# Development of a Precision Mite Management Program for the Control of the Ectoparasite *Varroa destructor* in Hives of *Apis mellifera* L.

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# Development of a Precision Mite Management Program for the Control of the Ectoparasite *Varroa destructor* in Hives of *Apis mellifera*

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## Abstract

The European honey bee, *Apis mellifera*, is an important pollinator of horticultural and agricultural field crops, providing ≈ 90% of all commercial pollination services (Genersch et al. 2010). The recent rise in colony loss due to Colony Collapse Disorder (CCD) has been a source of concern for both beekeepers and the apiculture industry. One of the factors implicated in CCD is infestation by the ectoparasitic mite, *Varroa destructor*. Initial efforts to control the mite relied heavily on regular application of miticides without regard to actual mite infestation levels. This approach has led to problems of resistance in the mite and contamination of the hive and hive-products. Because it is unlikely that miticides will be removed as an option for mite management, a precision mite management (PMM) approach using information on the spatiotemporal distribution of the mite to improve sampling and treatments is seen as a viable option, particularly with respect to treatment costs and impacts on the environment. The primary objective of this study was to develop an understanding of the spatiotemporal distribution of the Varroa mite and bee brood within hives for the purpose of developing a PMM approach for the mite.

Varroa mite populations were sampled from May to June, 2012 and February to October, 2013. Sampling was conducted with three commonly used sampling methods: soapy water roll (SWR), brood uncapping, and a modified sticky board; brood uncapping, however, was discontinued during the study due to hive the labor cost and harmful effects of this method to the hives. Similar trends in mite population levels were observed using the soapy water roll and sticky board sampling methods. Spearman's nonparametric analysis showed that there was a significant correlation ($\rho = 0.47$, $P<0.001$) in mite population levels for the soapy water roll and sticky board methods for sampling conducted from February to September, 2013 (the SWR method was not used in October). This was despite the fact that there was no significant correlation ($\rho = -0.03$, $P = 0.8548$) between the two sampling methods during the spring sampling period from February to April, 2013. The observed lack of correlation between the two sampling methods in early spring was likely due to the low population of brood in the hive, which caused the majority of the mites to remain on adult bees. Mites per 100 adult bees, therefore, appear to reflect mite population levels within the hive more closely than mite fall on sticky borad during the February to April sampling period. This suggests that the soapy water roll method is a better method for estimating mite population levels within the hive in the early spring compared with the sticky board method.

Geospatial analyses of the distributions of mite fall on the sticky boards were conducted using geostatistics and **S**patial **A**nalysis by **D**istance **I**ndic**E**s (SADIE). Both analyses showed that mite fall on the sticky board was generally aggregated and the aggregation increased with mite population levels. The average range of the variogram from geostatistical analysis was estimated at 4 sticky board cells; this range value was increased to 5 cells and was used to develop a systematic outside-range sampling protocol for mites on a sticky board. The results showed that the accuracy of the systematic outside-range sampling compared well with that of the traditional sticky board counting method in estimating total mite fall, but required only 60% of the effort (i.e., counting 63 instead of 105 cells).

SADIE analysis showed that there is an overall association between the distribution of mite fall on a sticky board and the distribution of brood within a hive. A greater degree of correspondence was also observed in the association of drone and mite distributions during May to June; greater correspondence in worker brood and mite associations was observed in August and September. These differences may be due to relative amounts of the two types of brood present within the hive. A test of the efficacy of precision application of Varroa mite treatment based on the association between drone brood and mite fall resulted in a significantly greater reduction in mite levels on the sticky board using a traditional miticide treatment method compared with the control and precision treatments ($\chi$2 =362.571; df = 2; P <0.0001); mite population levels with the precision method, however, were significantly reduced compared with the control.

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# CHAPTER 1

# Introduction and Literature Review

## 1.1 Introduction

The European honey bee, *Apis mellifera* L., is the most common species of commercialized bees in North American and is also one of the most important pollinators providing pollination services for ≈33% of food crops, worldwide (vanEngelsdorp and Meixner 2010). The introduction of the parasitic mites, *Acarapis woodi* and *Varroa destructor* into the United States has had a profound effect on populations of the honey bee and the beekeeping industry (Krause and Page 1995; Sammataro et al. 2000). The tracheal mite, *A. woodi*, is an internal parasite of the honey bee, which lives and reproduces in the respiratory passages (tracheae) of the adult bee causing a complex of disease conditions leading to significantly increased colony mortality (Wilson et al. 1997). The Varroa mite on the other hand is an external parasite that damages both immature and adult bees by feeding on the hemolymph. Feeding activities of both mites reduce the health of bees, shorten their lifespan and make the colony more susceptible to diseases (De Jong 1997). Both mites are also vectors for several viral pathogens.

In Virginia, annual honey bee colony losses have averaged close to 30% over the past decade since the introduction of the mites with the number of beekeeper colonies decreasing by ≈50% from 78,000 in 1985, the year the tracheal mite was first discovered in the state, to <35,000 in 1996 (Fell and Cobb 2009). At the same time the number of feral colonies, originally estimated at ≈80,000, declined to almost zero. Initial efforts to control infestation of both mite species focused on the use of chemical pesticides (Herbert et al. 1988, Witherell and Herbert 1988, Hoppe et al. 1989, Wilson et al. 1997, De Jong 1997). The high colony losses and serious consequences of mite infestations have led to the widespread use of chemical miticides such as *tau*-fluvalinate and coumaphos in managed hives. Not surprisingly, these miticides are the most commonly identified pesticide residues from honey bee colonies in N. America (Mullin et al. 2010). Beekeepers frequently treat their colonies on a regular basis with one of the two miticides, often without regard to actual mite infestation levels.

The excessive reliance on chemical controls has led to a number of problems, including the development of resistance in Varroa mite populations (Eischen 1995, Elzen et al. 1999) and numerous colony health problems. Evidence suggests that the use of miticides can have deleterious effects on the reproductive physiology of honey bees, reducing both the ability of colonies to raise queens, as well as the ability of drones to produce sperm (Fell and Tignor 2001, Haarmann et al. 2002). Sub-lethal effects from miticide use may also contribute to the increased problems of queen failure and colony loss (Burley et al. 2008). These problems are compounded by the potential for honey and wax contamination (Bogdanov 2006). Both *tau*-fluvalinate and coumaphos are lipid soluble and can be absorbed in hive products such as beeswax (Wallner and Fries 2003); both chemicals also have an estimated half-life of five years (Bogdanov 2006). Although a study by Fell and Cobb (2009) found that most Virginia honey samples are free from detectable miticide residues (<0.02 ppm), the continued reliance on the use of miticides increases the likelihood of honey contamination and human exposure. As such, the potential problems associated with miticide use point to the need for better management practices and reduction in the use of these chemicals.

A number of alternative approaches to Varroa mite control have been developed and include both hive and management modifications (screen bottom boards, drone brood removal, sugar dusting), the use of essential oils and organic acids (thymol, formic acid), and the use of bio-pesticides (sucracide) and biological control agents (entomopathogenic fungi). Efforts to select bees showing a lowered susceptibility to mites have also led to the release of mite-resistant and hygienic bee lines (Spivak 1996, Rinderer et al. 2001, Harbo and Harris 2005). The use of these alternate approaches has been adopted by many beekeepers; however serious drawbacks, such as high labor cost and poor overall efficacy, have hindered their widespread use (Rosenkranz et al. 2010).

The development of effective strategies for the management of Varroa mites requires an understanding of the biology, behavior, and ecology of the parasite. Research to date has provided knowledge of such factors as the mite development cycle, reproduction, feeding behavior and their ability to vector disease organisms. However, one area in which relatively little is known is the distribution of the mite within hives. We know that mites show a strong preference for drone cells for reproduction, and that the majority of mites in a hive at any one time are found in brood cells (Fuchs 1990). However, we do not have a good understanding of the spatiotemporal distribution of mites in relation to brood, and drone brood in particular.

Because the need to use pesticides to control mite populations within hives will undoubtedly always be present, a precision mite management (PMM) approach appears to be a viable option for keeping mite population in check and reducing pesticide loads and residue levels within hives. With PMM, miticides can still be applied but only when and where they are needed. However, for PMM to be used successfully an understanding of the spatiotemporal distribution of the mite is required along with the ability to manipulate and predict the distribution of this parasite. The purpose of the current study is to characterize the spatiotemporal distributions of the Varroa mite and bee brood, to measure the strength of their spatiotemporal relationship, and to determine whether knowledge of these relationships can be used for PMM.

However, before doing so an extensive review of the literature on both the honey bee and Varroa mite is provided.

## 1.2 European Honey Bee – *Apis mellifera*

### 1.2.1 History

Honey bees appeared during the early Cenozoic era (65.5 Myr to the present), particularly with the Miocene epoch, evolving the eusocial and thermoregulatory characteristics that would allow colonies to survive in a range of habitats (Ruijter 1987). There are now nine species of honey bee, the two most common being *A. mellifera* and *A. cerana*, the Asian honey bee. *A. mellifera* evolved around 1.25 Myr in Asia, eventually evolving into subspecies in the Middle East, some of which entered Africa while others, e.g., *A. mellifera mellifera* and *A. m. carnica*, invaded Europe (Garnery et al. 1992). From here humans took over, spreading *A. mellifera* throughout the world, from Australia to Chile (Lazar et al. 2006). Because of the high human population density in Europe there are very few wild populations of honey bees still in existence, with nearly all beehives tended by beekeepers (Büchler et al. 2010). As a result the economically important subspecies have flourished, while native subspecies, such as *A. m. macedonica* and *A. m. siciliana*, have gone extinct (Meixner et al. 2010) drastically reducing genetic variation in European bee colonies.

The modern honey bee used for mass pollination and honey production has been shaped over the years through selective breeding, ignoring important aspects such as disease resistance (replaced with synthetic antibiotics) and integration into the local ecosystem (Büchler et al. 2010). Instead traits such as low aggression, high yield and reduction of swarming were the focus of most breeding. Currently, with the emergence of pests such as *Nosema* spp., *Varroa* *destructor*, and a wide variety of pathogens infecting colonies, honey bee populations are struggling, impacting both natural ecosystems and the worldwide agricultural industry.

### 1.2.2 Economic Importance

Many angiosperms rely on the services provided by pollinators, allowing rapid and far reaching dispersal of their genetic material. Economically, insect pollination is valued at an estimated \$251.7 billion for worldwide agriculture and ~\$23.7 billion annually for North American agriculture (Gallai et al. 2007). Of the many pollinating insects, the European honey bee *A. mellifera* is by far the most important, acting as the primary pollinator for many crops, including cashew, which is valued at around \$280 million in Brazil alone (Kevan and Wojcik 2010), almond, which brings in over \$2 billion in California (Ratnieks and Carreck 2010), and cotton, which can have 20–30% higher yields in the presence of honey bees (Thomazoni et al. 2009). In fact, without the pollination services provided by honey bees crops such as almond, apple, asparagus, broccoli, Brussels sprouts, cabbage, cauliflower and honeydew would have crop yields reduced by ≥ 90% (Southwick and Southwick 1992). Overall, *A. mellifera* provides pollination services for over 90% of commercial agricultural crops, making them the world's most important pollinator (Genersch et al. 2010). Honey bees also provide hive products such as wax and honey. Wax is used for a variety of purposes, from candles to skin care products, while honey is primarily used as a sweetener; in 2007, honey was valued at around \$1.25 billion annually worldwide (vanEngelsdorp and Meixner 2010).

As agriculture and worldwide human population have boomed, so too has the demand for the pollination services of *A. mellifera*. In the last 50 years agriculture dependent on honey bee pollination has risen by more than 300%, and while commercial hives have increased by around

45% in the same timeframe, pollination enterprises are struggling to keep up with demand (Aizen and Harder 2009). A huge contributor to this problem is the recent increase in colony loss, coined "Colony Collapse Disorder" or CCD. Colony Collapse Disorder has been the cause of much concern to apiculture, agriculture and the general public. The true "cause" of CCD is an area of much contention, with over 60 different factors implicated as possibly contributing to the disorder (vanEngelsdorp et al. 2009). However, pathogens and diseases, many of which are transmitted by the ectoparasite *Varroa destructor*, are frequently viewed as the greatest contributor to the phenomenon and the most serious stressor of *A. mellifera* (Denmark et al. 1991; Ratnieks and Carreck 2010).

### 1.2.3 Taxonomy and Morphology

Kingdom: Animalia
  Phylum: Arthropoda
    Class: Insecta
      Order: Hymenoptera
        Family: Apidae
          Genus: *Apis*
            Species: *A. mellifera*

**Fig. 1.1:** A representative member of the species *Apis mellifera*, feeding on a wildflower. Image: David Cappaert, Michigan State University, Bugwood.org

Bees have all of the standard morphological characteristics of Hymenoptera; two sets of wings, which have interlocking hamuli during flight, large, compound eyes and tripartite ocelli. Because they are pollinators, bees have specialized plumose hairs covering the body, which provide sensory information, serve as a protective covering, and aid in the collection of pollen

(Snodgrass 1956). Hairs differ in function based on their location on the bee; for instance, on the legs there are antennae cleaners, wax spines and pollen basket arrays. The antennae cleaners are depressions full of sturdy hairs found on the forelegs that aid in the general upkeep of the antennae (Snodgrass 1956). Wax spines are modified hairs found on the middle pair of legs and are used for the movement of wax material produced in the abdomen to the mouth, where they can be manipulated into the comb or mixed with plant resin to form propolis (Kritsky 2010). The hind legs contain several modifications for the collection of pollen, including the auricle, rake, comb and pollen basket. These baskets, also called corbicula, are on the tibia of the hind legs and the hairs in the corbicula are arranged so as to facilitate the transportation of pollen collected from flowers (Snodgrass 1956). The comb collects pollen with specialized hair patterning, and the rake and auricle, which are protrusions from the leg above the comb, compact the pollen into the corbicula.

Pollen is transported in the corbicula back to the hive, where it is incorporated into a number of honey bee products, namely glandular brood food and bee bread (Kritsky 2010). Bees are able to manipulate the materials in their hive through specialized mouth parts. Rather than the slicing/chewing mouth parts of the predatory Vespidae, *A. mellifera* workers have lapping / chewing, which means they can both lick up liquids and use their shovel-like mandibles for scooping and smoothing out wax and propolis (Snodgrass 1956).

From these products bees acquire a range of necessary nutrients. Honey provides carbohydrates, while nectar provides water and sugar. However, nutrients necessary for survival come from pollen, such as many vitamins, proteins, fats, minerals and amino acids (Snodgrass 1956). Pollen can have a wide range of these nutrients, depending on the plant from which it originated, as well as climate, geography and season. Propolis is also collected from many types of plants, and its constituents can vary greatly between hives. Because of the geographic differences in pollen and propolis ingredients, bees which are transported across the country for pollination services may have a weakend ability to learn an area and collect the best pollen and propolis, though such a possibility has yet to be explored.

### 1.2.4 Social Organization

The caste system is one of the major defining characteristics of eusocial insects such as the honey bee; it allows for the efficient partitioning of resources, duties and time. By working together in a caste system, bees are able to accomplish multiple tasks concurrently. Caste is achieved not through genetic predetermination but primarily through diet (i.e., the feeding of royal jelly) and developmental cell size (Flanders 1960; Shi et al. 2011). The hive is made up of males, called drones, and two female casts: the queen, of whom there is usually only one, and workers, which can number in the tens of thousands. The primarily role of drones is to reproduce with queens from other hives and their population fluctuates greatly by season. The two female castes and the males all have differing developmental time periods, with workers taking an average of 280 hours (~12 days) to develop post cell capping, while drones take ~350 hours (~14.5 days) and queens take ~190 hours (~8 days) (Rosenkranz and Engles 1994). The queen is the only female in the colony with active ovaries; she uses pheromones to suppress worker ovary development, thereby limiting reproduction within the hive. While genetically similar, the queen is drastically dissimilar from workers, living a much longer life and having many unique physiological characteristics (Shi et al. 2011). Whether a larva develops into a worker or a queen is dependent upon the feeding of royal jelly, as any worker larva can become a queen if fed royal jelly during development, if feeding is initiated within the first three days of larval development (Flanders 1960).

Recent work has shown that the active ingredient in royal jelly responsible for the differentiation between worker and queen honey bee development is the protein royalactin (Kamakura 2011). Royalactin was found to induce a cascade of gene upregulation and protein and hormone (e.g. juvenile hormone) synethesis to shorten developmental time, increase body size and increase fecundity in honey bee larvae. These results were reproduced in larvae of *Drosophila melanogaster*, raising implications for royalactin's cross-species use (Kamakura 2011).

Honey bees are haploid-diploid, and therefore males, or drones, are produced through the lack of fertilization while the egg is in the oviduct (Flanders 1960). Drones serve the one purpose of inseminating queens from other colonies; therefore, they perform no important tasks within the hive, and the colony will cease rearing drones in the late summer, eventually ejecting the remaining males in the fall. Due to their lack of a supporting role inside of the hive, drones are more of a luxury for a hive and are typically raised in the spring after a substantial worker force has developed and food stores are at a level which can allow for the presence of drone brood. Larger hives will therefore typically have a higher drone population than smaller hives (around 5–7% of the total population) and drone cells are more likely to be found on the periphery of brood comb (Seeley and Morse 1976). The raising of drones occurs before virgin queens are reared, around four weeks before the peak swarming period in spring and again in late summer, so that mating aggregations can form in advance of queen arrival (Winston 1987). Drone comb cells are larger than those of worker bees and make up around 17–20% of the total comb area. Only occasionally do these cells actually contain drone brood since they are often empty or filled with food (Seeley and Morse 1976).

A drone will devote the majority of his life to finding a queen, which he does in mating aggregation areas that attract thousands of drones. Queens are attracted to these areas, which may be used for multiple years by hundreds of thousands of drones, though little is known about how drones and queens know to return to these same areas year after year. Males will make several mating trips each day after they have become sexually mature (~ two weeks) and a flight will usually last around half an hour (Winston 1987). As the queen flies within a drone congregation area she will leave a trail of queen mandibular pheromone, which drones follow aggressively. As soon as a drone mates with a queen his genitalia will detach and remain inside the queen, causing his death and helping to insure the successful implantation of his sperm into her spermatheca. A queen will typically mate with around ten to fifteen drones in her life, which can take multiple mating flights, but can occur in her first flight.

## 1.3. The Varroa Mite - *Varroa destructor*

### 1.3.1 Economic Importance

*V. destructor* is the most important pest of *A. mellifera*, particularly due to its pathogenic characteristics, ubiquity and economic impacts. In New Zealand alone Varroa mites cause an economic loss of between $400 million to $900 million annually (Zhang 2000). In addition, the German Bee Monitoring Project, a multiyear study involving over 1200 hives, noted *V. destructor* and two of the viruses it vectors were closely associated with colony collapse (Genersch et al. 2010). A serious mite infestation can lead to reductions in honey production and pollination, and the complete loss of colonies. An infestation, left unchecked, can cause the death of a colony in as little as three years, though in areas of ideal temperature for mite growth, such as Florida, this can be shortened to seven months (Denmark et al. 1991). Hive losses have been attributed to *V. destructor* throughout the world, from North America to Israel where the feral bee populations are nearly extinct from Varroa infestations (Soroker et al. 2011).

*A. mellifera* is so easily devastated by *V. destructor* due to its lack of any sort of co-evolution with the mites, which is why *A. cerana*, which evolved alongside *V. destructor*, shows few serious symptoms when in presence of mites (Le Conte et al. 2010). Many of the pathogens transferred by Varroa mites have been found in pollen, and there is concern that *A. mellifera* could transfer viruses into other pollinator species (Singh et al. 2010).

One of the more pressing issues, in both the media and academia, stemming from the recent worldwide spread of *V. destructor*, is what role the mite plays in Colony Collapse Disorder (CCD). CCD is characterized by a sudden loss of adult bees (alive or otherwise), even in the dead of winter, often while food stores and brood are still present in the hive. Frequently, the hive will have few signs of disease, at levels not sufficient to kill a healthy colony (vanEngelsdorp et al. 2009). Some authors (Cox-Foster et al. 2007, vanEngelsdorp et al. 2009) mentioned an unusual lag before wax-feeding pests and robber bees enter the hive as another defining symptom of CCD, yet to date this has not been scientifically tested or recorded.

Currently there is little conclusive evidence to claim that Varroa mites are the leading cause of CCD (Le Conte et al. 2010). They are, however, present in nearly every hive and transmit 15 serious pathogens, some of them having been blamed for CCD on their own, making the Varroa mite a logical culprit (Neumann and Carreck 2010). In addition, many beekeepers blame pesticides for the symptoms of CCD, and the two most common pesticides found in beehives, coumaphos and *tau*-fluvalinate, are placed in the hive for the control of Varroa mites.

