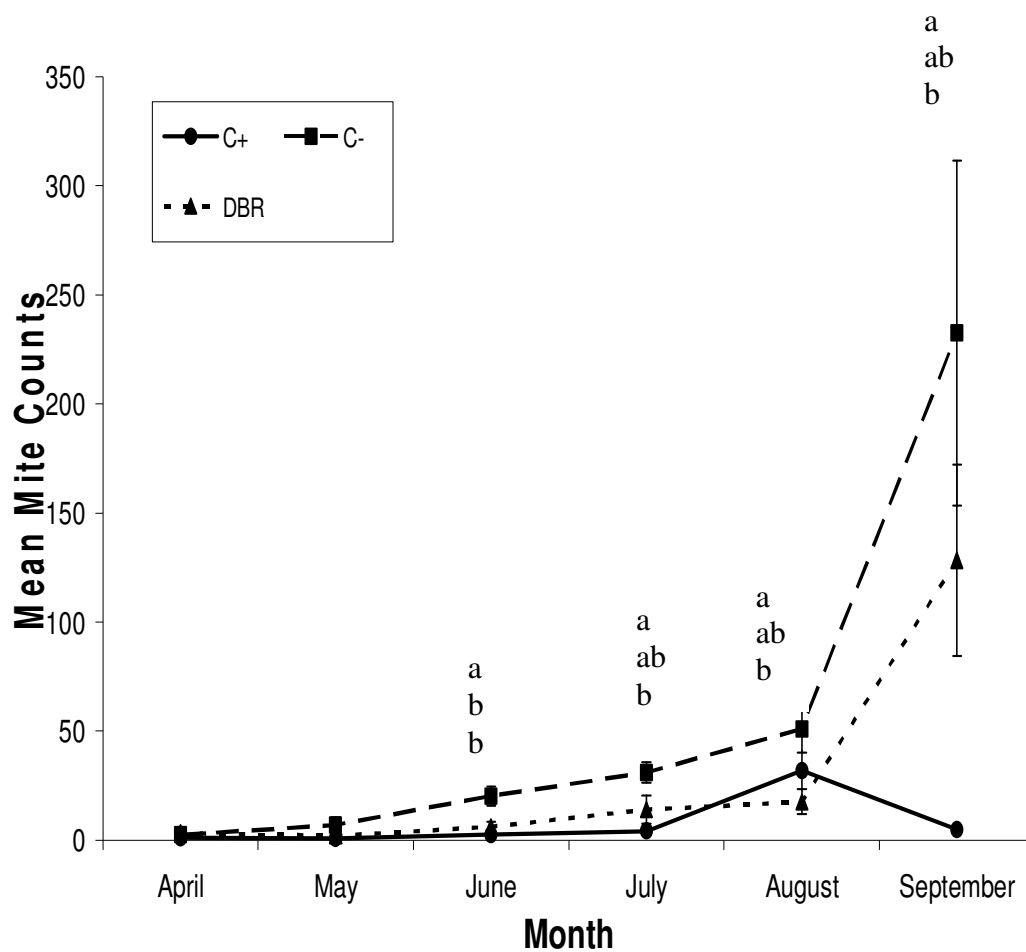


Figure 3.4

Comparisons of sugar shake mite counts in positive control, negative control, and drone brood removal groups (C+, C-, and DBR, respectively) during each month of the experiment (mean \pm SE). In June, the C- group had significantly higher mite counts than did the C+ or DBR group. In July, the DBR group was intermediate between the C- and C+ groups. In August, the C- group had significantly higher mite counts than the DBR group, and C+ group was intermediate between then two. In September, C- and DBR groups had significantly higher sugar shake counts than did the C+ group.

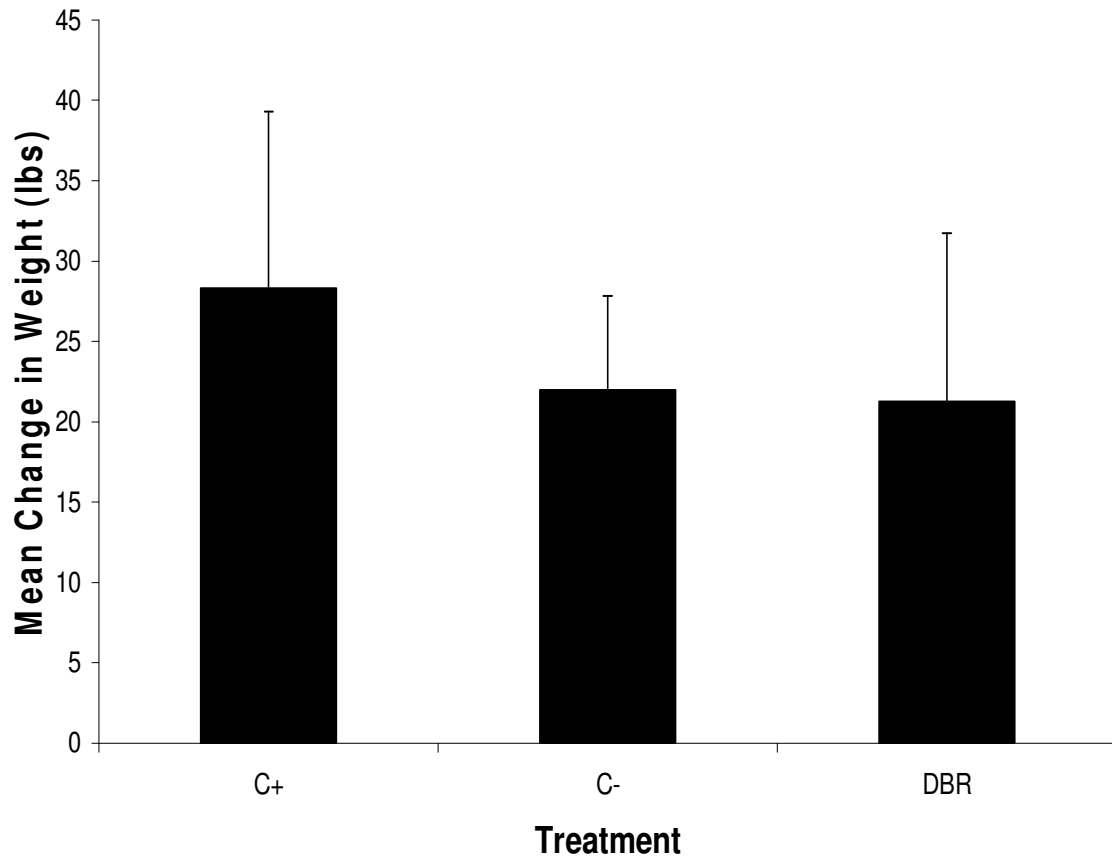


Figure 3.5

Comparisons of change in colony weight over the course of the study in positive control, negative control, and drone brood removal groups (C+, C-, and DBR, respectively; mean \pm SE). No significant differences were observed in changes in colony weight, indicating that treatments did not have harmful effects on colony productivity.