However, one of those chemicals, coumaphos, has recently been found to be more prevalent in non-CCD hives, making it an unlikely direct cause of CCD (vanEngelsdorp et al. 2009).

The term “Colony Collapse Disorder” was first coined in the US during the spring of 2007, when overwintering hives failed at an alarming rate (Le Conte et al. 2010). At the time, Varroa mites had not yet reached the Economic Injury Level for a pest of *A. mellifera*, bringing their influence over CCD into question (vanEngelsdorp et al. 2009). Nevertheless, it has been shown that colonies undergoing CCD have a significantly higher number of pathogens present in the hive than control hives, many of which are vectored by Varroa mites (vanEngelsdorp et al. 2009). The presence of these pathogens has yet to be shown to be the cause of CCD, and the possibility exists that their presence is a symptom of reduced immune response due to some other, as yet unknown, factor. However, one of the more damaging honey bee viruses, which has been implicated in CCD (Cox-Foster et al. 2007), Israeli Acute Paralysis Virus (IAPV), is also vectored by *Varroa destructor* (Prisco et al. 2011).

CCD has been reported in the US, Europe, the Middle East and Japan, making it a worldwide phenomenon, though seemingly only in the Middle to Northern hemisphere (Neumann and Carreck 2010; Fig.2). Cox-Foster et al (2007) calculated that some U.S. beekeepers experienced a 50 to 90% loss of colonies due to CCD. However, this is not the first appearance of drastic colony losses. In 1906 a spate of hive deaths on the Isle of Wight brought a great deal of media and scientific attention to colony loss, much as CCD has today. Years later (in 1921) the stumbling, flightless bees were diagnosed as suffering from *Acarapis woodi*, a tracheal mite, and much effort was undertaken to control the pest (Neumann and Carreck 2010). Although *A. woodi* is an important pest of the European honey bee, recent studies have shown that it was likely not the major cause of the 1906 collapse (Neumann and Carreck 2010). Rather the true culprit was most likely chronic bee paralysis virus, still a major pathogen of *Apis mellifera* today and one readily vectored by *V. destructor* (Kevan et al. 2006; Neumann and Carreck 2010).

While the losses experienced by beekeepers on the Isle of Wight in 1906 may have symptoms characteristic of chronic bee paralysis virus, there have been other losses in the past with symptoms more akin to those of CCD. Throughout the mid-late 1800’s hives were found in a state similar to that of absconded hives, with few bees, either alive or dead, however the queen was still present and there were ample food stores (Kulincevic et al. 1984). In 1973 beekeepers in 27 of the 50 United States reported sudden losses of around 17%, with those hives undergoing extreme dwindling of bee populations, though with ample food stores and very few dead bees present (Wilson and Menapace 1979). Beekeepers reported that 1973 was the peak of a steadily increasing rate of hive losses beginning in 1963, and samples of bees obtained by Wilson and Menapace showed a lack of any known harmful pathogens or parasites. At the time, the condition was referred to as “autumn collapse” or “disappearing disease” (Wilson and Menapace 1979). The cause of the sudden colony loss was just as mysterious then as it is now, though studies showed that the condition was not transferable, and was not caused by poor nutrition (Oertel 1965, Kauffeld et al. 1976). The true cause of disappearing disease, as with CCD today, may come from a combination of factors, rather than one factor alone.

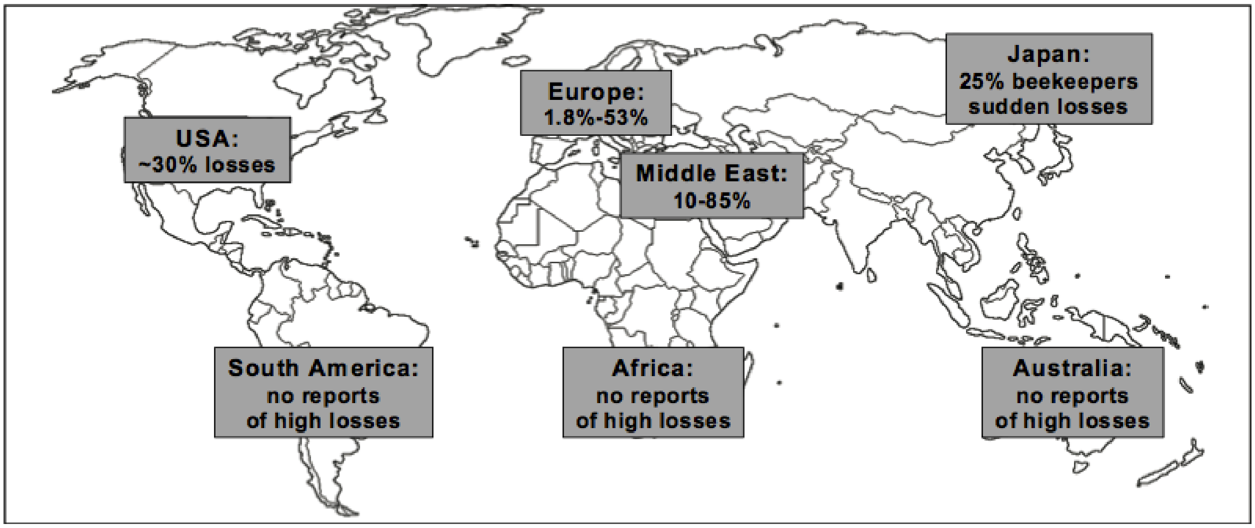

**Fig. 1.2:** The worldwide spread of *Varroa destructor* in hives of *A. mellifera* (Neumann and Carreck 2010).

While the cause and origins of CCD are unknown, the noticeable lack of CCD in the southern hemisphere correlates with Varroa populations. Dahle (2010) showed that the geographic distribution of CCD cases was strongly correlated with high Varroa infestation rates. In addition, Varroa mites are not found in Australia, and African and Africanized honey bees show high levels of resistance to the mites, mainly due to shorter adult lifespans and a lower worker brood fecundity for Varroa females (Martin and Medina 2004). Therefore there would appear to be a possible connection between Varroa infestation and CCD.

### 1.3.2 Discovery and Spread

The parasitic mite, *Varroa destructor* was first described in 1904 by Anthonie C. Oudemans from samples obtained in Java. At the time Oudemans named the new species *Varroa jacobsoni* after the collector, Mr. Edward Jacobson (Oudemans 1904). Nearly a century later *V. jacobsoni* was discovered, through molecular analysis, to be a complex of species and the name *V. destructor* was coined (Anderson and Trueman 2000). Interestingly, the specimens that Oudemans first described were in fact *V. jacobsoni*, but due to a lack of morphological difference between the two species, *V. destructor* and *V. jacobsoni* were assumed to be a single species. One may safely assume that research on *V. jacobsoni* from the 20th century was actually focused on *V. destructor*, due to the disproportionately higher economic importance of *V. destructor* compared with *V. jacobsoni* (Rosenkranz et al. 2010).

From Java, Varroa mites were found in Singapore in 1951, Japan in 1957, and Hong Kong and the Philippines in 1962 (Denmark et al 1991; de Guzman et al. 1997,). Varroa quickly spread to the Americas through Paraguay in 1971, most likely from *Apis* hives brought in from Japan, and from Paraguay moved to Brazil a year later. It is unknown whether these mites were *V. destructor* or *V. jacobsoni*, though evidence would point to the latter as they caused little to no harm to bee hives in South and Central America (de Guzman et al. 1997; Oldroyd 1999). The spread of Varroa in Europe had a similar timeline to that in Asia, first being found in Russia in 1952 and then spreading rapidly throughout the European nations (de Guzman et al. 1997). When and where the first Varroa mites were found in the US is uncertain, though it is generally accepted that mites first appeared in 1987 in packages in Wisconsin bought from Florida; likely originally brought into Florida illegally from Europe (de Guzman et al. 1997). The most recent entrance of Varroa into a previously Varroa-free country was in 2000, when mites were discovered in New Zealand (Zhang 2000). The only country to currently be Varroa free is Australia, likely due to extremely strict import policies (Le Conte et al. 2010).

### 1.3.3 Host Shift

At some point in the mid-20th century *V. destructor* shifted from its original host, *A. cerana* to *A. mellifera* (Anderson and Trueman 2000; Rosenkranz et al. 2010). This shift is thought to have taken place during the first half of the 20th century and was most likely facilitated by the movement of hives from Korea to Eastern Russia, where *A. cerana* was the dominant bee (Le Conte et al. 2010). *A. cerana* and *V. destructor* coevolved; *A. mellifera*, however, did not and therefore lacked any natural defenses against *V. destructor* (Rosenkranz et al. 2010). This made *A. mellifera* a much more susceptible host for *V. destructor*, allowing for the rapid proliferation of the mite throughout the world when introduced to areas with *A. mellifera*.

The discovery of *V. destructor* was due to the higher susceptibility of *Apis mellifera* to infestation by the mite compared with *A. cerana.* Anderson (1994) observed that while *V. jacobsoni* collected from Java parasitized both *A. cerana* and *A. mellifera*, it was unable to reproduce on colonies of *A. mellifera*. Anderson and Sukarish (1996) suspected a host shift when they noted *V. jacobsoni* had begun to reproduce successfully in hives of *A. mellifera*. This fact lead the researchers to postulate that perhaps *V. jacobsoni* was in fact a complex of species, and that this shift was due to the introduction of a species separate from *V. jacobsoni* (Anderson and Trueman 2000). Through mtDNA comparison Anderson and Trueman (2000) found that there was a 6.2% difference between two clades of *V. jacobsoni* sampled from 33 countries, leading them to define *V. jacobsoni* as, at the least, two separate species, with the new species, *V. destructor*, being the predominant pest of *A. mellifera* (Denmark et al. 1991; Anderson and Trueman 2000; Rosenkranz et al. 2010). These two species differ genetically, yet morphological differences are slight, with size and shape being the only visible differences (*V. jacobsoni* is slightly smaller and less oval, Anderson 2000).

### 1.3.4 Taxonomy and Morphology

Males and females of *Varroa destructor* are highly sexually dimorphic. Adult females are red in color (Fig. 3), weigh roughly 0.1 mg, and can reach up to 1.00–1.77mm in length and 1.5–1.99 mm in width; males are ≈0.5 mm and circular with a slight yellow coloration (Denmark et al. 1991; Sammataro et al. 2000; Rosenkranz et al. 2010; Fig. 4).

Kingdom: Animalia
  Phylum: Arthropoda
    Class: Arachnida
      Subclass: Acari
        Order: Parasitiformes
          Suborder: Mesostigmata
            Family: Varroidae
              Genus: *Varroa*
                Species: *V. destructor*

**Fig. 1.3:** Adult female *Varroa destructor* Photograph by Scott Bauer, USDA.

Unlike Hexapods, *V. destructor* is divided into two sections, the frontal head and mouth area, known as the gnathosoma, and the body or idiosoma, which consists of the dorsal and ventral shields. The gnathosoma is placed under the idiosoma, facing ventrally, and contains two chelicerae and two pedipalps used for sensory detection (Rosenkranz et al. 2010). The chelicerae in the male are called the spermatodactyl and serve the purpose of sperm transfer into the female. In the female the last of three chelicerae digits can be manipulated to cut a wound in the host for hemolymph extraction, sometimes behind the bee’s head but usually near the proventriculus on the bee’s abdomen (Rosenkranz et al. 2010). All mites found feeding on adult bees are female, as males never leave the brood cell and appear to serve the sole purpose of mating. On the female, the idiosoma is heavily sclerotized, as they are more likely to be exposed to bee hygienic behaviors and the elements than males. Even with the heavy scleritozation of the idiosoma, female mites are able to deform their bodies with the help of membranes between the sclerites, allowing them to move into a variety of small spaces, such as under *A. mellifera* abdominal sclerites (Rosenkranz et al. 2010). The idiosoma of the female is much wider than it is long, giving them a distinct oval shape, while in males, it is nearly spherical and weakly sclerotized. Female mites are also much stronger than the males, with short legs and simple apoteles, claw-like structures specialized for holding on to the host (Colin et al. 1999).

**Fig. 1.4:** A male and female Varroa mite, with five life stages of the female shown. From upper left to lower right: Protonymph, deutonymph, deutochrysalis (stationary form), recently molted female, fully mature female and fully mature male (Rosenkranz et al. 2010).

In terms of mating, the female reproductive organs are made up of two systems, the standard ovary, spermatheca, uterus and vagina, situated ventrally in the center of the body, and the other sperm reception area, the gonopores, which are located on the side of the body between the third and fourth pair of legs (Alberti and Hanel 1986). The male's reproductive opening is located in the center of its body, between the second pair of legs. During mating, the male ejaculates immature sperm, which complete their development inside the spermatheca of the female (Rosenkranz et al. 2010). Mating is facilitated through pheromones produced by young females, and can take place multiple times with many partners within the cell.

Mating is external, with the male using his chelicerae to place his spermatophore into the gonopores of the female where it will move to the spermatheca to be stored for future use (Rosenkranz et al. 2010). Here sperm from multiple partners can mix, providing a source of genetic variation. Despite the possibility of mixing, inter-population variation is generally low, most likely due to female mites' sole male offspring, necessitating the invasion of the cell by multiple foundress mites in order to produce multiple males (Donze et al. 1996; Salvy et al. 1999). Multiple foundresses present in the same cell are not generally beneficial for Varroa mites, with two or three being the maximum in one cell before competition for food becomes deleterious (Salvy et al. 1999). However, Hamilton (1967) hypothesized that the low level of genetic variation could be adventitious, reducing the number of harmful recessive alleles. That being said, an isomer, (Z)-8-heptadecene, has been identified as having a repressive effect on female mite fecundity, and is found in brood cells that are overly infested (Milani et al. 2004). The origin of this isomer is still uncertain, but is hypothesized to be released by the stressed bee larvae.

*V. destructor* mites do not have antenna, but seem to use their front pair of legs for sensing the environment around them, utilizing a pit filled with sensory hairs on each leg (Rosenkranz et al. 2010). These hairs may serve to detect volatile chemicals, such as those found in royal jelly, brood pheromones, as well as humidity and temperature. Their bodies are covered in hairs, many of which are thought to provide chemo- and mechanoreceptive sensory information (Rosenkranz et al. 2010). As they spend the vast majority of their existence inside bee hives, vibrations and temperature, while perceived, do not play a critical role in host finding, or even in orientation.

### 1.3.5 Additional Information

Other than *V. destructor* and *V. jacobsoni* there are two other species in the genus *Varroa*, *V. underwoodi* Delfinado-Baker and Aggarwal and *V. rindereri* De Guzman and

Delfinado-Baker (Delfinado and Baker 1974, Rosenkranz et al 2010). Of the four species of Varroa only *V. destructor* poses an economic threat, mainly due to its pathogenic characteristics (Rosenkranz et al. 2010). Spread of the mite is facilitated mainly through the movement of hives by humans, but can also occur from other pollinators, such as bumble bees, flies and beetles. In the *Apis* genus *V. destructor* can be found on three species (*A. cerana*, *A. koschevnikovi* and *A. mellifera*) and ten subspecies (Denmark et al 1991). Anderson (2000) noted that even after the naming of *V. destructor, V. jacobsoni* may still represent a complex of species, with haplotypes from the Philippines showing significant genetic difference from both *V. jacobsoni* and *V. destructor*.

The full mitochondrial genome of *Varroa destructor* was mapped by Navajas et al. (2002) using the Korean mitochondrial haplotype found in Europe, North, South and Central America, the Middle East, Africa and Asia.

### 1.3.6 Varroa Life Cycle

The life cycle of *Varroa destructor* begins with the laying of the first egg, a male, 60–96 hours after brood cell capping; at no time is the mite separate from its host (Ifantidis 1983; Sammataro et al. 2000; Rosenkranz et al. 2010). Only one male, which never leaves the cell, is produced from each female with all subsequent eggs being female. A single adult Varroa female produces from one to three female eggs per reproductive cycle, of which she may have up to three, every 30 hours (Schulz 1984). The first female offspring typically reaches the imago stage ~220 hours after the cell is capped (Martin 1994). Fecundity is normally higher in drone brood cells, with a single mother mite producing around 1.45 viable female offspring in worker brood (Martin 1994) and 2.0–2.2 in drone brood (Martin 1995a), most likely as a result of the drone broods' longer development period. Martin (1995b) found that the maximum number of viable offspring produced by a single mother Varroa mite was 16 in drone brood and eight in worker brood.

The foundress female places fecal pellets on the sides of the cell where her offspring will mate after maturing, usually five to six days after emergence for males and six to seven for females (Donze and Guerin 1994). Males emerge before the females, and congregate on the fecal pellet where they await females in order to mate, which generally occurs around 190 hours after the egg is laid (Donze et al. 1996; Oldroyd 1999). The male repeatedly mates with the first female until a second female matures, typically preferring the youngest female in the cell (Oldroyd 1999). Some level of young mite death is to be expected, with up to 20% of males dying before mating in worker brood and 10% in drone brood (Martin et al. 1997); 5% of females die within worker brood cells and 18% in drone brood cells (Lobb and Martin 1997). The reasons for these deaths have not been described, though competition for food and space may play a role.

Competition does not include cannibalism, which is a trait of a very early level of sociality (Donze and Guerin 1997). Mites also clean the cell and female mites perform some level of parental care, not only through the packing of the fecal pellets but also through maintenance of the wound on the bee larvae's fifth segment, from which she and her young gain access to hemolymph. Immature Varroa mites lack the sclerotized chelicerae of their mother, and are therefore unable to feed on their own, necessitating this basic form of parental care (Rosenkranz et al. 2010).

During the relatively short development period, a mite goes through five instars before sexual maturity, starting as a pharate larva and continuing into mobile protonymph, pharate deutonymph, mobile deutonymph, pharate adult (deutochrysalis) and finally adult (Donze and Guerin 1997). Instars alternate between quiescence and active feeding. The female's adult body shape appears during the deutochrysalis stage, though pigment is lacking. Sexual maturity is achieved with the development of the imago stage (Rosenkranz et al. 2010).

When a female leaves a cell as an adult she will become a phoretic passenger on an adult bee, eventually being transported to another cell containing a bee larva where she can reproduce (Rosenkranz et al. 2010). This phoretic period is dangerous for the female, however, as she may go an extended time without food. It has been shown in laboratory tests that a population of female mites will become 2–3-fold less fertile with only a 7–18 hour period without food (Rosenkranz and Sturmer 1992). On average, a female Varroa mite will reproduce successfully in two to three different cells during the course of its lifetime, though the maximum observed is eight (Fries and Rosenkranz 1996; Martin and Kemp 1997). Martin et al. (1997) argued that a female mites' failure to reproduce in its birth cell is most likely a physiological issue and, while she will enter several other cells and attempt to mate, she will continue to be sterile. There is some evidence for temporary infertility, however, as some female mites that failed to mate have been shown to be successful in new cells (Rosenkranz et al. 2010).

When invading a cell the female first crawls past the 5th instar and hides inside the food pellet that has been packed into the bottom of the cell in order to elude detection. The mite begins to feed on the larva once the larva has consumed the food pellet, normally around six hours after cell capping for worker brood and 20 hours after for drone brood (Ifantidis et al. 1999). Garrido and Rosenkranz (2003) showed that *A. mellifera* brood provided a stimulus that affected both the sexual cascade of offspring as well as the female mites' timing of reproduction. Female mites that had already begun a normal reproductive cycle were removed from the cell and placed into a freshly capped cell, at which point they began the cycle anew, producing a male and then subsequent females (Garrido and Rosenkranz 2003). The first example of an insect kairomonal primer was discovered by Garrido and Rosenkranz (2004), in the form of volatiles produced by the bee larvae that induce oogenesis in adult female mites immediately upon entering the cell.

### 1.3.7 Orientation and Drone Brood Preference

In general, orientation is controlled by chemical signals (Rosenkranz et al. 2010). It is thought that adult female mites can sense chemicals given off by adult nurse bees and 5th instar brood, both of which play integral roles in the life cycle of the mite. The mites may utilize the pheromones that bees release to communicate job roles, following the chemicals associated with nursery bees in order to find transportation to uncapped brood cells. However, this behavior has yet to be confirmed (Rosenkranz et al. 2010). There is also no evidence that mites recognize one another through chemical means, or produce any kind of aggregation pheromone (Salvy et al. 1999).

Males of *A. mellifera* are the preferred host for *Varroa destructor* (Fuchs 1990; Reich et al. 1998; Wantuch and Tarpy 2009); a higher percentage of female mites raised on drone brood are fertile compared with those raised on worker brood (Calderone and Lin 2001). In fact, the discrepancy between infestation rates of drone and worker brood can be as high as eight to one (Fuchs 1990).

There are many possible reasons for the preference for drone brood. Preference may be chemically controlled or may simply be a result of the extra attention paid to drone brood by nurse bees, providing ample opportunity for mites to infest drone cells. However, all larvae have chemicals in their cuticle that have been shown to attract adult *V. destructor* females (Rickli et al. 1994). Larvae have been shown to release esters that induce cell capping in adult bees, with the highest concentration of esters and differing hydrocarbon combinations being released during the 5th instar (Aumeier et al. 2002; Rosenkranz et al. 2010,). These chemicals are released for longer periods of time and at higher amounts by drone brood, though in laboratory tests these have not been shown conclusively to attract mites (Zetlmeisl and Rosenkranz 1994). *V. destructor* mites are also attracted to semiochemicals released from the food packed into the bottom of the brood cell, such as 2-hydroxyhexanoic acid, as well as the brood cocoon (Nazzi et al. 2004). Some chemicals can also repel mites, such as octanoic acid, which can be found in high quantities in royal jelly, possibly playing a role in the low infestation rate of queens (Rosenkranz et al. 2010).

In addition, drone brood remains capped for a greater period of time than worker brood, allowing mites to fully tan and mate for around 24 hours longer than in worker brood. Lobb and Martin (1997) found that there was a two to three fold greater number of mites falling from worker brood than from drone brood, regardless of population level. As stated above, mites may produce semiochemical signals, which help them to avoid detection by nurse bees, and the shorter capping period for worker brood may inhibit the production of these chemicals. Such a reduction in chemical production would be aided by the lack of adequate time for mites to fully develop. In fact, this hypothesis is supported by the level of maturation observed in fallen mites, with a greater number of lighter (i.e., less mature) mites falling from worker brood than drone brood. In addition, most of the mites that fall from worker brood are usually still alive, as opposed to those falling from drone brood that are mostly deceased and most likely have completed their reproductive cycle (Lobb and Martin 1997).

### 1.3.8 Hive Susceptibility to Mite Attack

Whether a hive is more or less susceptible to a high mite population is dependent on a few, basic characteristics of both the hive and the mites. A hive that has a healthy population of drone brood will obviously attract more *V. destructor* than one without (Rosenkranz et al. 2010). The aggression level of the bees' hygienic behavior also plays a role, as do environmental factors such as weather, season and the availability of naturally occurring food. Mites prefer a cooler temperature compared with *A. mellifera*, and this may play a role in the observed lower Varroa populations in hives found in the tropics than those in more temperate areas (Rosenkranz et al. 2006) and in the preference for drone brood displayed by Varroa females (drone brood are found more commonly on the periphery of the hive, Caron 2013).

Bee behavior is also a common factor in mite attraction to a hive. When colonies are strong, but nectar is low, bees often engage in "robbing," where they enter hives other than their own and steal food to bring back to their own colony. The robber bee also brings mites back to its hive causing an interesting phenomenon where the healthiest hives in an area will also have the highest Varroa levels when food is scarce (Goodwin et al. 2006).

Other aspects of the hive can attract or repel mites. The length of the brood cell has been shown to have a significant impact on infestation rates, with shorter cells having significantly fewer invading female mites (Kuenen and Calderone 2000); the same holds for thinner cells (Piccirillo and de Jong 2003). Age also has a significant effect on mite attraction, with older cells attracting a higher number of mites, perhaps due to the semiochemicals released by the many cocoon remnants left behind by previous developing bees (Rosenkranz et al 2010).

### 1.3.9 Mite Population Seasonality

It is generally accepted that mite populations will peak around late summer to early fall, quickly dropping during the winter and gradually increasing again during spring and early summer. While population levels may differ among hives and apiaries (Lee et al. 2010), population growth tends to follow the above trend (Sakofski et al. 1990). This is true for other harmful mite pests as well, such as another ectoparasite of honey bee brood, *Tropilaelaps mercedesae* (Lou et al. 2011). Swarming in spring and early summer may reduce mite levels and retard population growth in individual hives; however the timing of swarming can shift drastically with the weather and mites will travel on swarming bees, which could increase the local mite population growth should the swarm find a suitable destination within the original apiary (Wilde et al. 2005). Integrated Pest Management (IPM) control methods follow the above seasonal fluctuation closely, timing treatment to match either low or high mite levels. For example, Rosenkranz and Renz (2003) found that up to 90% of the mite population can be found within brood cells during the summer months. The vast majority of in-hive mite treatments are applied in the late summer/early fall. Timing treatments in this manner improves treatment efficiency through two means. First, by targeting mites when their population is at its peak, the treatment interacts with the greatest number of mites possible. Second, reducing the number of mites in the hive as the colony begins raising winter bees, which will lower the mite population for the following spring and increase survival of winter bees.

### 1.3.10 Pathogens Vectored by *V. destructor*

*Varroa destructor* has been shown to indirectly transmit around 15 different pathogens during pupal feeding (Kevan et al. 2006; Prisco et al. 2011, Li et al. 2014). These pathogens vary in virulence, but often a hive will have multiple pathogens at once, compounding damage to the colony. Mites not only vector pathogens to honey bees but also to one another through the use of the same feeding wound on the bee pupae (Chen et al. 2004b). This implies that as the mite population increases, and therefore the number of mites feeding on the same wound, so too will the rate of pathogen transmission. In addition, many pathogens, such as Deformed Wing Virus, can be transmitted vertically through infection of the queen, and subsequently her eggs (Chen et al. 2004b). Vertical transmision raises questions for pathogen persistence within the hive, even after mite populations have been reduced.

Mites have been shown to suppress the natural immune system of the bees, allowing any latent viruses to establish and multiply within the bee host (Yang and Cox-Foster 2005). Also of great concern is the ability of *Apis mellifera* to further facilitate transmission via pollen of these pathogens to other pollinator species, such as deformed wing virus in bumble bees (Genersch et al. 2006). The exact role of each of these pathogens in CCD remains controversial, but several are frequently found in CCD hives. The following table presents a list of the pathogens indicated to be transmitted by *V. destructor*, many of which act synergistically in a hive, increasing damage:

**Table 1.1:** Viruses transmited by *V. destructor*, life stage of the honey bee impacted, and symptoms.

| Family | Name | SI* | Symptom(s) | Source(s) |
|---|---|---|---|---|
| Cripaviridae | Chronic Bee Paralysis (I & II) | Ad | **I:** Covering of thin grease, loss of mobility, wing detachment, lack of hair; **II:** dysentery, abdomen filled with fluid | Williams 2000; Kevan et al. 2006; Ribière et al. 2010 |
| Dicistroviridae | Acute Bee Paralysis | A | Tremors (adult), death | Bailey et al. 1963; Genersch and Aubert 2010 |
| | Black Queen Cell | P | Queen pupae turn black, death | Williams 2000; Chen et al. 2006, Kevan et al. 2006 |
| | Cloudy Wing | Ad | Wings become cloudy | Chen et al. 2006, Kevan et al. 2006) |
| | Kashmir Bee | Ad | Shortned lifespan | Bailey et al. 1976, Genersch and Aubert 2010 |
| | Israeli Acute Paralysis | Ad | Shortned lifespan | Maori et al. 2007, Genersch and Aubert 2010, Prisco et al. 2011 |
| | Sacbrood | L, Ad | **L:** liquefied innards, death **A:** Cease brood attendance and pollen consumption, precocious nectar foraging | Bailey and Fernando 1972, Williams 2000, Shen et al. 2005, Chen et al. 2006, Kevan et al. 2006 |
| | Thai Sacbrood | L, Ad | **L:** liquefied innards, death **A:** Cease brood attendance and pollen consumption, precocious nectar foraging | Kevan et al. 2006 |
| Iflaviridae | Deformed Wing | Ad | Shortened lifespan, crippled wings, discoloration, swollen abdomens | Ball 1993; Kevan et al. 2006, Yang and Cox-Foster 2007 |
| | Kakugo | Ad | Heightened aggression | Kevan et al. 2006, Genersch and Aubert 2010 |
| | Slow Bee Paralysis | Ad | Paralysis of forelegs, death | Williams 2000, Chen et al. 2006, Kevan et al. 2006, Miranda et al. 2010 |
| | *Varroa destructor* Virus-1 | Ad | Shortened lifespan, crippled wings, discoloration, swollen abdomens | Zhang et al. 2007 |
| Secoviridae | Tabacco Ringspot | Ad | Muscle and nerve function degredation | Li et al. 2014 |
| **---** | Bee Viruses X and Y | Ad | Shortened lifespan | Kevan et al. 2006; Genersch and Aubert 2010 |

*SI = Life stage of bee impacted by virus (A = all, L = larvae, P = pupae, Ad = Adult)

### 1.3.11. Further Damage to Bee and Hive

The act of feeding by *V. dFpriestructor* causes a multitude of other problems for both developing and adult bees. Schatton-Gadelmayer and Engles (1988) showed that weight reductions in emerging worker bees was a common result of Varroa feeding, and Duay et al. (2003) found that infestations by one female mite can lead to a 10% decrease in normal body weight for emerging drones. Berg et al. (1997) found that reproductive success of drone bees was significantly correlated with their weight, implying that a Varroa infestation can lead to a reduction in hive population. This finding is supported by Duay et al. (2002) who found that drones who had been infested with only one mite during pupal development produced 24% less sperm, and those infested with two mites produced 45% less and had difficulties flying as adults. The use of coumaphos to control mites may then act alongside the mites to lower drone sperm production and vitality (Fell and Tignor 2001).

Worker bees infested during development show a host of problems as well. Through the reduction in hemolymph protein levels, life span can be shorted, making overwintering nearly impossible for colonies in temperate climes (Amdam et al. 2004). Infested worker bees also have trouble with associative learning, most likely due to a reduction in stimuli response, such as to sugar water and odor, causing navigational issues and impairing their ability to return to the hive after foraging (Kralj et al. 2007). Both infested worker and drone bees have much reduced immune systems upon emergence from the cell, exposing them to pathogens that would normally not pose a problem, such as the microbe *Escherichia coli,* causing significantly reduced life span (Yang and Cox-Foster 2007).

For the hive as a whole there are problems with reduced swarming, population numbers and survival. Villa et al. (2008) found that in the years directly after the introduction of Varroa, hives experienced a 47% drop in number of swarms per year, and a 29% reduction in survival. These detrimental effects, when combined with poor management, high levels of pesticide exposure, other pests (e.g. *Nosema* spp.) and even climate change can be fatal to a hive, often without easily diagnosable symptoms. Attempts have been made to quantify the Varroa population level needed to cause serious harm to a hive, with counts ranging from 7% infestation to 30% (Rosenkranz et al. 2010). Few agree, and the only definitive strategy for protection of hives is constant monitoring for both mite population and pathogen symptoms.

### 1.3.12. *Varroa* Mite Sampling

Three common methods are used for sampling Varroa mites within honey bee hives (Barlow and Fell 2006). The merits of each approach are debatable, but for large-scale beekeepers none is currently economically practical or ideal.

Sticky Board: These boards come in a variety of sizes, but most are made of a tough paper with a grid printed on one side, which is then covered in an adhesive material. The grid cells can be of varying size and number, for instance 12 x 18 cells on the sticky board for Ostiguy and Sammataro (2000) and 21 x 27 for our research (Figure 5). The sticky boards are placed on the bottom of the hive for about 72 hours (3 days). During this time mites that die or are removed through bee hygienic activities fall to the bottom of the hive and adhere to the board.

By counting the mites present on the sticky board bee keepers and researchers are able to roughly assess the necessity of treatment. The current treatment threshold for the southeastern United States instructs beekeepers to only treat for *Varroa* mites if a daily count of 59–187 were attained (Delaplane and Hood 1999). There are drawbacks to the sticky board method, most important being the time and effort associated with counting the mites. Measuring the mite fall in a hive is also a poor method for assessing the total population level of Varroa within a hive, with several studies finding conflicting levels of accuracy and precision (Calatayud and Verdu 1993; Branco et al. 2006).

**Fig. 1.5:** An example of our modified 21 x 27 sticky board

Roll Method: The roll method is one of the most commonly used mite population assessment tools. The method is rather simple: around half a cup (~300) of adult honey bees is collected in a clear glass jar and media such as ether or sugar is used to remove mites from the bees by slowly rolling the bees back and forth inside the jar. The contents are then poured through a filter, usually on to a white substrate, where the mites can be easily counted. Frequently the bees will also be examined for mites that were not dislodged (Azizi et al. 2008). Azizi et al. (2008) compared several different media and found that ether was the most effective at removing mites from adult honey bees. The obvious disadvantage of this method is that the bees die during the process. Therefore, sugar is the most commonly used substrate in the roll method, allowing the majority of the bees to be returned to the hive unharmed.

The accuracy of the roll method depends on mite density, number of bees sampled, and time of year (Azizi et al. 2008). Accuracy typically decreases with mite density (Lee et al. 2010), though for the average beekeeper so too does the necessity of rigorous accuracy. If rigorous accuracy is desired then, depending on hive strength, the number of bees required for the sample may be so great that hive health may be negatively impacted. In addition, estimating apiary-wide mite density is labor intensive and time consuming (Lee et al. 2010). Notwithstanding these concerns, small-scale beekeepers employ the roll method more than any other mite sampling technique, most likely due to a perceived ease of use and clarity of understanding in comparison to the sticky board method. The current treatment threshold for the Southeastern United States is 15–38 mites per 100 adult bees (Delaplane and Hood 1999) and 5-6 mites per 100 adult bees in Virginia (Keith Tignor, pers. comm.).

Drone Brood Uncapping: Mite numbers can also be assessed via direct inspection of drone brood cells, though this method is time consuming and involves the destruction of brood and is therefore primarily only used for research purposes. When uncapping cells, beekeepers may use a variety of tools, though the most common is a capping scratcher, which opens and removes brood from multiple cells simultaneously. Mites do move between the removed brood quickly, however, and accurate counting of mites per cell is hindered by the use of this tool. Uncapping a single cell at a time and counting the mites within is more time consuming and labor intensive but also lends a greater degree of accuracy to the inspection. However, accuracy is highly variable and there exists no universally agreed-upon threshold for advising mite treatment. The only threshold which has been published for the US (15 percent infestation) was developed using a computer model, and no field-relevant thresholds exist in the literature (Wilkinson and Smith 2002).

### 1.3.13. Methods of Varroa Control

Natural: The most basic form of Varroa control is that of evolved tolerance. *Apis cerana*, the original host of *Varroa destructor*, evolved with the mite and is able to co-exist with little serious damage to colonies. Tolerance against Varroa mites can simply be defined as a colony's ability to survive without human intervention, while infested with Varroa mites. While Varroa mites and *A. mellifera* only recently came into contact with each other, there are naturally occurring hives of *A. mellifera* that are more tolerant of the mites. These hives were first discovered in France in 1994 and were dubbed "Varroa surviving bee," or VSB (Büchler et al. 2010). VSB hives have been recorded to live up to fifteen years; however, the most interesting aspect is that the mite populations reduced in number over time, while those in the control colonies, treated with traditional apiculture practices, including acaricides, increased (Le Conte et al. 2007).

Natural resistance manifests as physiological and/or behavioral characteristics, for example the advanced detection abilities of *A. cerana* (Büchler et al. 2010) or the "grooming dance" where adult bees infested with mites request help through a specialized dance (Peng et al. 1987). These traits are heritable and are found in *A. mellifera*, though to a lesser amount than *A. cerana* (Thakur et al. 1996), leading researchers to undertake selective resistance breeding, discussed below. Indeed, there are several genes that are up-regulated in VSB bees which code for olfaction and neural response, suggesting that VSB bees are better able to "sniff out" infested cells (Navajas et al. 2008). Martin et al. (2002) found several compounds that bees may use to locate capped cells that are infested with Varroa females. The bees remove the cells containing Varroa females that are reproductively successful; those that are not are ignored (Harbo and Harris 2005).

Beyond olfaction, VSB hives have several other traits worth investigating, including differential post-capping stage length, thermoregulation and increased swarming, all of which disrupt Varroa mites (Büchler et al. 2010). Perhaps these VSB colonies are simply the future of the Varroa and *A. mellifera* relationship. However, as it now stands *A. mellifera* is, overall, a poor remover of mites. A study by Peng et al. (1987) showed that while *A. cerana* was able to remove 97% of introduced mites, *A. mellifera* could only remove 28%, though this has not been tested in recent years. As it is disadvantageous for the mites to kill their host, it is reasonable to assume that Varroa and *A. mellifera* will eventually balance out, existing together just as *A. cerana* does with Varroa. The time required for such a relationship to develop is difficult to determine, though there would be a strong evolutionary pressure on the mite to reduce virulence. However, the tendency for beekeepers to help weak hives, or hives less able to cope with mite infestations, alive through supplemental feeding and mite treatment removes much of the evolutionary pressure that would otherwise occur if hives were left unmanaged (Seeley 2007). Mites can also move with ease from one hive to the next in an apiary setting and will not perish with the death of a hive (Seeley 2007).

<u>Selective Breeding for Resistance – Behavioral</u>: At this point, breeding bees for behavioral resistance to mites has seen little success even with VSB traits being heritable. For example, de-Grandi-Hoffman et al. (2002) performed a study where they crossed queens and drones from hives with low mite reproduction (average of 2.4 offspring / female mite) with those with high mite reproduction (average of 4.2 offspring / female mite). Their results showed no overall reduction in mite fecundity. Resistance also frequently involves a trade-off of some sort, in the case of *A. mellifera* in reduced honey production, a major issue for apiculture (Le Conte et al. 2007). In addition, selecting for resistance takes a bit of guesswork, as researchers are

frequently only able to see results rather than the resistant mode of action. For example, the discovery by Harbo and Harris (2005) that VSB bees removed only cells containing reproductively successful mites led the authors to postulate that perhaps what they had been selecting for in breeding programs was not mites that had low fecundity, rather they were selecting for bees with advanced hygienic behaviors.

There have been a few encouraging results in breeding programs, specifically using hives that have had no treatments against Varroa mites whatsoever, a method given the term the “Bond” test after the film “Live and Let Die” (Büchler et al. 2010). With the Bond test, hives are monitored and those that survive the best are bred together. The thought behind the Bond test is that applying acaricides basically removes elements of natural selection, which is unhelpful in developing mite resistant bees.

Fries et al. (2006) left 150 hives without mite treatment for 6 years and observed a form of co-adaptation, where winter bee mortality rates decreased, adult bee mite infestations decreased and swarming rate increased. These hives were then compared with control hives which had been treated for mites and the Bond test hives were found to have an 82% lower infestation rate (Fries and Bommarco 2007). They observed that the Bond hives had fewer mites in sealed brood cells, and fewer brood cells overall. It should be noted that these hives were in an island in the Baltic Sea, so whether the results are relatable to warmer climates is unknown, still, the results are promising.

Another heritable mechanism by which mites are removed from the colony is grooming, where bees either groom themselves, autogrooming, or others, allogrooming (Rinderer et al. 2010). During grooming, bees locate and masticate Varroa females that are either phoretic passengers or directly feeding on adult bees. Grooming behavior can be measured by the number of mutilated mites found on the bottom of the hive, and has been shown to negatively correlate with mite populations, i.e. the higher the number of masticated mites on the hive floor, the lower the mite population in a hive (Arechavaleta-Velasco and Guzmán-Nova 2001). However, this measurement is both time consuming and can be inaccurate, as not all mites that are groomed are injured, some are just dropped, and live mites will fall in high numbers during warm weather (Rinderer et al. 2010). In addition, mites with injuries are not always due to grooming, as once they have fallen onto the sticky board, mites may be predated upon by other pests, such as wax moth larvae (Szabo and Walker 1995) and may show signs of damage from developmental issues.

Mechanisms that sound promising but have yet to show any significant and/or economic potential include increasing the phoretic period of mites in order to allow more time for grooming (Rinderer et al. 2010) and decreasing the capped development time for brood in order to reduce the amount of time that mites have to breed (Büchler et al. 2010). There are cuticular compounds of bees that have been shown to affect mite reproduction (Garrido and Rosenkranz 2004), but no research has yet approached this topic from a breeding standpoint.

Selective Breeding for Resistance - Physiological: As discussed earlier in the Varroa Morphology section, mites use semiochemicals released by larval food and cocoons to locate brood. Mites are unable to reproduce in worker cells of *Apis cerana*, leading some investigators to hypothesize that these chemicals may be different between the two species and perhaps able to be bred into *A. mellifera* (Rinderer et al. 2010). This line of research is young, and little progress has been made. Wang et al. (2007) found that *A. cerana* worker brood had much lower levels of copper and zinc than the drone and worker brood of *A. mellifera*, nutrients necessary for insects' survival, and higher concentrations of free amino acids, which cause harm to the mites. However, more work needs to be done on the heritability of these traits.

Breeding for Resistance - Success and Failure: The successes found in breeding programs are somewhat bittersweet, with colonies bred for low Varroa populations surviving over control hives, but without a mechanism yet identified. The two characters which have been most successfully selected for in breeding programs are low mite population growth and increased hygienic behavior on the part of the bees (Büchler et al. 2010). For most characters, however, it is very difficult to tease out whether the true mechanism of resistance is some form of Varroa tolerance by the bees or whether it is a lower virulence of the Varroa themselves.

Chemical Treatment - Conventional Acaricides: The most commonly used method for Varroa control is the within-hive application of synthetic acaricides. Application is through the use of strips treated with the acaricide, usually placed in the center of the hive body between two frames of brood. Synthetic chemicals have a host of drawbacks, some of which are quite serious. Acaricides leave residues in bee by-products which some fear may come to affect humans (though no evidence of an impact on humans yet exists), can directly harm bees and, as in the case of antibiotics, have been found to contribute to the reduction of the natural honey bee gut bacteria necessary for their survival (Williams, pers. comm.). Residues are obviously of great concern to apiculturists, and the fact that bees are notoriously good at filtering trace elements out of their products makes their existence even more worrisome (Bogdanov et al. 2003). However, the greatest problem with conventional acaricides is the development of mite resistance (Büchler et al. 2010). Resistance can arise in a population rapidly, especially when acaricides are used improperly by the beekeeper, and with little extra cost to the mite. Martin et al. (2002) showed that mites resistant to Apistan®, a common acaricide, had no reduction in fitness compared to susceptible Varroa.

Apistan®'s active ingredient is the pyrethroid *tau*-fluvalinate, which interferes with mites' sodium channels in their central nervous system (CNS), prolonging the depolarization of the nerve axon, paralyzing and killing the mites. It is a "hard" acaricide, much like the organophosphate coumaphos and the formamidine amitraz (Rosenkranz et al. 2010). Organophosphates inhibit acetylcholinesterase, which allows acetylcholine to flood the CNS, causing all of the mites' nerves to fire at once, again inducing paralysis and, eventually, death. The mechanism of action of Amitraz is still not fully understood, though it is thought to have a wide variety of effects on mites including interfering with octopamine receptors in the mite CNS, once again causing hyperexcitation of nerves, paralysis and death. Resistance develops to specific pesticides, like Apistan®, but resistant mites will also show resistance to most other pyrethroids, causing issues for the application of a wide variety of brands (Rosenkranz et al. 2010). "Hard" acaricides are very effective, at least before resistance develops, and are relatively cheap and easy to use. Their benefits must be weighed against their drawbacks, and as resistance has become more commonplace, they have become less and less effective.

<u>Chemical Treatment - Alternatives</u>: Studies have examined the use of "natural" chemicals to suppress Varroa populations, hopefully avoiding mite resistance development. These include compounds like organic acids and essential oils (Mert and Yucel 2011, Adamczyk et al. 2005), propolis (Damiani et al. 2010, Garedew et al. 2002) and chemical components of the hive that may alert nurse bees to the presence of Varroa females (Martin et al. 2002), though little research exists on the latter.

Organic acids have several benefits. Some of these acids are produced naturally by the bees and can be found in honey, so, at small concentrations, there is little fear of direct damage to the adult bees themselves. Contamination is also therefore not an issue. Formic acid can actually penetrate sealed brood cells and kill the mites within, which even conventional acaricides cannot (Rosenkranz et al. 2010). In addition, there is no evidence that mites will develop resistance to organic acids. Formic acid is typically applied as a vapor, with a saturated sheet placed on the top frames of the hive, allowing vapors to move down through the hive bodies. Oxalic acid is trickled between the frames as a liquid and essential oils, such as thymol, are applied as saturated blocks placed on the top of the brood frames.

The major drawbacks of using organic acids are climate/location of hive and unpredictability. Several organic acids, such as oxalic acid, may cause harm to the brood, and therefore can only be used in temperate areas where there is a broodless period during winter (Emsen and Dodologlu 2009). Organic acids, as well as essential oils, can linger and cause harm to the bees if the concentrations are too high for evaporation due to the temperature within the hive, necessitating a need for extreme care when measuring out dosage and knowledge of the macro- and microclimates of the hive (Rosenkranz et al. 2010). Environmental conditions do not appear to affect the overall lethality of organic acids (Sammataro et al. 2008).

Propolis is a highly viscous material collected by forager bees and is used to coat the inside of the hive, block any holes and provide anti-microbial benefits. It is made up of plant resins (which can come from any number of plant species) and wax (Simone-Finstrom and Spivak 2010). The most interesting aspect of propolis is its inherent anti-microbial properties and how these can be applied to *Varroa destructor* control. Propolis has already been shown to have a deleterious effect on American Foulbrood, and at higher concentrations of active antimicrobial compounds propolis may compare favorably with common antibiotics, against which AFB has shown resistance (Bastos et al. 2008).

Garedew et al. (2002) have observed 100% mortality of *Varroa destructor* after 5 seconds of exposure to a 10% concentration of active antimicrobial compounds found in propolis. At even lower concentrations (0.5%) mites were shown to be weakened significantly, encouraging results when faced with continually increasing resistance rates to synthetic compounds. An IPM approach that includes propolis extract application combined with a greater understanding of in-hive *V. destructor* spatial distribution may provide a safer (i.e., less negative effects on *A. mellifera* and reduced harmful residual compounds in the hive and honey) method of treatment than conventional practices. In addition, propolis can contain around 300 active ingredients, making the development of resistance in mites or other pests much more difficult than with the few active ingredients in synthetic compounds (Simone-Finstrom and Spivak 2010).

Damiani et al. (2010) determined that a spray of 10% propolis extract killed 78% of Varroa mites in a hive, yet caused no harm to the bees. Spraying is most likely the best method of application, as Damiani et al. (2010) also showed that mixing propolis extract in with the bees' food caused death of bees but not mites. Due to complexity and expense, the practicality of propolis will have to be researched further.

Biological Control: Accurately predicting the outcome of introducing a species into a new territory is nearly impossible, though when successful it can have an incredibly lasting effect, essentially helping to integrate the pest, which is likely itself introduced, into the new ecosystem's equilibrium. However, this process is made more difficult by hive dynamics. Bees are extremely efficient at removing intruders, and organism that predate on Varroa mites would have to be able to avoid detection by bees as well. Candidates for biological control of Varroa mites include predatory mites (*Startiolaelaps* sp.), parasitoids and entomopathogens (Chandler et al. 2010). The most likely candidate is a fungal entomopathogen, *Metarhizium anisopliae*, which would attack the mites but not the bees. Whether these fungi actually work or not is an area of contention, with some researchers claiming great success while others show no significant results (Rosenkranz et al. 2010).

Cultural Practices: There are many beekeeping practices and methods that have been shown to reduce Varroa populations, though several demand high investments of time and labor and some stray into the realm of questionable science, such as magnets and water with mysterious properties. The most effective, and the most time intensive, is simply pulling three to four brood frames out of the hive at a time and applying high heat (bees can withstand higher temperatures than mites) or formic acid to the brood cells, before replacing them in the hive (Rosenkranz et al. 2010). This method may work for small operations, and has been shown to reduce mite numbers drastically without chemicals, but on a large scale is likely unfeasible (Fries and Hansen 1993). Drone brood frames can also be removed at the beginning of spring, reducing Varroa populations 2–3.5 fold less than control hives (Charrière et al. 2003), though this practice alone will rarely control Varroa mites adequately (Wilkinson and Smith 2002).

An area with much promise is that of Integrated Pest Management (IPM). IPM programs take every form of control into consideration, using several in concert to control the population levels of a pest. For Varroa mites this can include management practices, breeding, pesticide use and biocontrol. An aspect of Varroa control that has received little attention from researchers is that of the spatial distribution of the Varroa mites within the hive, how it relates to the brood, and in what manner the spatiotemporal relationship between mites and their hosts can be used effectively in a precision mite management program.

### 1.3.14 Integrated and Other Pest Management Approaches

Integrated Pest Management (IPM) utilizes all available and economically feasible methods for the control of a pest. The ideal goal is a multifaceted approach to pest control which will reduce the need for potentially harmful methods of management, such as synthetic pesticides, while simultaneously increasing control efficiency (Overton 1996). The basic nature of IPM is comprehensive and inclusive, and therefore nearly any method of control may be used in an IPM program. Frequently, IPM is used interchangeably with other types of pest management, such as ecologically based pest management (EBPM) and precision management (PM). The three management approaches are not synonymous; however they are highly connected, and may be used in tandem.

EBPM evolved out of a backlash against the use of pesticides and their many inherent risks (Overton 1996). In a sense, EBPM is a resurgence of traditional pest management practices which emphasize using natural control to maintain a balance between a pest and the agricultural system. As with IPM, EBPM utilizes a wide array of control methods, including chemical, but does so in a way that supports the means by which a pest would be suppressed naturally in the system. Therefore, a successful EBPM program must be based on extensive knowledge of the pest and its host and how their relationship fits in with the natural ecosystem (Overton 1996). Obviously modern agriculture (i.e. monocropping) represents a huge disruption of the natural order, but this is a necessary evil which must be overcome.

The three major areas of insect management which EBPM programs utilize are biological control, narrow spectrum pesticides and the development of resistant plants. These represent more technologically and conceptually advanced forms of the earlier methods implemented before the development of synthetic pesticides, and all EBPM programs must adhere to the goals of safety, durability and profitability (Overton 1996). An extensive knowledge base of both the insect and its host crop, as mentioned above, is necessary for achieving these goals. The creation of such a knowledge base is difficult, as ecosystems represent complex, volatile relationships between a wide variety of factors. To this end, exhaustive sampling and experimental rigor is paramount to the successful implementation of an EBPM program.

Precision Agriculture (PA) is a broad term which involves the use of Global Positioning Systems (GPS) and other sensing technologies to better understand the dynamics of a particular geographic area (Srinivasan 2006). The knowledge gained through these sensing technologies improves the decision making process for growers, from fertilizer application to treatments for pests. While pest control makes up only a portion of PA, it still represents a critical aspect of successful precision farming. As with EBPM, a PA program relies on a comprehensive understanding of the relationship between a pest and its host. PA differs from EBPM, however, through its emphasis on predicating where a pest will be within the system at any given time (Srinivasan 2006). Therefore, much emphasis is placed on the spatiotemporal dynamics of the pest and host, and the timing of treatment application.

The goals of PA are the lowering of costs through the reduction in the use of treatments and resources such as fertilizers and pesticides, a decrease in the environmental impact of farming, and an increase in yields and food quality (Srinivasan 2006). Growers implementing PA follow a five step process to reach these goals, beginning with data collection. Data collection is by far the most important aspect of a successful PA program, as all management decisions are based on the knowledge gained through sampling the agricultural area in question. Data collection not only refers to the standard crop monitoring and soil sampling, but spatial mapping as well (Srinivasan 2006). Farmers then go on to run diagnostics on the data collected, analyze the data and build a management plan, implement the management plan in the field and then evaluate the results. The process is cyclical, as farmers continually collect data on the management plans they implement, allowing for the improvement of their PA program. Therefore PA is evolutionary in nature and is consequently much more complex than traditional agricultural practices (Srinivasan 2006). As the understanding of the variability inherent in the agricultural landscape has spread, however, farmers have embraced PA as a means to increase their yields while simultaneously increasing the efficacy of treatments and farming practices.

PA has, for obvious reasons, been specifically designed for use in agricultural (e.g., field) systems, but the basic principles of sampling and predictive modeling can be applied to other systems as well. The following proposed research objectives discuss the application of PA, as part of a Precision Mite Management (PMM) program for Varroa mite management in commercial and recreational honey bee hive operations.

## 1.4. Research Rationale and Objectives

An understanding of the relationship between in-hive *V. destructor* and bee brood spatial distribution would facilitate the creation of an integrated pest management program that beekeepers, whether professional or hobbyist, could use to control mite populations in their hives. However, there is currently little known about the in-hive association of *V. destructor* and bee brood, beyond the mites' well-known preference for drone brood, and no research has been published mapping the in-hive spatial distribution of *V. destructor* populations. To date, research on the spatiality of Varroa mites has focused on inter-hive and intra-bee distribution (e.g., Bowen-Walker et al. 1997) with a few studies investigating Varroa distribution within infested brood cells (e.g., Donze and Guerin 1997). Unfortunately the findings of these studies have been contradictory, additionally confounded by their relative paucity.

The hypothesis of this research is that through the characterization of Varroa mite spatiotemporal distribution and the relationship between both mite and honey bee brood spatiotemporal distribution, mite sampling can be improved and a Precision Mite Management program can be developed which will increase mite treatment efficacy. The main objectives of this research, therefore, are:

1. To characterize the spatiotemporal distribution and population levels of Varroa mites in honey bee hives. Specifically, this objective will:
    a. Assess the spatiotemporal distribution of Varroa mites on sticky boards.
    b. Assess the relationship between three common Varroa mite sampling methods, i.e., roll method, brood uncapping, and stick boards.
    c. Compare the standard sticky board sampling method to a sticky board sampling that incorporates information on spatiotemporal distribution of the Varroa mite.
2. To determine the extent to which the spatiotemporal distribution of Varroa mites on sticky boards is correlated with the spatiotemporal distribution of drone and worker brood.
3. To determine whether information on the relationship between the spatiotemporal distribution of Varroa mites and honey bee brood can be used effectively in a Precision Mite Management Program.

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# CHAPTER 2

## Spatial Distribution and Sampling of Varroa Mites in Honey Bee Hives

### 2.1 Introduction

*Varroa destructor* Anderson and Trueman, originally a member of the *V. jacobsoni* complex, is an ectoparasite of the European honey bee, *Apis mellifera* Linnaeus. Following its introduction to North America in 1987, the Varroa mite quickly spread throughout the United States and Canada (de Guzman et al. 1997). The mite originally fed on the developing drone brood of *Apis cerana*, the Asian honey bee; however during the mid-20th century it shifted from *A. cerana* to *A. mellifera* (Smirnov 1978). Unlike its attack on *A. cerana*, *V. destructor* is able to reproduce inside both worker and drone brood of *A. mellifera* and, therefore, populations of the mite tended to increase more rapidly. Feeding by *V. destructor* can reduce honey bee size, productivity, and lifespan. In addition, the mite is known to transit over ten different viruses (Kevan et al 2006). As such, since its nearly ubiquitous infestation of apiaries in the U.S., the Varroa mite has been implicated as one of the possible major contributing factors to Colony Collapse Disorder (CCD) and is currently viewed as the most damaging pest of *A. mellifera* (Denmark et al. 1991; Ratnieks and Carreck 2010).

Initial efforts to control infestation of *V. destructor* and the tracheal mite, *Acarapis woodi*, another highly destructive pest of honey bees, focused on the use of chemical pesticides (Herbert et al. 1988; Witherell and Herbert 1988; Hoppe et al. 1989; Wilson et al. 1997; De Jong 1997). Beekeepers relied heavily on the use of miticides to control population of the two mites and would often treat colonies on a regular basis without regard to actual mite infestation levels. The excessive reliance on chemical controls led to a number of problems, including the development of resistance in Varroa mite populations (Eischen 1995, Elzen et al. 1999), deleterious effects on the reproductive physiology of honey bees, reduction in the ability of colonies to raise queens and the ability of drones to produce sperm, as well as queen failure and colony loss (Fell and Tignor 2001, Haarmann et al. 2002; Burley et al. 2008). In addition, problems with miticide use have been compounded by the potential for honey and wax contamination (Bogdanov 2006). The problems associated with miticide use in honey bee hives have indicated a need for better management practices that would lead to a reduction in the use of these chemicals.

Individual management tactics, such as the use of pesticides and natural enemies have had a long history in pest population control (Knipling 1979), but long-term pest suppression with these tactics often has only been achieved within an integrated pest management (IPM) framework (Dent 1991). As an informational science, IPM requires an understanding of the entire ecosystem of the pest, which includes having knowledge of its biology and interactions with the environment at different spatial scales (Knipling 1979, Dent 1995, Rosen et al. 1996).Such knowledge of the spatial structure of a pest population, or of its effects, can be used to create distribution maps for management support systems, which then can lead to optimized pesticide application programs via spatially precise and targeted applications. This approach to management, which relies on the spatial variability in the management entity of interest, and which generically is referred to as precision agriculture or site-specific agriculture, has been adapted to IPM in a new approach known as precision IPM or PIPM (Weisz et al. 1995, Midgarden et al. 1997, Ellisbury et al. 1998, Blom and Fleischer 2001, Blom et al. 2002). The PIPM approach not only has the potential to reduce pest management effort, but also to reduce pesticide load on the environment (Weisz et al. 1995). In addition, the information obtained from an understanding of the spatial distribution of the pest can be used to develop more efficient sampling (Kemp et al. 1989, Schotzko and O'Keeffe 1989, Midgarden et al. 1993) and to obtain spatially independent samples that satisfy the assumption of random sampling for the design and analysis of experimental data (Williams et al. 1992, Wright et al. 2002).

Sampling is a primary component in the development of any IPM program (Ellsworth and Martinez-Carrillo 2001). With respect to management of the Varroa mite, beekeepers must first sample their hives to assess within-hive mite population levels before any decisions can be made to apply a treatment. Three main sampling methods are generally used for this purpose: the roll, brood uncapping, and sticky board methods (e.g., De Jong et al. 1982; Fries et al. 1991; Ellis and Baxendale 1994; Brødsgaard and Brødsgaard 1998; Calderone and Turcotte 1998; Branco et al. 2006). Each of the three sampling methods examines a different subset of the mite population within a hive, with the roll, brood uncapping, and sticky board methods focusing on mites on adult bees, mites within cells, and mites falling through the hive, respectively (Branco et al. 2006). The roll method consists of placing a half cup of adult bees (~300) into a wide-mouthed jar containing sugar, soapy water, or alcohol (e.g., ethanol) and rolling the jar back and forth to dislodge the mites. The advantage of using alcohol or soapy water is that an accurate bee-mite ratio may be obtained; however, the bees will usually die during sampling, which can be a concern if the hive is weak. Accuracy of the roll method also is dependent on the mite population level, number of bees sampled, and time of year (Azizi et al. 2008); for these reasons the roll method may not be the most reliable for making management decisions.

The brood uncapping method, which is based on a presence-absence sampling of at least 100 cells, assesses the number of mites within brood cells and is used to estimate percent mite infestation (Charrière et al. 2003). Because this method is highly destructive, labor intensive, and time consuming, it is not commonly used by beekeepers, but may be suitable for research.

The sticky board method assesses the number of mites falling through the hive, either from natural death, hygienic removal by bees, or because of the inability of the mite to maintain its grasp on the brood frame. Sticky boards are placed on the bottom of the hive, and are covered with an adhesive substance, which traps mites upon contact. Boards can be purchased from bee keeping supply companies, but can also be made by using posterboard and an adhesive. Ostiguy and Sammataro (2000) modified the sticky board to include a grid of 2.89-cm$^2$ square cells in a 21 x 27 configuration. One third of the cells are white in color, and only mites on these cells are usually counted to assess daily mite fall (DMF). The DMF value is then used to form treatment options based on a threshold unique to the geographical location of the apiary (e.g. Delaplane and Hood 1999; Barlow and Fell 2006).

The goals of this study were threefold: 1) to examine the temporal trend in the estimates of mite population levels within honey bee hives using the roll (mite/100 bees), brood uncapping (percent of infested cells) and sticky board (mite fall) methods; 2) to characterize the spatiotemporal distribution and assess the degree of aggregation of mite fall on sticky boards, and 3) to determine whether the information on the spatiotemporal distribution of mite fall can be used to improve the current sticky board sampling method.

## 2.2 Materials and Methods

### 2.2.1 Apiaries and Hives

Hives used in the study were located at three apiaries owned and operated by Virginia Tech at Montgomery Co., Virginia: Moore Farm, Prices Fork Research Center (PFRC), and Kentland Farm. The Moore Farm apiary is located in a predominately rural area within 3.22 km of suburban center on Prices Fork Road and is ≈3.5 km from the Virginia Tech main campus.

Prices Fork Research Center is a collection of research facilities on Prices Fork Road, and is located ≈6.0 km from Virginia Tech campus. Kentland Farm, which is located 14.5 km from Virginia Tech campus is a large research farm used by a variety of departments within the College of Agriculture and Life Sciences. The apiary is located within a 0.5 ha wooded area on the farm and is surrounded by an electric fence.

### 2.2.2 Mite Sampling

Sampling of mite population was conducted in 2012 and 2013 at the three apiaries. In 2012, mite population levels were assessed every 6 weeks from May to August using three sampling methods: soapy water roll, brood uncapping, and a modified sticky board. Each of these three methods have been used to estimate total mite populations within honey bee hives (e.g. Branco et al. 2006) and to make treatment decisions (e.g. Rosenkranz et al. 2010). Six hives were sampled, with two hives located at each of the three apiaries. In 2013, sampling was conducted every four weeks between February and October. Thirteen hives were sampled using the soapy water roll method: 5 at PFRC, 4 at Moore Farm and 4 at Kentland Farm. Nine hives were sampled using the uncapping brood method: 4 at PFRC, 2 at Moore Farm and 3 at Kentland Farm. Thirty one hives were sampled using the sticky board method: 14 at PFRC, 9 at Moore Farm and 8 at Kentland Farm. The hives used for the sampling with the soapy water roll and brood uncapping methods were included in the number of hives used for the sticky board sampling.

The soapy water roll entailed placing a half of a cup of adult bees (~300) into a wide mouth clear jar that was about half full of soapy water. The jar is inverted repeatedly to dislodge mites from bees. Bees were removed and rinsed individually in a separate jar of clean water.

Mites in both the soapy water and rising jar were counted with the naked eye. Sampling using the uncapping brood method was conducted by first randomly selecting three frames from each hive and dividing each side of each frame into four equal-sized quadrates. One quadrat was selected from each side of each frame and cells along the diagonal of the selected quadrat were uncapped. The contents of the cells were removed and inspected for adult female Varroa mites using a magnifying glass. Results were recorded as presence-absence to obtain percent infestation with respect to 100 cells.

Sticky boards were acquired from Great Lakes IPM (Vestaburg, MI). Commercially available sticky boards (Fig. 2.1A) are made of poster board and are covered in an adhesive substance (Tangle-Trap) which mites adhere to as they fall to the bottom of the hive. Printed on each sticky board is a 15 × 21 grid of black and white (1.8 cm × 1.8 cm ) colored cells (i.e., 315 cells) with white cells pre-selected randomly and covering one-third (105 cells) of the board . The pattern of black and white cells on the sticky board, however, is fixed for all sticky boards and only mites that fall into the white cells were counted.

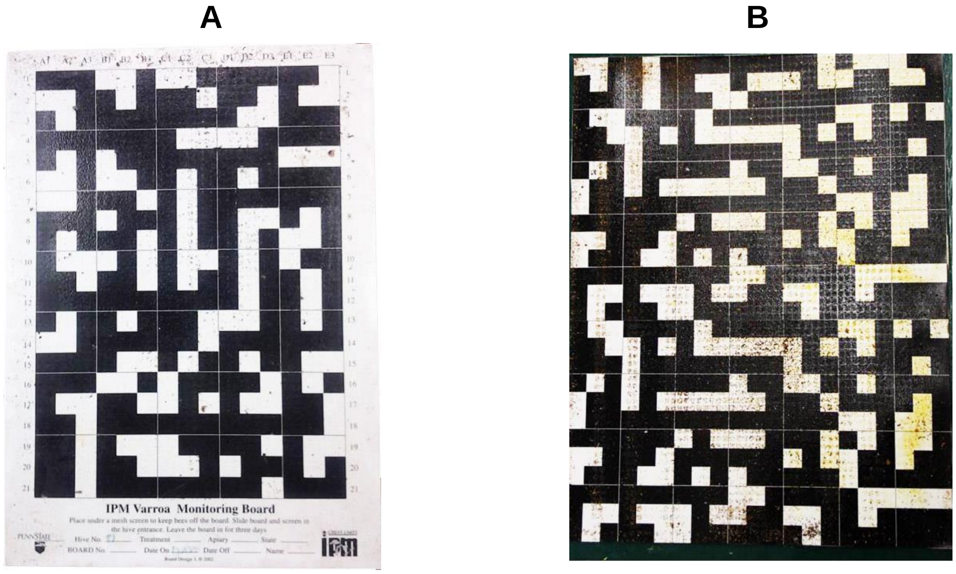

**Fig. 2.1: (A)** Commercial or standard sticky board, and (**B**) a modified sticky board used for sampling Varroa mite fall in honey bee hives. Note the areas of white and black cells, delineating where a beekeeper should (white) and should not (black) count mites.

Because the standard sticky board does not cover the entire bottom board of a hive, mites that fall at the edges of the hive were not collected. Therefore, for the current study, a modified sticky board (Fig. 2.1B) was developed to cover the entire hive bottom board by cutting off the borders of two standard sticky boards, rotating the cut boards 90°, and joining them at the edges. Modification of the standard sticky board increased the size of the board from 15 × 21 cells to 21 × 27 (567) cells. The number of white cells on the modified board was held at one-third of the total number of cells on the board (i.e., 189 cells).

At the start of each sampling period, sticky boards were placed on the bottom boards of the study hives and left in place for 72 h, after which the boards were collected and the number of imago-stage female mites counted and recorded. A plastic screen was placed over each sticky board to reduce the chance of bees accidentally adhering to the board.

### 2.2.3 Mite Spatial Distribution

Spatial distributions were assessed using two methods of geospatial analysis, variography and **S**patial **A**nalysis by **D**istance **I**ndic**E**s or SADIE (Rossi et al. 1992; Perry 1995; Thomas et al. 2001). For each sticky board, variography was used to develop an experimental semivariogram for the number of Varroa mites present on the sticky board after three days within a hive. This analysis characterized the degree of spatial dependence (autocorrelation) between Varroa mite counts with distance and/or direction on the sticky board. The semivariogram was developed by applying the equation,

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2, \qquad (1)$$

to the Varroa mite data, where $\hat{\gamma}(h)$ is the estimated semivariance for counts of Varroa mites ($z$) at all sampling points ($x_i$) on the sticky board separated by lag distance $h$, with $N(h)$ representing the number of pairs of samples separated by lag distance $h$. The graph or semivariogram generated by applying equation 1 shows the estimated semivariance values ($\hat{\gamma}(h)$) at different lag distances ($h$) between sampling points. If Varroa mite counts at $z(x_i)$ and $z(x_i + h)$ were autocorrelated, equation 1 would result in a small semivariance relative to pairs of points that were uncorrelated (Robinson and Metternicht 2006).

Once the semivariogram was developed, a variogram model (e.g., spherical, exponential, or Gaussian) that best described the spatial structure of Varroa mites on the sticky board was fitted to the data. The best fit model was determined based on the highest coefficient of determination ($r^2$) and/or lowest residual sums of squares (RSS) for each of the fitted models (Cressie 1993, Park and Tollefson 2005, Robinson and Metternicht 2006). The shape of a variogram model is described by three parameters, the nugget ($C_0$), sill ($C_0 + C$ [variance above the nugget]), and range ($A$) (e.g., Fig. 2).

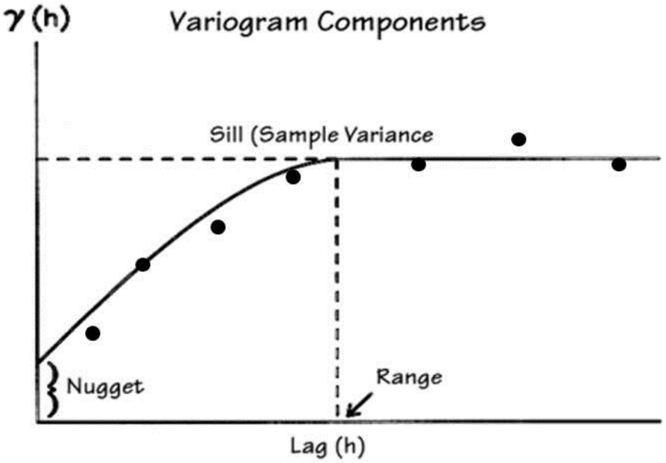

**Fig. 2.2.** An example variogram the nugget, sill, and range.

The nugget is the value of the semivariance at which the lag distance equals zero (i.e., where the model intercepts the *y*-axis of the semivariogram graph); the sill is the value of the semivariance at which the model levels off and is equivalent to the variance of the data (Farias et al. 2004); the range is the average distance at which the semivariogram levels off, or the lag distance beyond which spatial dependence decreases. The nugget-to-sill ratio, $C_0/(C_0 + C)$, was used as a measure of the degree of spatial dependence in Varroa mite count data and to compare semivariograms developed for different sticky boards and dates (Cambardella et al. 1994, Farias et al. 2004, Liu et al. 2006). Values of the nugget-to-sill ratio <0.25, 0.25–0.75, and >0.75 typically are used to indicate strong, moderate, and weak spatial dependence, respectively. The shape of the fitted variogram model was also used to determine whether the spatial structure of Varroa mites on each sticky board was uniform, random, or aggregated (Schotzko and O'Keefe 1989). In the case of an aggregated variable, for example, the semivariance is expected to increase with an increase in the separation distance between pairs of points up to the range after which it will level off at the sill (Farias et al. 2004). All of the above analyses were carried out using the geostatistical software $GS^+$ 9.0 (Gamma Design Software, Plainwell, MI).

A test of the null hypothesis of a random pattern in the distribution of Varroa mites on the sticky board was carried out using SADIE (Perry 1995, Thomas et al. 2001). In addition to an index of aggregation ($I_a$), SADIE provides the value $P_a$, which represents the probability that the data are not distributed randomly. Values of $I_a > 1$, $I_a = 1$, and $I_a < 1$ indicate aggregation, randomness, and regularity in the spatial distribution of the data. If the value of $I_a > 1$ is associated with a value of $P_a < 0.025$, the aggregated spatial distribution is considered significantly different from random; likewise, if the value of $I_a < 1$ is associated with a value of $P_a > 0.95$, the regular spatial distribution is considered significantly different from that expected from a random pattern.

### 2.2.4 Comparison of Sticky Board Mite Estimation Methods

The spatial pattern of mite counts on sticky boards collected during July to September 2013 was recorded in an Excel spreadsheet. For comparison, only cells corresponding to the standard sticky board were considered. We chose to focus our estimation method comparison on the months of July, August and September because these are the times of the year during which mite levels are highest, and in late summer colonies raise winter bees, and therefore beekeepers sample and treat during these months to reduce mite loads on overwintering bees (Amdam et al. 2004). Three methods were used to estimate the total number of mites on each sticky board: 1) Total Count, whereby mites in all of the cells on the board were counted; 2) White-Cell Count, a method adapted from Ostiguy and Sammataro (2000) in which only mites in white-colored cells on the sticky board were counted and the value multiplied by three; and 3) Systematic Outside-

Range Count, which utilized information on the spatial distribution pattern of mite counts on the sticky board. The logic of the Systematic Outside-Range method was based on the idea that in order to obtain unbiased estimates of population totals, independent samples must be taken at random so that every sample location has an equal chance of being selected (Pedigo 1998). For aggregated variables, random, independent samples can only be obtained at distances greater than the range of spatial dependence (i.e., the range of the variogram) to ensure that these samples are not autocorrelated (Frank et al. 2011). Therefore, for the systematic counting method the average range for the variograms of mite distribution for all of the sticky boards collected from hives from July to September was calculated. Mites in the first cell outside the estimated average range, $i$ = range + 1, were then counted along with mites in every subsequent $i$th cell on the board. The total number of mites on the board was estimated as:

$$TM = MC * \left( \frac{TC}{CC} \right) \qquad (3)$$

where $TM$ is the estimated total number of mites on the board, $MC$ is the number of mites obtained from the systematic count, $TC$ is the total number of cells on the sticky board, and $CC$ is the number of cells that were counted.

### 2.2.5 Statistical Analysis of Nonspatial Data

The strength of relationship between the overall trends of mite population levels for the hives sampled in 2013 using soapy water roll and sticky board sampling was tested using Spearman's nonparametric correlation (Zar 2010). Because it was only possible to use the brood uncapping method at three out of the nine sampling periods in 2013, the data from this method could not be included in the analysis and were not considered further. The relationship between mean mite fall over a 3-day period for the 31 study hives and the index of aggregation ($I_a$) from the SADIE analysis was examined using simple linear regression analysis in Excel 2010 (Microsoft Corp).

The White-Cell and Systematic Outside-Range counting methods for estimating mite population levels on sticky boards were compared to determine their accuracy and effort level in respect to the total mite count. The data from the hives studied in each of the three months July, August and September were analyzed separately. For each month and for each hive the difference in mite count estimated for each of the methods from the total count was calculated. A nonparametric Wilcoxon paired sample or signed rank test (Zar 2010) was used to compare the two sets of difference values for all of the hives sampled during the month. All statistical analyses were carried out using JMP 7.0.1 (SAS Institute 2013) at an $\alpha = 0.05$ significance level.

## 2.3. Results

### 2.3.1 Mite Population Levels

The trends in mite population levels estimated using the soapy water roll and sticky board methods were similar for sampling conducted in 2012 and 2013 with increasing mite populations from spring to summer. Summary data for sampling conducted in 2013 are shown in Fig. 2. No soapy water roll sampling was conducted in October because of low ambient temperatures.

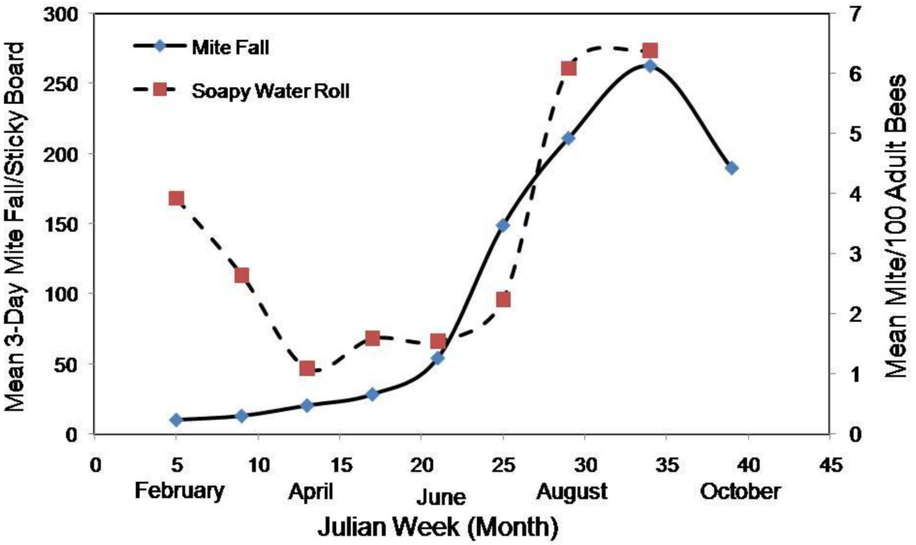

**Fig. 2.3** Varroa mite population trends in honey bee hives estimated using sticky board (mean mite fall over a three-day period) and soapy water roll (mean number of mites per 100 adult bees) sampling methods. Time is shown as both Julian week with month of sampling.

Spearman's nonparametric analysis showed that there was no significant correlation (ρ = -0.03, P = 0.8548) in the estimate of mite population between the soapy water roll and sticky board sampling methods during the spring (February to April). The analysis, however, showed that there was a significant correlation (ρ = 0.72, P < 0.001) between the two sampling methods for the combined summer and fall periods (March to September). Overall, a significant correlation (ρ = 0.47, <0.001) in mite population was observed for the two sampling methods for the entire study period from February to September.

### 2.3.2 Mite Spatial Distribution

Figure 2.4 shows examples of the variograms and interpolated mite surfaces generated for the sticky board collected in July, August and September from on of the study hives.

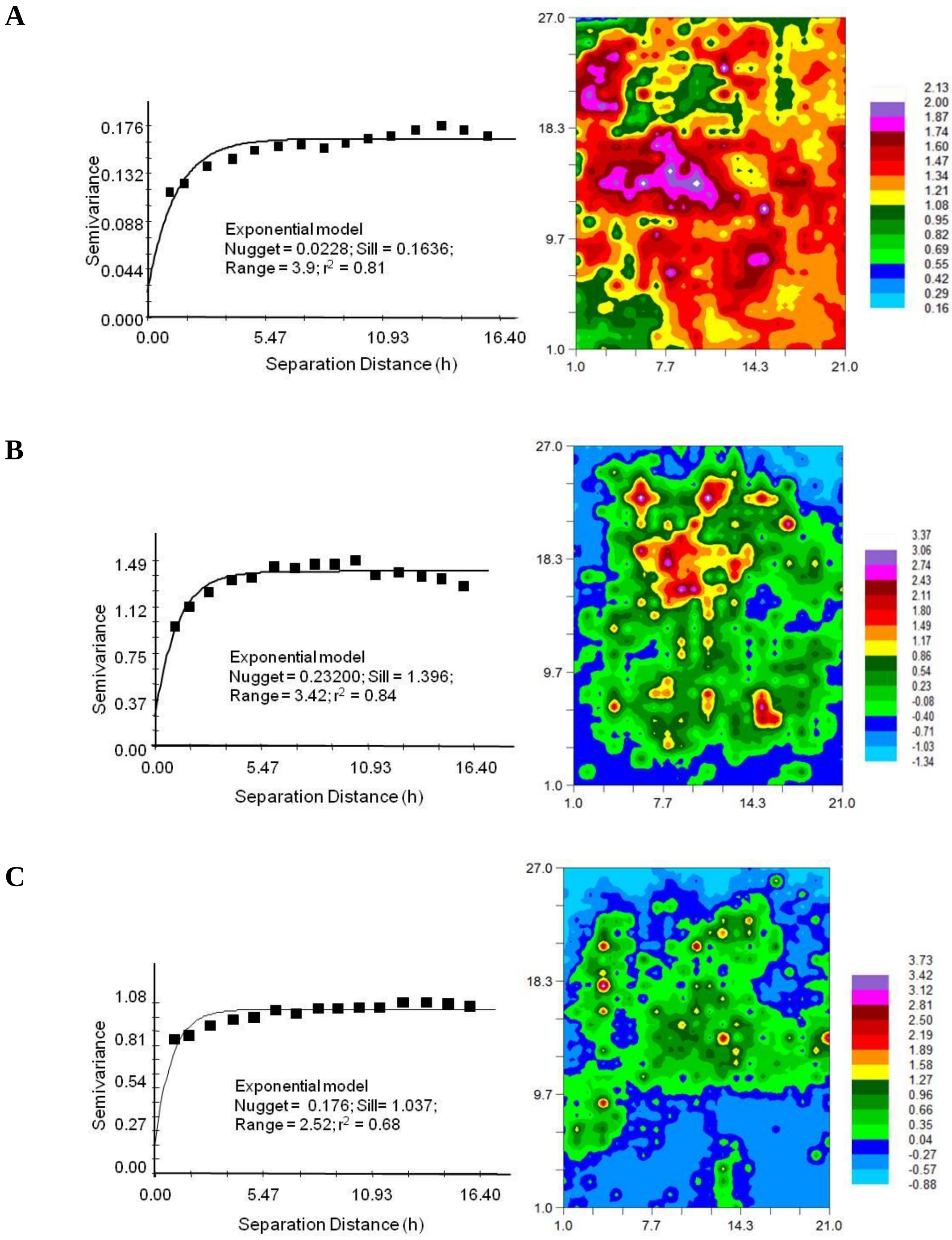

**Fig. 2.4** Examples of variogram models and interpolated surfaces for mite count data on a sticky board for sampling conducted in July (A), August (B), and September (C) 2013.

Summary results of the geostatistical analysis of mite counts on sticky boards at each of the sampling periods in each year are shown in Table 1. In general, mite fall on sticky boards tended to be aggregated throughout the 2012 and 2013 sampling periods as seen by the high occurrence of the exponential and spherical variogram models, the nugget-to-sill ratios indicating strong to moderate aggregation, and the $I_a$ values > 1.0 from the SADIE analysis (Table 1). Not unexpectedly, the mean Index of Aggregation, $I_a$, was positively related to mite fall ($R^2 = 0.9152$; Fig. 2.5).

The average range for the variograms of mite distribution on sticky board for July, August, and September was estimated at 4 cells. Because of this, the Systematic Outside-Range counting method was based on counts of mites in every 5th cell on the board. The overall mean number of mites estimated by the Total, White-cell, and Systematic Outside-Range counting methods are shown in Table 2.2 along with the number of cells that were counted in making the estimate. Wilcoxon paired sample test showed that there was no significant difference between the White-cell and Systematic Outside-range counting methods in the estimate of total mite fall on sticky boards at any of the sampling periods July, August, and September ($P > 0.05$). However, 40% more cells were counted using the White-Cell method compared with the Systematic Outside-Range method for a similar level of accuracy.

**Table 2.1. Results of geostatistical and SADIE analyses of the distribution of Varroa mites on sticky boards from honey bee hives in Southwestern Virginia**

| Year Period | Month | No. of Hives [a] | Mean (± SE) mites per sticky board[b] | Mean (min–max) Variogram Range[c] | Mean (min–max) $r^2$ | Mean (min–max) $C_0/ C_0+C$ | Mean (min–max) $I_a$ |
|---|---|---|---|---|---|---|---|
| **2012** | | | | | | | |
| Spring | May | 6 | 50.67 ± 16.08 | 2 (1–2) | 0.21 (0.07–0.38) | 0.17 (0.07–0.23) | 1.42 (0.91–2.14) |
| Summer | July | 6 | 97.67 ± 35.39 | 1 (1–2) | 0.21 (0.02–0.89) | 0.14 (0.11–0.19) | 1.30 (0.94–1.65) |
| Fall | August | 6 | 223 ± 80.34 | 4 (1–10) | 0.57 (0.09–0.97) | 0.30 (0.17–0.50) | 2.19 (1.09–3.74) |
| **2013** | | | | | | | |
| Spring | February | 12 | 10.25 ± 1.71 | 3 (1–16) | 0.15 (0.00–0.58) | 0.24 (0.03–1.00) | 1.26 (0.84–1.92) |
| | March | 12 | 13.08 ± 2.45 | 4 (1–16) | 0.13 (0.00–0.41) | 0.34 (0.19–1.00) | 1.30 (0.83–2.18) |
| | April | 13 | 19.62 ± 4.99 | 2 (1–3) | 0.14 (0.00–0.40) | 0.19 (0.00 –0.31) | 1.20 (0.75–1.78) |
| Summer | May | 13 | 28.46 ± 6.65 | 4 (1–16) | 0.19 (0.00–0.50) | 0.29 (0.05–1.00) | 1.29 (0.85–2.36) |
| | June | 13 | 54.31 ± 17.36 | 4 (1–16) | 0.14 (0.00–0.61) | 0.31 (0.08–1.00) | 1.29 (0.92–2.46) |
| | July | 29 | 178.59 ± 40.91 | 4 (1–21) | 0.41 (0.00–0.98) | 0.24 (0.09–0.50) | 1.88 (0.89–3.78) |
| Fall | August | 13 | 210.85 ± 41.10 | 3 (1–7) | 0.62 (0.13–0.93) | 0.25 (0.12–0.50) | 1.87 (1.12–2.73) |
| | September | 23 | 269.65 ± 35.06 | 5 (1–10) | 0.64 (0.05–0.98) | 0.35 (0.05–0.50) | 2.26 (1.12–2.73) |
| | October | 23 | 185.87 ± 33.11 | 6 (2–10) | 0.58 (0.09–0.98) | 0.30 (0.09–0.50) | 2.13 (1.16–3.57) |

[a] The number of sticky boards sampled at each month is equal to the number of honey bee hives.

[b] Mean for each month is the total mite count from all sticky boards (hives) sampled divided by the number of sticky boards (hives).

[c] Range of the variogram model in units of cells. Sticky board cell dimension is 1.8 cm × 1.8 cm

$C_0$ = Nugget; $C_0+C$ = Sill; $C_0/ C_0+C$ = Nugget-to-Sill ratio; Values < 0.25 = strong aggregation; 0.25– 0.75 = moderate aggregation; > 0.75 = weak aggregation.

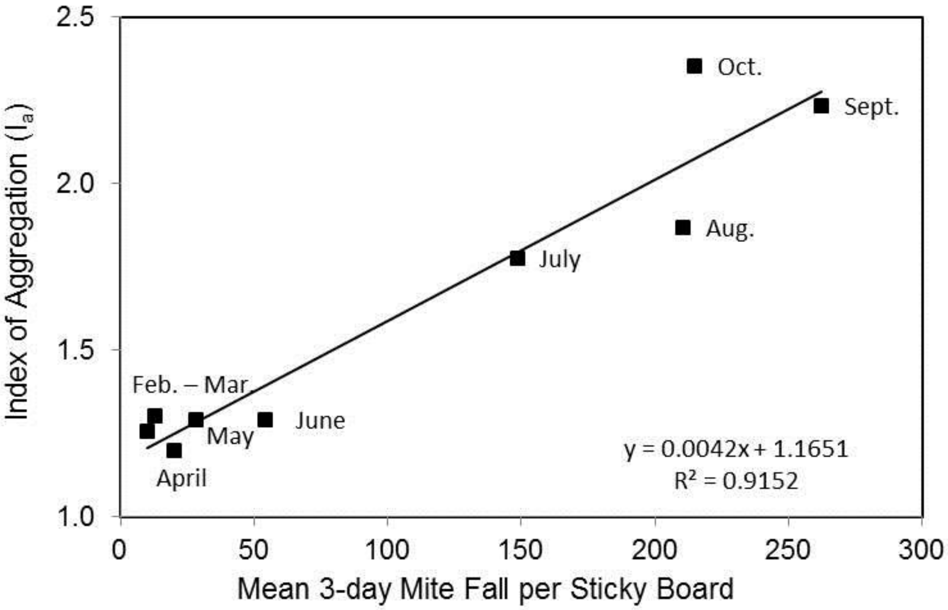

**Fig. 2.5** Relationship of mean mite fall over a three-day period and Index of Aggregation ($I_a$) for sticky boards sampled from February to October 2013.

**Table 2.2. Overall mean (± SE) number of Varroa mites per sticky board and number of cells counted for three different sampling methods.**

| Month | Number of Hives | Sampling Method<br>Mean (± SE) number per sticky board | | |
|---|---|---|---|---|
| | | **Total Count** | **White-Cell Count** | **Systematic Count** |
| July | 29 | 133.69 (± 29.95) | 132.48 (± 29.12) | 133.27 (± 29.16) |
| August | 13 | 152.38 (± 32.14) | 157.15 (± 31.67) | 158.46 (± 31.43) |
| September | 23 | 210.09 (± 28.76) | 208.04 (± 28.31) | 208.69 (± 30.07) |
| Number of cells counted | | 315 | 105 | 63 |

## 2.4. Discussion

Through their annual contribution of pollination services, valued at around $20 billion in the U.S., honey bees have become an integral part of our modern agricultural economy (vanEngelsdorp and Meixner 2010). Varroa mites are considered the most damaging pest of *Apis mellifera* in much of the world since their switch from *A. cerana* sometime during the 20th century (Rosenkranz et al 2010; Smirnov 1978). *V. destructor* has also been implicated in the recent increase in colony losses, which has been referred to as Colony Collapse Disorder or CCD (Potts et al 2010). For these reasons many beekeepers overreact upon discovering even a single mite within one of their hives. Before the pros and cons of treatment options can be assessed, beekeepers must sample to obtain a good understanding of the level of mite infestation within their hives.

The characterization of both the number of mites falling through the hive as well as the number of mites per 100 adult bees provides some context for all of the results of the current study. Fig. 2.3 shows mite fall for sampling conducted over several months in 2013 compared with the percentage of bees carrying mites. The honey bee queen ceases to lay eggs in late fall and will not begin laying again until mid-late winter (Caron 2013). The lack of brood cells available for invasion by the Varroa mite would explain the higher levels of mites on adult bees during February and March. As brood levels increase throughout the spring and early summer (Caron 2013) we see a reduction in the percentage of adult bees carrying mites as the mites disembark and invade brood cells. Mite levels on adult bees will increase during the late summer as mite population increases, while the brood population, especially drone, begins to decline (Caron 2013). Mite fall presents a much smoother curve, with the number of mites falling through the hive increasing steadily until October, where it begins to drop off, most likely due to the decrease in bee population as the hive prepares for winter.

The two methods of mite population estimation were correlated throughout the year, though there appears to be a lack of correlation in the trends during the spring. This difference is likely due to the roll and sticky board methods assessing two different subsets of the mite population. The soapy water roll method examines mites on bees and the sticky board method looks at mite that fall through the hive, which may include mites falling off of adult bees, or out of cells. The lack of correlation between the two sampling methods during spring has implications for mite monitoring, as beekeepers who employ both methods during this period may obtain differing estimates of mite population levels. Branco et al. (2006) also found significant correlation between the roll method (when paired with brood uncapping) and sticky board counts, though sampling in their study only took place during the summer and autumn. The authors put forth two conditions, which must be met in order to achieve correlation between the two methods: 1) hives must be "broodright," meaning the hive must have a minimum of 3000 brood cells (Brødsgaard and Brødsgaard 1998), and 2) hives cannot be in the process of collapsing (Branco et al. 2006). Both of these conditions relate to the need for high brood levels within the hive for there to be a reasonable level of correlation between the two sampling methods. While brood levels were not assessed in the current study in early spring, it is likely that the hives did not have sufficient brood for the roll and sticky board methods to correlate as brood rearing had only recently be reinitiated and hive growth was slow due to low ambient temperatures.

Mite fall dynamics were mapped across the twelve months of the study (May, July and August 2012; February – October 2013, Fig. 2.5) and for both years, the degree to which the mites were aggregated on the sticky boards increased with mite population, a pattern which has been seen many times before in other population sampling studies (Taylor 1984). Mite aggregation is to be expected as they exist in a confined area and must reproduce within a limited number of cells, all of which must have developing brood. Earlier in the season the amount of brood present in bee colonies will increase rapidly, peaking around late spring / early summer (Caron 2013). While the brood is spread throughout the hive we see that mite aggregation is low, most likely due to the large amount of brood available for infestation. Later in the season bee colonies will reduce brood rearing as they prepare for winter (Caron 2013), reducing the area that brood take up within the hive. This would likely act to concentrate the mites, explaining the rapid increase in the degree of aggregation later in the season.

Multiple methods of assessing spatial distributions exist, and the use of several methods can improve analysis (Madden and Hughes 1995; Perry et al. 2002, Queiroz et al. 2010). Variogram model shape, the Nugget-to-Sill Ratio (NSR) and the Index of Aggregation ($I_a$) all showed that there was some degree of mite aggregation on the sticky boards throughout the 2012 and 2013 seasons (Table 1). The $I_a$ was strongly positively correlated with mite fall, and this may have extensive implications for the control of *V. destructor* by setting the stage for precision mite management protocols that could take advantage of mite aggregation to more precisely apply treatments. The NSR was not significantly correlated with mite fall (data not shown; $r^2 < 0.1$), however variogram analysis relies primarily on the magnitude of counts, while SADIE examines the shape of clusters for those counts (Perry et al. 2002; Perry and Dixon 2002).

To date there have been no attempts to improve the standard sticky board counting method described by Ostiguy and Sammataro (2000). This method is based on counting a randomized one third (105) of the cells on the sticky board, and this count is used to calculate a daily mite fall by leaving the sticky board in the hive for three days. This method is accurate and simple to employ, however beekeepers still show resistance to counting such a large number of cells. We hypothesized that a smaller number of cells could be counted and, thus, less effort would be required if the cells selected for counting were done so based on an understanding of the spatiotemporal dynamics of Varroa mites on the sticky board. Two different counting methods were tested using the standard, commercially available sticky board design, with the total cell count as a measure of accuracy: counting a set third of the cells (White-Cell Count) and counting every fifth cell, based on the average monthly variogram range of 4.0 (Systematic Outside Range Count) estimated by this study. As stated above, the variogram range indicates the distance at which spatial autocorrelation breaks down and has been used in previous studies to form sampling and mapping protocols (Weisz et al. 1995, Park and Tollefson 2005, Frank et al. 2011). Sampling outside of the range insures that samples are independent and that each cluster will have an equal chance of being sampled (Pedigo 1998). We focused our comparison of the sampling methods in July, August and September, the three months during which mite populations are typically highest and beekeepers are most likely to sample (Rosenkranz et al. 2010). The results showed that there was no significant difference between the standard White-Cell Count and the Systematic Outside-Range Count. However, the Systematic Outside-Range Count required 40% fewer cells than the standard method. We also investigated the rate at which all of the counting methods on the standard sticky board incorrectly informed beekeepers as to treatment decisions based on a treatment threshold of 40 mites per day (Barlow and Fell, 2006). Both the White-Cell counting (standard) method and the Outside-Range methods had an overall misinformation rate (telling beekeepers to treat when they should not and vice versa) of 3%. Most beekeepers in Virginia have fewer than 100 hives (Richard Fell, Pers. Comm.), and such a low misinformation rate should not be a cause for concern for small beekeeping operations. Therefore, although both the standard sampling method and the method based on the geospatial information were similar in terms of accuracy and treatment decision, the latter method is seen as an improvement of the standard sampling method because of the 40% reduction in sampling effort.

Sampling is the first line of defense against *V. destructor*, and having an accurate method of sampling mites is the only way in which a beekeeper can be correctly informed as to the treatment needs for their hives (Branco et al 2006; Rosenkranz et al. 2010). Improper application of acaricides and other treatments for Varroa mites can result in wastage of time and money and may lead to the buildup of harmful chemicals in hive products and materials as well as furthering the development and spread of mite resistance (Eischen 1995; Elzen et al. 1999; Fell and Tignor 2001; Haarmann et al. 2002; Burley et al. 2008; Mullin et al. 2010). An understanding of mite spatiotemporal dynamics can aid in sampling protocols and may be used to improve treatment site location, potentially reducing the use of exogenous materials, and their drawbacks, in the control of *V. destructor*.

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# CHAPTER 3

## An Assessment of the Spatiotemporal Association of Honey Bee Brood and Varroa Mites on Sticky Boards

### 3.1. Introduction

*Apis mellifera*, the European honey bee, is an important pollinator, providing around 90% of all commercial agricultural pollination (Genersch et al. 2010). Honey also represents a significant contribution to the global economy, valued in 2007 to be around $1.25 billion annually (vanEngelsdorp and Meixner 2009). These benefits to the economy are threatened by the recent increase in colony decline. There has been much debate about the factors responsible for colony decline, with over 60 different factors implicated as possibly contributing to the disorder (vanEngelsdorp et al. 2009). However, pathogens, many of which are transmitted by the ectoparasite *Varroa destructor*, are frequently implicated as the greatest contributor to the phenomenon and the most serious stressor of *A. mellifera* (Denmark et al. 1991; Ratnieks and Carreck 2010).

*Varroa destructor* mites are ectoparasites and feed on the hemolymph of both developing and adult honey bees. Adult female mites hitch rides on adult bees until they sense an open cell which contains a 5th instar and is only a few hours away from capping. At this point, the mite will crawl into the cells of developing brood, hide in the brood food at the bottom of the cell and, around 60 hours after capping, begin to lay eggs (Ifantidis 1983). The foundress mite and her offspring will feed on the developing bee, reducing lifespan, size and productivity. Varroa mites also transmit a variety of diseases, some of which can bring about the collapse of a colony in a brief period of time (Kevan et al. 2006). For these reasons, *V. destructor* is considered the most serious pest of *A. mellifera* and a multitude of treatment protocols have been developed to combat the mite (Rosenkranz et al. 2010).

Treatments for Varroa mites include the use of biological controls, cultural methods and simple solutions such as application of powdered sugar to the hive frames (Rosenkranz et al. 2010). The most common treatments for Varroa mites, however, are synthetic acaricides (Herbert et al. 1988; Witherell and Herbert 1988; Hoppe et al. 1989; Wilson et al. 1997; De Jong 1997), primarily with the active ingredients coumaphos, *tau*-fluvalinate and amitraz (Mullin et al. 2010). These chemicals are applied through the placement of impregnated strips between frames within the hive, typically two per five frames of bees, and are left in the hive for a number of weeks. Synthetic acaricides are cheap, easy to use, and effective; however there are serious drawbacks to their use. Any lipophilic chemical applied to a bee hive will likely become sequestered within the wax, and little is known about the long-term effects of wax contamination and the build-up of residues within the hive (Bogdanov et al. 2003), however exposure to coumaphos has been demonstrated to negatively impact drone sperm viability (Burley et al. 2008). Varroa mites have also rapidly developed resistance to acaricides such as *tau*-fluvalinate, which began losing efficacy only a few years after its introduction to the commercial market (Hillesheim et al. 1996). Resistance develops to specific pesticides, e.g., *tau*-fluvalinate, but resistant mites will also show resistance to most other pyrethroids, causing issues for the application of a wide variety of brands (Rosenkranz et al. 2010). In addition, the development of new chemistries is a slow and arduous process and mites will often develop resistance far more quickly than a new product can be introduced to the market (Hillesheim et al. 1996).

Potentially exacerbating both the build-up of harmful chemicals within hive materials and the development of resistance in mites is the lack of knowledge on mite spatiotemporal patterns within the hive. As the season progresses, mite populations fluctuate in response to brood dynamics, and a better understanding and the ability to predict these fluctuations will allow the applicator to apply treatments in a more precise and environmentally friendly manner. Mites have been shown to have a preference for drone brood (Fuchs 1990); however the current information on mite aggregation within the hive is minimal (Fuchs 1998; Martin 1995b) and no studies have examined the spatiotemporal relationship between mites and their host, as well as the potential for such information to be used in a Precision Mite Management (PMM) program. The main objective of this study was to investigate the relationship between the spatial distribution of mite fall on sticky boards and honey bee brood on frames.

## 3.2 Materials and Methods

### 3.2.1 Apiaries and Hives

Hives used in the study were located at three apiaries owned and operated by Virginia Tech at Montgomery Co., Virginia: Moore Farm, Prices Fork Research Center (PFRC), and Kentland Farm. The Moore Farm apiary is located in a predominately rural area within 3.22 km of a suburban center on Prices Fork Road and is ≈3.5 km from the Virginia Tech main campus. Prices Fork Research Center is a collection of research facilities on Prices Fork Road, and is located ≈6.0 km from the Virginia Tech campus. Kentland Farm, which is located 14.5 km from the Virginia Tech campus is a large research farm used by a variety of departments within the College of Agriculture and Life Sciences. The apiary is located within a 0.5 ha wooded area on the farm and is surrounded by an electric fence to prevent bear attack on the hives.

### 3.2.2 Mite Sampling

Assessment of Varroa distribution took place over two periods, every six weeks from May to August 2012 and every four weeks from February to October 2013. Bee brood distribution was also assessed during the same mite sampling period in 2012 and and every four weeks from May to October 2013. Bee brood distribution was not assessed earlier in 2013 to avoid exposing frames of brood to cold ambient temperatures.

For an explanation of sticky board sampling methods see Chapter 2.

### 3.2.3 Mite Spatial Distribution

For an explanation of mite spatial distribution assessment methods see Chapter 2.

### 3.2.4 Assessing Bee Brood Distribution

The brood frames within each hive were photographed, front and back, using a digital camera. Frames were mounted on a frame and camera holding device designed for this purpose (Fig. 3.1).

**Fig. 3.1**. Camera and brood frame holding device. On the left is the frame holder; on the right is the tripod for the camera.

To capture an image of the brood, the frame was secured in the frame holder of the device; the camera was held steady on the tripod and aimed at a 13° angle to compensate for the upward angle of the brood cells. The back of the frame holder was painted black to eliminate any white space caused by holes in the comb.

The digital photographs of brood frames were imported into Iphoto (Apple Corporation) for cropping. The natural colored image of the cropped brood frame was then split into the red, green and blue channels using the image processing software TnTmips Pro 2009 (Microimages, Inc.). Once imported into TnTmips, the cells on each frame image were categorized so as to distinguish capped brood cells from other cells, such as those containing pollen, honey or uncapped brood. A supervised feature classification process was used to identify the brood cells on the frame.

*Feature Classification*: The process of feature classification of an image can be conducted using either an unsupervised or supervised approach (Avery and Berlin 1992; Richards 1993). In an unsupervised classification, the analyst specifies the maximum number of classes into which the image data are to be grouped and the classification algorithm in the image processing software (classifier) sorts the pixels into the required number of classes. Examples of unsupervised classifiers are K-means and Isoclass (Richards 1993, Tou and Gonzalez 1974). In a supervised classification, the analyst uses prior sample information to specify the classes into which the image (raster) data are to be grouped and the classifier sorts the pixels into the predefined classes. The analyst uses the sample information to create a training data set of the known classes (e.g., worker brood) that trains the classifier to recognize other pixel in the image as coming from the same multivariate distribution as pixels in the predefined class. The training set is created by selecting representative sample pixels in the image for each feature of interest using observed information and/or experience as a guide. This sampling process creates a statistical distribution for each known feature class from the pixels that are selected. Therefore, careful selection of representative pixels in the samples for each feature training class is important to minimize variation within the sample. A common supervised classifier is the Gaussian Maximum Likelihood algorithm (Thomas et al. 1987, Richards 1993, Tou and Gonzalez 1974). This algorithm computes the probabilities of a multivariate pixel belonging to the defined feature distributions and assigns the pixel to the feature class that has the highest probability.

The Gaussian Maximum Likelihood algorithm was used in this study to classify the images of the brood frames based on assigned feature classes. As a first step in the process, one or more region of interests (ROI) were created to isolate pixels other than those for the freature of interest. For example, a region of interest was created to isolate pixels around cells known by experience to be drone brood cells from other cells, such as those containing honey. Samples of pixels from drone brood cells and from other cells were then selected to create a training set with two feature classes: drone brood and other. Feature classification was then used to place all of the pixels in the image into one of the two feature classes.

*Classification Accuracy*: Several methods are available for determining classification accuracy (e.g., Van Genderen et al. 1978, Rosenfield et al. 1982, Hudson & Ramm, 1987, Congalton 1991, Richards 1993), but the most common methods rely on the data in the confusion or error matrix generated as part of the classification process. Congalton (1991 p. 36) defines a confusion matrix as “a square array of numbers set out in rows and columns which express the number of sample units (i.e., pixels, clusters of pixels, or polygons) assigned to a particular category relative to the actual category as verified on the ground.” Although not the case for all confusion matrices, rows represent the pixels selected for each feature class from the data, while the columns report the classification of these data in each feature class. The probability that ground truth pixels in a class are correctly classified (*omission error*) is obtained by dividing the number of correctly classified pixels in that class by the total number of ground truth pixels selected for that class. This accuracy estimate is called the *producer's accuracy* and it indicates to the producer of the classification how well the classification was for each class with the ground truth data provided. On the other hand, the probability that ground truth pixels classified in the map actually represents that class on the ground (*commission error*) is determined by dividing the total number of correct pixels in that class by the total number of pixels that were classified in that class. This accuracy estimate is known as *user's accuracy* since it provides an estimate of the reliability users should have in the classification. Finally, by dividing the sum of the major diagonal elements of the matrix (total correct pixels) by the total number of pixels, an estimate of *overall accuracy* of the classification is obtained.

Another measure of accuracy that is derived from the confusion matrix is the Kappa statistic ($\kappa$) (Hudson & Ramm 1987, Congalton 1991, Richards 1993). Unlike overall accuracy that considers only the elements of the major diagonal of the error matrix, $\kappa$ indirectly incorporates the off-diagonal elements and hence often does not agree with the *overall accuracy* estimate.

Supervised classification converted the digital images (rasters) of each side of each brood frame into a binary matrix with 1 and 0 representing pixels where brood was found and all other pixels, respectively. Once all of the images for an individual hive body were converted into matrices, we resampled each image to insure that the matrices were of a uniform size. At this point, the matrices were converted into text files and imported into MatLab to be combined into a single 2-D matrix representing brood distribution within the hive. The combined brood matrix was overlaid and compared with the Varroa mite population distribution (e.g., Fig. 3.2).

For each sampling period, three brood distributions were made per hive, one for all bee brood, one worker brood, and one of only drone brood. In this way mite distribution could be compared against the two types of brood, and to brood as a whole, to determine which was most strongly associated with the distribution of mite fall on the sticky board.

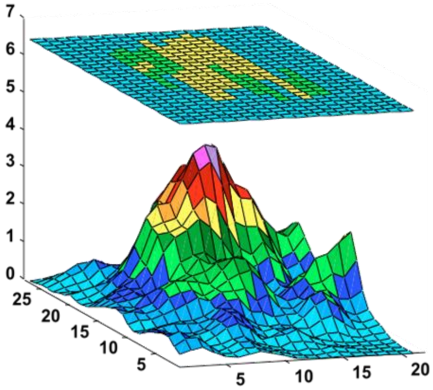

**Fig. 3.2.** Example of the overlay of the distributions of brood (top distribution) and mite fall (bottom distribution) for comparison of the spatial distributions. Shades higher on the color spectrum indicated greater intensity.

### 3.2.5 Association of Mite and Brood Distributions

The degree of association between the two distributions for bee brood and Varroa mite (e.g., Fig. 3.2) was quantified in SADIE by the index of association or dissociation ($I_m$) between pair-wise spatial data sets (Thomas et al. 2001). Values of $I_m > 0$ indicate spatial association and negative values ($I_m < 0$) indicated disassociation between counts in the two data sets (Thomas et al. 2001). The probability ($P_m$) of a significant association or disassociation between the two distributions was indicated when $P_m < 0.025$ and $P_m > 0.975$, respectively.

### 3.2.6 Statistical Analysis.

A Correspondence Analysis (Greenacre 1993; Hair et al. 2010) was conducted to examine the relationship between the index of aggregation ($I_m$) from the comparison of the spatial distributions of worker, drone, and combined brood and mite fall on sticky boards across months (May–September) for sampling conducted in 2013. The correspondence graph that is generated from the analysis shows the relationship between month and brood type in a 2-D space with different lines representing the two variables (Fig. 3.5). The closeness of the lines and the angle formed by the lines indicate the degree of correlation between the variables. An acute angle (i.e., < 90°) between the lines for two variables indicates a positive correlation between the variables; an obtuse angle (> 90°) between the two variables indicates negative correlation. An angle of 0° or 180° indicates a correlation of 1.0 or -1.0, respectively, while an angle of 90° represents a correlation of zero. Because the analysis does not consider values less than 0.0, the aggregation index was transformed as $I_m + 1$ for analysis.

## 3.3 Results and Discussion

### 3.3.1 Image Classification

Feature classification was used to process a total of 3,671 brood frame images from 14 honey bee hives. Fig. 3.3 shows an example of a brood frame at different stages during the feature classification process. An error matrix, generated as part of the feature classification of each image showed that the classifications were highly accurate. Overall Kappa were >95% (Table 3.1).

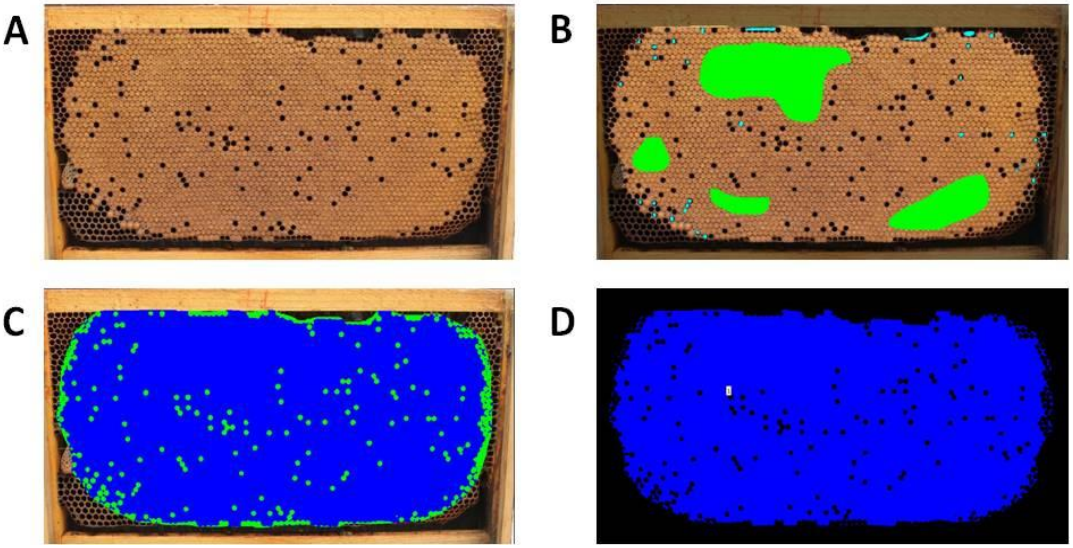

**Fig. 3.3.** Example of a brood frame at different stages in the feature classification process. (**A**) unclassified brood frame: (**B**) the frame after masking and selection of representative pixels of two features (brood and empty cells); (**C**) image after feature classification, and (**D**), classified frame with brood pixels represented by 1 and all other pixels represented by 0.

**Table 3.1.** Kappa accuracy values from the supervised feature classification of the raster images of honey bee brood frames.

| Apiary | Hive | All Brood | | Worker | | Drone | |
|---|---|---|---|---|---|---|---|
| | | Number of Images ($n_B$) | Mean % Kappa (± SE) | Number of Images ($n_W$) | Mean % Kappa (± SE) | Number of Images ($n_D$) | Mean % Kappa (± SE) |
| Prices Fork | 1 | 91 | 98.0 (± 0.3) | 86 | 98.9 (± 0.2) | 57 | 98.3 (± 0.4) |
| | 3 | 175 | 98.3 (± 0.2) | 153 | 98.9 (± 0.1) | 104 | 98.2 (± 0.4) |
| | 15 | 104 | 95.8 (± 0.5) | 86 | 98.1 (± 0.4) | 80 | 96.1 (± 0.8) |
| | 22 | 86 | 95.8 (± 0.7) | 83 | 95.9 (± 0.8) | 23 | 97.6 (± 0.7) |
| | J1 | 147 | 96.7 (± 0.3) | 133 | 97.1 (± 0.3) | 60 | 96.8 (± 0.7) |
| Kentland | 5 | 70 | 98.9 (± 0.2) | 61 | 98.9 (± 0.2) | 41 | 99.1 (± 0.2) |
| | 10 | 110 | 98.0 (± 0.3) | 83 | 98.6 (± 0.3) | 85 | 98.6 (± 0.3) |
| | 39 | 33 | 97.8 (± 0.6) | 31 | 98.1 (± 0.4) | 8 | 95.4 (± 1.9) |
| | 57 | 75 | 97.5 (± 0.4) | 62 | 98.7 (± 0.2) | 42 | 96.3 (± 0.7) |
| | 63 | 118 | 96.8 (± 0.5) | 113 | 97.5 (± 0.5) | 54 | 96.9 (± 0.7) |
| Moore Farm | 4 | 94 | 98.5 (± 0.2) | 92 | 99.0 (± 0.2) | 50 | 98.5 (± 0.3) |
| | 8 | 81 | 98.1 (± 0.3) | 72 | 98.6 (± 0.2) | 61 | 98.5 (± 0.3) |
| | 88 | 159 | 97.7 (± 0.4) | 139 | 98.6 (± 0.3) | 81 | 98.0 (± 0.4) |
| | 92 | 158 | 96.7 (± 0.7) | 133 | 96.9 (± 0.8) | 97 | 98.3 (± 0.4) |

The quality of the images that result from feature classification of remote sensing data depends on several factors (Brewster et al. 1999). The extremely high accuracy levels for images of the brood frame that resulted from the feature classification in this study is not surprising given the ability of the analyst to create regions of interest on the image to mask unwanted features and the fact that there were only two features on interest in each image (i.e., the specific brood type and other). Accuracy is generally expected to decrease as the number of features of interest increases (Brewster et al. 1999).

### 3.3.1 Mite Fall and Brood Association

Overall, a positive association was observed between the spatial distribution of mite fall on sticky boards and the spatial distribution of brood within the hive (Table 3.2). Additionally, the degree of mite and all brood association increased with date possibly due to the increase in mite population (Fig. 3.4) and the simultaneous decrease of brood rearing that occurs in late summer / early fall (Winston 1987). A graphical example of the association between the spatial distributions of brood and mite fall is shown in Fig. 3.5 as overlay images.

The results of the correspondence analysis are shown in Fig. 3.6. There appears to be a positive correlation in the index of association ($I_m$) between spatial distribution of brood and mite fall within hives for sampling conducted in June and May; similarly, a positive correlation was observed in $I_m$ for sampling in May and July. However, there appears to be no correlation between $I_m$ for sampling conducted in June and July, as indicated by the 90° angle between the two variables (Fig. 3.6). A strong positive correlation was observed in $I_m$ for sampling conducted in August and September. Overall, there appears to be a negative correlation between the $I_m$ for sampling conducted during the period May, June and, July (Summer) and August and September (Early Fall). Fig. 3.6 also shows a strong correlation between $I_m$ for worker and all brood (worker and drone brood) with respect to mite fall within the hive. In addition, the $I_m$ for both worker and all brood appear to be related to sampling in August and September, while those for drone brood tend to be related to the periods of sampling in May, June, and July.

The amount of drone brood within a hive will change throughout the season, with drone levels higher in spring and summer than in fall (Winston 1987). Here we show that during late spring and early summer, when there is assumed to be a greater amount of drone brood within hives, there exists a greater correspondence between the $I_m$ for drone brood and mite fall than there does between the $I_m$ for worker brood and mite fall and the $I_m$ for all brood and mite fall. This would suggest that when drone brood are present, mites are more highly spatially associated with the drone brood than with worker brood, and when hives slow the production of drone brood, mites shift over to the more abundant worker brood. As we demonstrated in Chapter 2, mites may also become concentrated within the hive as overall brood production slows later in the season, lowering the number of cells available for infestation at the point of highest mite population. Therefore, a Precision Mite Management program which targets drone brood in the spring to knock down mite levels as they build up and worker brood in the fall to reduce the mite load on winter bees may have a greater impact on overall mite levels and colony health than would a single treatment of the whole hive during the fall.

**Table 3.2: Mean association indices between the spatial distribution of Varroa mite fall on sticky boards and the spatial distribution of worker, drone, or all brood.**

| Year | Period | Month | Number of Hives | Mean mite fall per hive (± SE) | Mean $I_m$ (± SE) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Mite:Brood | Mite:Worker | Mite:Drone |
| 2012 | Spring | May | 2 | 32.5 (± 11.5) | 0.32 (± 0.27) | 0.32 (± 0.28) | 0.29 (± 0.29) |
| | Summer | July | 3 | 87.5 (± 61.5) | 0.07 (± 0.05) | 0.02 (± 0.08) | 0.04 (± 0.05) |
| | Fall | August | 3 | 188 (± 24.3) | 0.42 (± 0.05) | 0.41 (± 0.07) | 0.41 (± 0.06) |
| 2013 | Spring | May | 16 | 39.9 (± 11.3) | 0.02 (± 0.06) | -0.03 (± 0.07) | 0.15 (± 0.06) |
| | Summer | June | 13 | 61.8 (± 17.3) | 0.11 (± 0.07) | 0.07 (± 0.08) | 0.13 (± 0.06) |
| | | July | 13 | 177.9 (± 68.8) | 0.23 (± 0.05) | 0.16 (± 0.06) | 0.24 (± 0.07) |
| | Fall | August | 13 | 227.5 (± 41.1) | 0.21 (± 0.04) | 0.22 (± 0.04) | -0.06 (± 0.05) |
| | | September | 13 | 294.5 (± 57.7) | 0.27 (± 0.08) | 0.26 (± 0.09) | 0.17 (± 0.08) |

$I_m > 0$ and $I_m < 0$ indicate spatial association and disassociation respectively.

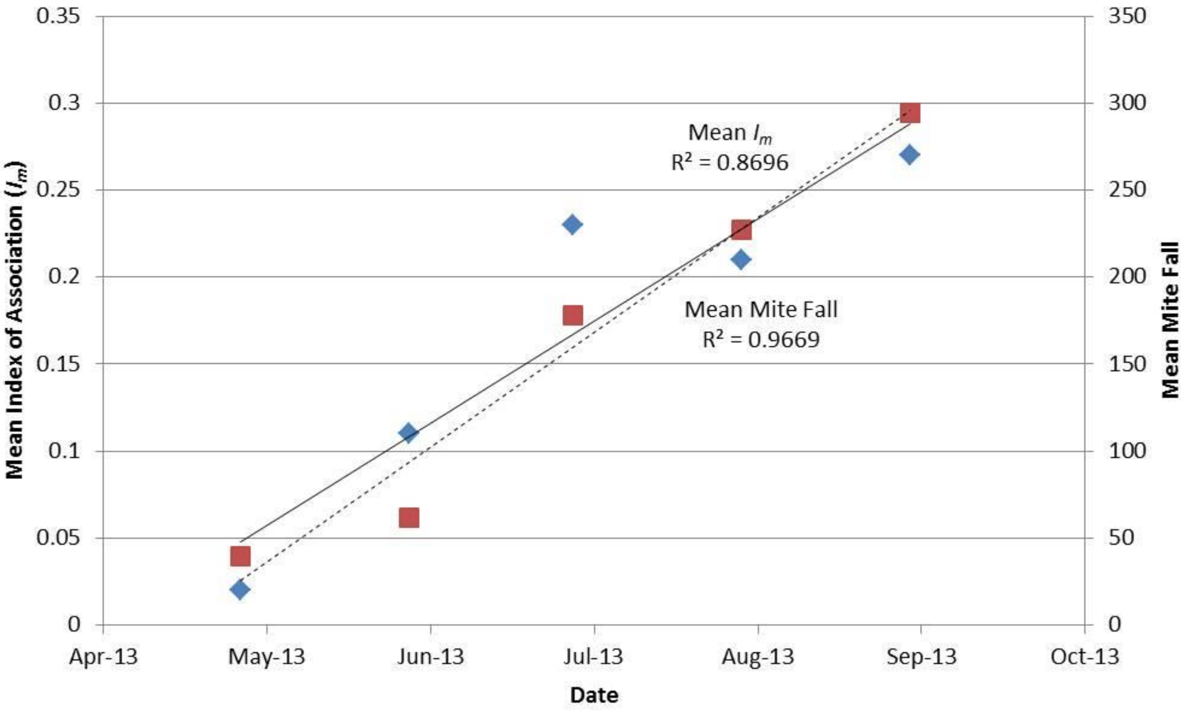

**Fig. 3.4** Relationship of mean Index of Association ($I_m$) and date and mean mite fall and date for hives sampled from May to September 2013.

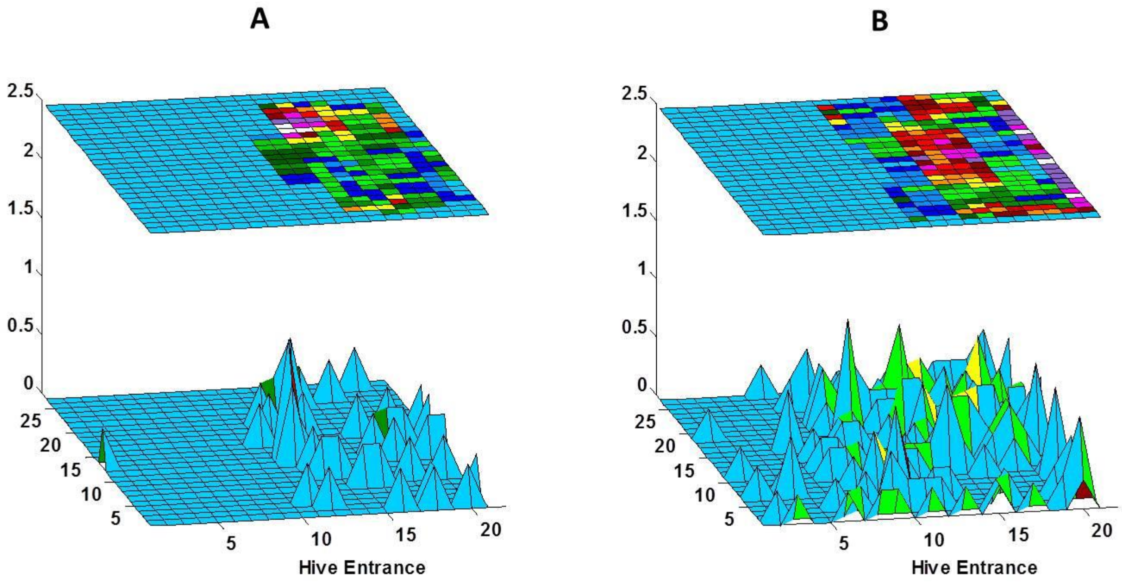

**Fig. 3.5** Examples overlays of brood and mite fall spatial distributions for (**A**) May all brood, $I_m$ = 0.5955; and (**B**) August all brood; $I_m$ = 0.4177. Shades higher on the color spectrum indicate greater intensity, with white as the peak.

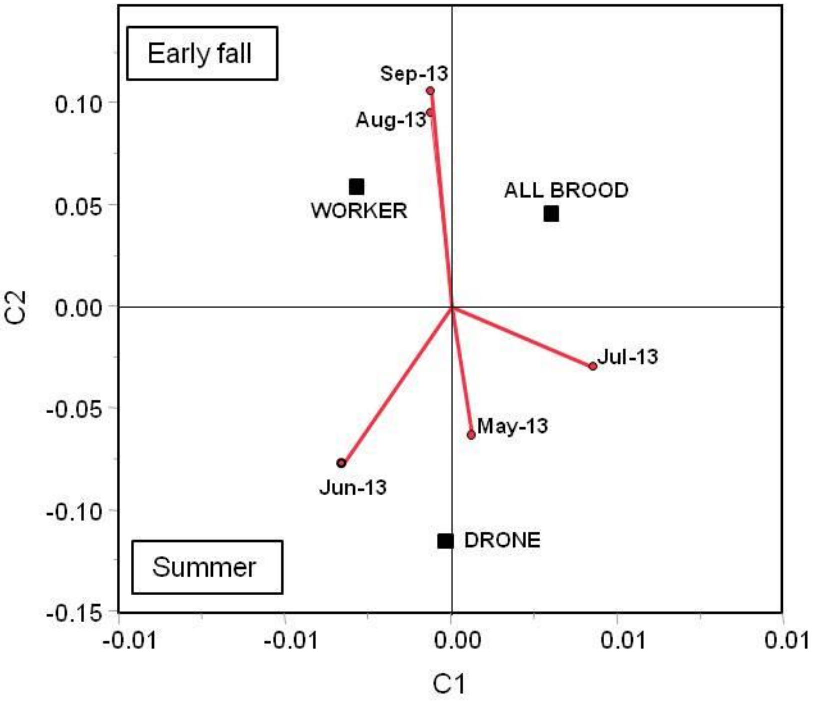

**Fig. 3.6** Correspondence graph showing the relationship between brood type and month of sampling for the index of association ($I_m$ + 1) between the spatial distributions of mite fall and brood for year 2013.

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# CHAPTER 4

# Manipulation of Mite Distribution and Evaulation of Targeted Treatment for Precision Mite Management

## 4.1. Introduction

*Varroa destructor* mites have been considered one of the most damaging pests of *Apis mellifera*, the European honey bee, since the mite first invaded hives of *A. mellifera*, sometime in the mid-20th century (Smirnov 1978). The mites feed on developing bee brood, reducing lifespan, productivity and size and can transmit viruses to the bee during feeding. Some of these viruses, such as the Israeli Acute Paralysis Virus, have been implicated in the set of symptoms recently termed Colony Collapse Disorder or CCD (Cox-Foster et al. 2007). Honey bees are responsible for over 90% of commercial pollination services (Genersch et al. 2010), and therefore represent billions of dollars in the worldwide economy (Gallai et al. 2007). Regardless of their role in CCD, damage from Varroa mites poses a serious threat to this worldwide agricultural commodity and because of this there have been several methods developed for mite control.

The most common method of control utilized by both small scale and large scale beekeepers is the use of synthetic acaricides (Rosenkranz et al. 2010). Typically, synthetic acaricides are applied as plastic strips impregnated with the chemicals and are placed directly into the hive, between frames of comb. These chemicals target the central nervous system of the mite and induce over excitation of nerves, leading to paralysis and death (Rosenkranz et al. 2010). Labor and financial costs for synthetic acaricide treatments are low, and they can have a significant effect on mite populations. However, there are serious drawbacks to their use. Synthetic acaricides are lipophilic, which causes them to be sequestered in hive materials such as beeswax and propolis.  The build-up of these materials within the hive may lead to serious issues for the honey bees; with recent research showing that exposure to a common Varroa mite treatment, coumaphos, can harm drone sperm viability (Burley et al. 2008).  Mites have also developed resistance to the commonly used acaricides, often quite rapidly (Hillesheim et al. 1996; Elzen et al. 1999).

A wide variety of alternative methods have also been developed for the control of *V. destructor*, though all have drawbacks when used alone or incorrectly.  Some organic acids, for example, have the benefit of being produced by bees naturally, meaning that at low levels we can assume that they are more or less safe and contamination will be a lower concern compared to synthetic acaricides (Rosenkranz et al. 2010).  In addition, there is the potential for development of resistance to organic acids by the mite.  Organic acids are fairly volatile, however, and efficacy is dependent on temperature, humidity and hive location.  Some organic acids, such as oxalic and formic acid, can also cause health problems for both the brood and the applicator (Emsen and Dodologlu 2009).  Cultural and mechanical methods can also help control Varroa mites, and do so in a way which bypasses any concern over resistance development, or harm to the brood/applicator.  One of the most common cultural methods for Varroa control is drone brood trapping and removal.  Mites have been shown to prefer drone brood over worker brood (Fuchs 1990) and, therefore, a beekeeper can place two frames of drone brood foundation into their hive and freeze or heat the frames once they are full of capped drone brood, killing the mites inside.  The thought is that a large concentration of drone brood will attract a large proportion of the mite population and through the sacrifice of the brood, mite levels can be reduced.  This approach has been shown to work for small, hobbyist beekeeper operations (Fries and Hansen 1993), but the labor cost is likely too high for large numbers of hives.

Many of the aforementioned drawbacks to mite control methods may be reduced or eliminated through the use of a Precision Mite Management (PMM) program as part of an Integrated Pest Management strategy (IPM). The main tenant of an IPM program is the use of multiple control measures in order to reduce pest populations to a sustainable and non-harmful level (Overton 1996). This approach would include conventional methods such as acaricidal strips, but would also include cultural practices, such as drone brood removal. In addition an extensive understanding of the spatiotemporal relationship between Varroa mites and honey bee brood could benefite control practices. As the season progresses, mite populations fluctuate in response to brood dynamics, and if we were better equipped to predict these fluctuations then we may be better able to apply treatments in a more effective manner.

Thus far, we have found that mites and brood are spatiotemporally associated (Objective 2), whether mite distributions are predictable, stable and manipulatable still needs to be determined. If we can manipulate frames of drone brood and shift the mite population distribution in a similar manner, then we will know that the relationship between drone brood and mites is stable and predictable. Ideally, the relationship between mite fall and drone brood could be exploited for mite control by using a targeted acaricidal strip treatment in early fall, following a drone brood removal event in late spring.

## 4.2 Materials and Methods

### 4.2.1 Apiaries and Hives

For an explanation of the apiaries used in these studies, see Chapter 2. Three hives were located at both the PFRC and Kentland Farm apiaries, while two were located at the Moore Farm apiary, for a total of eight hives. For hives used in the drone manipulation study, queens were confined to one full depth hive body through the use of a metal queen excluder. Control and traditional hives in the precision treatment study varied in size, though all those treated in the targeted manner had their queens confined to one full depth hive body.

### 4.2.2 Drone Manipulation

Two frames containing drone foundation were placed in each of the eight hives. The sampling schedule differed between hives, depending on the speed at which the worker bees drew out the comb and when the queen deposited eggs within the cells. For four of the eight hives, mite distribution, as well as the distribution of the bee brood, was assessed before and after manipulation of drone frame placement. The remaining four hives acted as controls, and were sampled in the same manner as the experimental hives without frame manipulation.

After the initial mite and brood distribution sampling, the two frames of drone brood in each hive were frozen overnight and placed back in the hive, either in the same position (control hives) or on the opposite side of the hive (experimental hives). Bees will remove the dead brood and mites from the cells, allowing the queen to repopulate them with eggs. As before, sticky boards were placed in the hives once a majority of the cells contained mature drone larvae and would be capped at some point during the three day period that the sticky boards were present in the hives.

### 4.2.3 Mite Population and Distribution

For an explanation of mite distribution estimation see Chapter 2.

### 4.2.4 Brood Distribution

For an explanation of brood distribution estimation see Chapter 3.

### 4.2.5. Comparison of Traditional and Precision Miticide Treatment

As a test of the efficacy of a precision mite management treatment program acaricidal (amitraz) strips were placed in twelve hives. Six hives were treated as per the manufacturer's instructions (2 strips per 5 frames of bees), while the other six had a single strip of amitraz placed between the two frames of drone comb within each hive. An additional six hives acted as controls. Strips were left in the hives for a period of six weeks, as per the manufacturer's instructions, and mite populations were assessed using sticky boards before and after strip application. The treatments were compared using a chi-squared contingency analysis with the null hypothesis of no association between the three treatment strategies and observation time (Pre-treatment and Post-treatment).

## 4.3 Results and Discussion

There are many varieties of pest control programs, utilizing a wide breadth of treatment materials and strategies. Precision agriculture relies on the use of pest spatiotemporal dynamics understood through both experience and information, and communication technologies, to better target treatment methods and reduce environmental impact and the use of harmful chemicals (Srinivasan 2006). The goal of this study was to test the efficacy of using a precision agricultural approach to control *V. destructor* within honey bee hives.

Thus far (Chapter 3) we have shown that mite fall distribution is associated positively with brood and this study's goal was to test the feasibility and efficacy of applying knowledge of this relationship to Varroa control. Due to the known mite preference for drone brood (Fuchs

1990) we based our sampling on the spatial relationship between the Varroa mites and drone brood. First we tested whether the relationship between drone brood and mite distributions was predictable and stable.

Predictability of the mite–drone spatial relationship was tested by shifting frames containing primarily drone brood from one side of the hive to the other, and measuring the spatial association between mite fall and drone and worker brood before and after the shift. Results were variable (Table 4.1) with control hives showing equal mean mite–drone and mite–worker $I_m$ values before the shift, and a greater mean mite–worker $I_m$ after the shift, while experimental hives showed a strong mite–worker disassociation before the shift, and a strong mite–drone association after the shift.

**Table 4.1.** Mean Index of Association ($I_m$) before (B) and after (A) drone brood shift.

| **Treatment** | **Before** | | **After** | |
|---|---|---|---|---|
| | **Mite:Drone** | **Mite:Worker** | **Mite:Drone** | **Mite:Worker** |
| Control | 0.05 | 0.05 | 0.06 | 0.13 |
| Experimental | 0.05 | -0.15 | 0.11 | 0.04 |

Four hives were used for each shift treatment, and therefore these results are possibly due to the small sample size. In addition, the timing of the shift was entirely dependent on when bees had drawn out the comb, and the queen had filled the cells with eggs. This led to disparity in shift timing between the hives, and as a result, the amount of drone brood on shifted frames was variable. Due to these factors and the small sample size, it is difficult to draw many conclusions, but clearly mite distribution can shift in response to drone brood movement, and with a greater sample size trends may become apparent. An example of a strong mite distribution shift can be seen in Fig. 4.1.

We tested the efficacy of applying a within-hive treatment for Varroa mites in a precision manner, coupled with an early season drone brood removal, by placing a single strip containing Amitraz between two frames of drone brood. The results show that there was a significant association between treatment strategy and observation time with respect to mite fall levels within the hives (Fig. 4.2; $\chi2$ =362.571; df = 2; $P <0.0001$). The traditional method had a significantly greater reduction in mite levels compared with both the precision and control method. However, mite population levels with the precision method were significantly reduced compared with the control (Table 4.2). Additionally, the traditional method calls for 2 acaricide-impregnated strips per 5 frames of bees, which led the hives treated in the traditional manner to be treated with an average of 3 strips per hive. The traditional method resulted in an average mite population reduction of 70%, which means that there was an average reduction per strip of acaricide of 23%. This is the same degree of mite reduction as the precision treatment, however the precision treatment used only a third of the treatment material. Therefore we can state with confidence that the precision treatment increased treatment efficacy over the traditional treatment method.

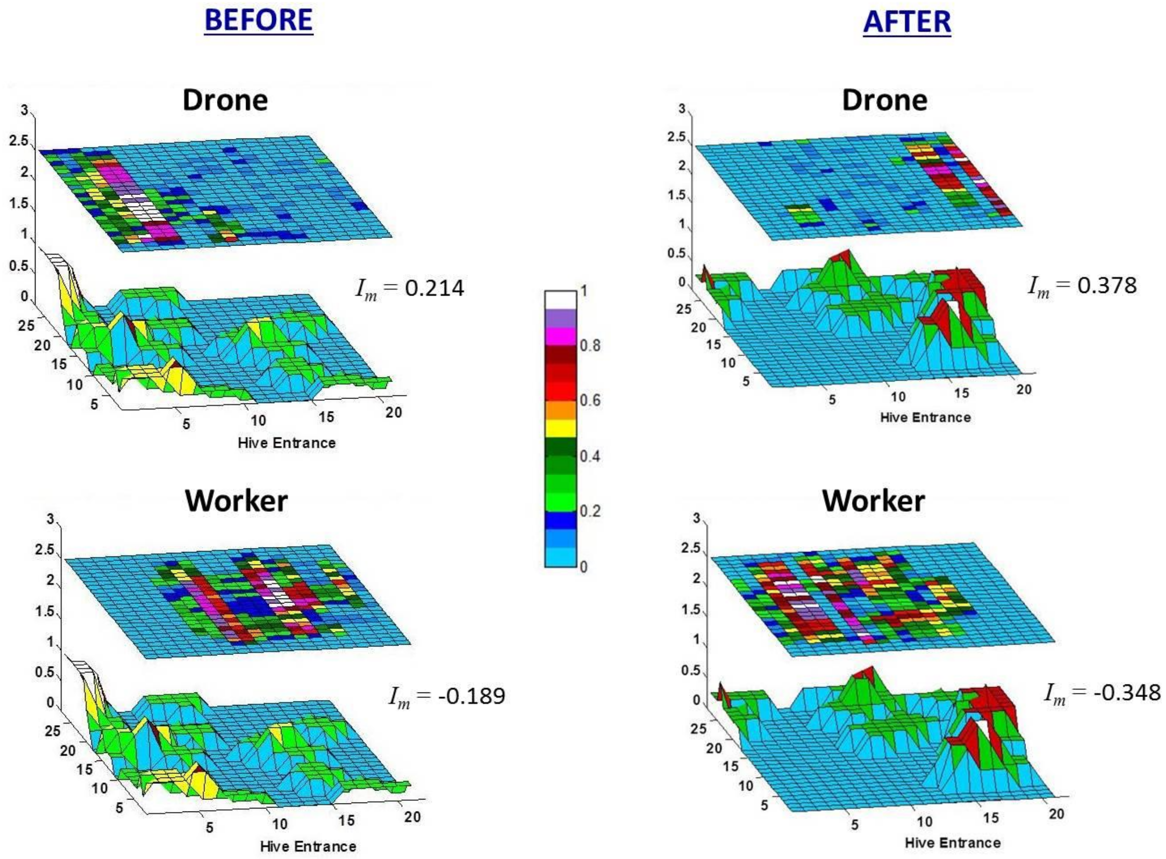

**Fig. 4.1** Example images of the spatial distributions of drone and worker brood and mite fall on sticky boards showing a successful mite population shift using frames of drone brood. A generalized color scale is displayed in the center.

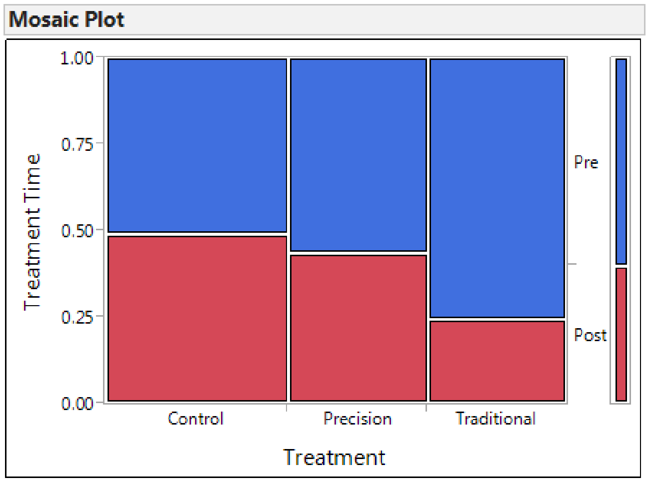

**Fig. 4.2.** Mosaic plot from the contingency analysis of the association between Treatment time (Pre and Post) and Treatment (Control, Precision, and Traditional) on mite fall numbers within honey bee hives.

**Table 4.2.** Mean mite fall after a 72-hour period before and after strip treatment

| Treatment | Number of Hives | Mite Fall | | % Reduction in mite fall[a] |
|---|---|---|---|---|
| | | Before | After | |
| Control | 6 | 278 | 265 | 4.6 a |
| Traditional | 6 | 308 | 101 | 70 b |
| Precision | 6 | 235 | 181 | 23 c |

[a] Percentages followed by the same lowercase letter are not significantly different (P>0.05)

These results are promising for a precisions mite treatment protocol, however, as with the mite distribution shift experiment, the sample size was lower than would be ideal and greater trends may be revealed with an increase of the sample size. This study was also performed late in the season (September – October) when hives were available for use. Because of the late timing, drone brood levels within the hive were much lower than in late spring and early summer and, as we showed in Chapter 3, mite and drone brood association is lower later in the season than in spring and summer. Precision management not only refers to the precise application of treatments in space, but also the precise application of treatments in time (Srinivasan 2006). For a Precision Mite Management program to be evaluated fully, precision application would need to be tested using a range of time periods, such as late spring, mid-summer and early fall. However, these results show that a precision treatment, along with an early season drone brood removal, can have an important effect on mite populations within the hive.

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# CHAPTER 5
## Summary

*Varroa destructor* (Anderson and Trueman) (Parasitiformes: Varroidae) is an ectoparasitic mite which feeds on both adults and developing brood of *Apis mellifera* (Linnaeus) (Hymenoptera: Apidae). Mites have been shown to vector around 13 different viruses, some of which can cause hives to collapse in short periods of time (Kevan et al. 2006; Li et al. 2014). Feeding by Varroa mites can also reduce bee size, lifespan and productivity (Rosenkranz et al. 2010). Honey bees are responsible for over 90% of commercial pollination services (Genersch et al. 2010), and therefore represent billions of dollars in the worldwide economy (Gallai et al. 2007). Many treatments exist for the control of *V. destructor* however the improper use of synthetic acaricides has led to the development of mite resistance, and the spread of potentially harmful chemical residues within hives. Alternatives to synthetic acaricide treatments exist, but have their own set of drawbacks and often work poorly when implemented alone (Rosenkranz et al. 2010). The goal of this research was to investigate the spatiotemporal relationship between Varroa mites and honey bee brood, and determine the feasibility of the use of this relationship to improve mite sampling strategies and for the development of a Precision Mite Management program.

### Chapter 2

Sampling is the first line of defense which a beekeeper may employ again Varroa mites (Ostiguy and Sammatarro 2000). By understanding the mite levels within a hive, treatment methods may be implemented in an efficient manner (Rosenkranz et al. 2010). To this end, we examined the correlation between the mite level estimation of two common sampling methods, the soapy water roll and sticky boards. There was significant correlation later in the year, but in early spring the estimates of the two sampling methods were highly divergent. We also measured mite aggregation on sticky boards. Mites were found to be aggregated on sticky boards throughout the 2012 and 2013 sampling periods, with a strong positive relationship between numbers of mites falling through the hives and mite aggregation. A sampling method incorporating geospatial information on the mite fall was developed using commercially available sticky boards, and while accuracy was similar between the standard counting method and the geospatial counting method, the effort was 40% lower in the latter. We focused our counting method comparisons on the July – September time period, due to this being the preferred time of sampling for beekeepers (Rosenkranz et al. 2010).

## Chapter 3

Precision agriculture programs implemented for the control of a pest rely on a deep understanding of the relationship between the pest being treated and its host (Srinivasan 2006). One aspect of the Varroa mite and honey bee relationship which has yet to be properly explored is that of the spatiotemporal distribution. By mapping mite and brood distributions treatments can be applied in a more targeted manner. Here, we showed that by taking photographs of hive frames, a digital matrix of capped brood cells can be generated using geospatial software with a high degree of accuracy. Additionally, mite fall distribution was found to be strongly associated with brood distribution throughout the sampling periods for both 2012 and 2013, with the correspondence between drone brood distribution and mite fall distribution higher in the spring and summer than in the fall, while worker brood and mite fall were most highly associated in the fall.

## Chapter 4

Drone brood has been demonstrated to be more attractive to mites than is worker brood (Fuchs 1990), and as they contribute nothing to the hive directly, they are considered more expendable than worker brood (Winston 1987). Therefore we focused our exploration of the feasibility of a Precision Mite Management plan on drone brood. Before spatiotemporal relationships between a pest and host can be used in a treatment program the stability of the model must be demonstrated (Srinivasan 2006). To test the stability of mite and drone brood spatiotemporal distribution we shifted frames containing primarily drone brood from one side of the hive to the other, and measured the association between mite fall and worker and drone brood before and after the shift. Results were mixed, with some hives displaying strong associations and mite shift, while others showed little association between mite fall and drone brood, or even apparent repulsion. Such confusing results are likely due to the overall small sample size of the shift study. In addition, we examined the impact of a late season synthetic acaricide targeted treatment application, along with an early season drone brood removal, on mite levels, and compared this impact to that of the traditional synthetic acaricide treatment method. Both the traditional and the precision treatment methods lowered mite levels and were significantly different than the control; however the traditional method reduced the mite fall to a greater degree. These results are not surprising, however, as the traditional method calls for two strips of acaricide per five frames of bees, which meant that some hives were treated with up to six strips.

## Future Research

A wide variety of treatment methods utilizing the mite and drone brood spatiotemporal associations should be explored for the control of *Varroa destructor*. These could include synthetic acaricides, organic acids, cultural and mechanical control and biological control agents. One of the more interesting biological control agents is *Metarhizium anisopliae*; a fungus which rapidly overtakes Varroa mites but will leave honey bees relatively unharmed (Kanga et al. 2002). The major issue thus far with the use of *M. anisopliae* to control Varroa mites has been the need for frequent reapplication and the relatively poor spread of the control agent throughout the hive (Rosenkranz et al. 2010). Efficacy of *M. anisopliae* treatments could be increased if application could be centralized on frames primarily containing drone brood, preferably a day or two before capping to get the maximum number of invading mites. Treatments need to also be tested at different times of the year, so as to find the most efficacious time to target Varroa mites on brood. In addition, when using synthetic acaricides, it would be beneficial to test the pesticide residue level of the traditional strip treatment method agains that of the precision strip treatment method.

Finally, a study should also be undertaken to examine treating drone and worker brood at different times of the year. Drone brood should continue to be the target of treatments such as synthetic acaricides, but worker brood could also be treated in later summer with less damaging methods, such as organic acids / essential oils. In this manner a Precision Mite Management program could be devised which takes advantage of not only the times of the year when mite fall and drone brood are highly associated, but also when mite fall is highly associated with worker brood.

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