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Essential oil treatments to control *Varroa destructor* Anderson and Trueman 2000 (formerly *Varroa jacobsoni* Oudemans 1904) (Mesostigmata: Varroidae)

Maria Mercedes Vargas-Sarmiento
West Virginia University

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Essential Oil Treatments to Control *Varroa destructor* Anderson and Trueman 2000
(formerly *Varroa jacobsoni* Oudemans 1904)
(Mesostigmata: Varroidae)

María Mercedes Vargas-Sarmiento, B.Sc

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Dr. James Amrine, Chair
Dr. Linda Butler
Dr. William Bryan

Department of Entomology

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ABSTRACT

Varroa destructor Anderson and Trueman is the most serious pest of the honey bee *Apis mellifera* L. Varroa mites transmit several viruses, bacteria and fungi to the bees, which results in a condition called parasitic mite syndrome. There are no completely reliable treatments available to control the mite. Fifty-one beehives were artificially infested with varroa mites by using three different methods. Observations on mite population growth during three infestation attempts were made. All colonies were inspected for the presence of the small hive beetle, *Aethina tumida* Murray, no beetles were found. Thirty-five colonies were used to establish the efficacy of essential oils and Apistan® to control *V. destructor*. Three essential oil treatments were delivered into the hives using different application methods. Eight percent Wintergreen in mineral oil was delivered by using a modified bottom board. A 2:1 blend of lemongrass and spearmint oils in a 50:50 sucrose to water solution was administered to the bees with Boardman feeders and quart Mason jars with perforated lids. A combination of paper towels and grease patties containing lemongrass and wintergreen were placed into the hives. Treatments were applied for 29 days. Apistan® was the best treatment. There were no differences between essential oil treatments and their controls. The essential oils tested did not appear to control varroa mites under the conditions of the experiment. It was also observed that lemongrass essential oil may alter hygienic behavior in honey bees, and wintergreen evaporated from a modified bottom board produced behavioral disturbances in bees.

To: Marc Clinton Apgar for his unconditional love and support.

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INTRODUCTION

The varroa mite, *Varroa destructor* is considered the most serious pest affecting the Western honey bee *Apis mellifera* L. (Fries *et al.* 1994). Since the appearance of the mite on *A. mellifera*, honey yields have been reduced, and decreases in both apiary and feral honey bee colonies have reduced crop pollination and agricultural yields (Fries *et al.* 1994). The mite is found worldwide, except for Ireland, Australia, and Central Africa (Oldroyd 1999, Delaplane 1997, Fries *et al.* 1994).

Recently, genetic studies have demonstrated the existence of genotypic variations among populations of the honey bee parasite, *Varroa jacobsoni* Oudemans 1904. These studies also confirmed reproductive isolation of varroa populations in Asia and this pest was renamed *Varroa destructor* Anderson and Trueman 2000. Past research on *V. jacobsoni* is probably referable to *V. destructor* (Anderson and Trueman 2000).

Typical efforts to control the mite include biomechanical methods, organic acids and pesticides. Biomechanical methods that involve brood manipulation have proven to be inefficient or impractical for large-scale beekeeping operations (Delaplane 1997). The misuse of pesticides and organic acids has resulted in mite resistance, bee health problems, and the contamination of honey bee products (Pettis and Shimanuki 1999, Colin *et al.* 1997, Lodesani *et al.* 1995, Imdorf *et al.* 1999).

Essential oils are good alternatives for control programs targeting varroa mites. Several essential oils have shown acaricidal activity in screening tests, but it is necessary to prove their efficacy and bee compatibility in field trials (Imdorf and Bogdanov 1999).

The objectives of this work were to find efficacious essential oils and adequate delivery methods to control varroa mites, without affecting bee health or hive products.

1. Literature Review

1.1 Varroatosis.

The infestation of brood of *Apis mellifera* L. by *Varroa destructor* is known as varroatosis (Ball 1988). In general, varroatosis reduces the life span of bees, including the queen, and reduces the bee population. *Varroa destructor* feeds on the hemolymph of adults and immature honey bees causing a reduction of up to 50% in the protein content of the hemolymph, a 30% reduction of hemocytes, weight loss, and wing and limb deformity in adults (Ball 1988, Strick and Madel 1988). However, there is little evidence that feeding activities of mites alone lead directly to the death of the colony (Martin 1997). *Varroa destructor* transmits several viruses, which together with the feeding injury caused by the mite results in a condition called Parasitic Mite Syndrome (PMS) (Shimanuki *et al.* 1994). Acute paralysis virus, chronic bee paralysis virus, sac brood virus, and deformed wing viruses, have been isolated from bees collected from colonies infested with *V. destructor*, which suggests that the mite acts as a vector of these viruses (Martin 1997, Ball 1988, Bowen - Walker *et al.* 1999). Fungi and bacteria have also been isolated from bees attacked by varroa mites. According to Strick and Madel (1988) varroa mites participate in the transmission of the bacterium *Hafnia alvei*, which can cause septic infections, and is considered to be a moderate pathogen of bees. Other pathogens associated with *V. destructor* are *Ascosphaera apis*, and several fungi in the genus *Aspergillus*, which cause symptoms of chalkbrood and stonebrood disease, respectively (Ritter 1988).

Colony symptoms depend on the number of mites and presence of viruses (Amrine, personal communication). High infestations are characterized by the presence of malformed workers and drones with malformed wings that are unable to fly (Anonymous 1995) ([Appendix 1](#)). A parasitized colony of *A. mellifera* can be destroyed within five years, and sometimes within only a few months depending on the climate, presence of viruses and degree of infestation (Korpela *et al.* 1992). It has been observed that overwintering infested colonies are restless and do not form clusters, which reduces their chance of survival during the winter (Langhe and Natsgii 1977).

According to Robaux (1986) factors that result in mite spread are: worker and drone drift, swarming, superposition of colonies, honey robbing, frame exchange, and migratory beekeeping. Undoubtedly, beekeeping practices promote the transmission of the parasite.

The occurrence of mites in apiaries does not mean that mites are only present in commercial beekeeping operations. Varroa mites were found in feral hives until most wild bees died out (Sherman *et al.* 1998, Ritter 1988).

1.2 Biogeography¹ of *Varroa destructor*.

The mite originally recognized as a honey bee pest, *Varroa jacobsoni*, was first described by Oudemans in 1904 from mites parasitizing *Apis cerana* Fabricius in Java, Indonesia (Anderson and Trueman 2000). Until spring 2000, specimens classified as *Varroa jacobsoni* were reported in hives around the world. The belief was that this species infested the hives of *A. mellifera* and *A. cerana*. But, in 1989 morphometrical studies revealed enough morphological differences to suggest that *Varroa jacobsoni* is a complex of several species (Delfinado-Baker and Houck 1989). More recently, genetic variation was found in *Varroa jacobsoni* that can only be explained by at least two species and the pest of western honey bees and *Apis cerana* in Northern Asia was given the name *V. destructor* (Anderson and Trueman 2000).

1.2.1 The original concept: *Varroa jacobsoni* distribution.

Varroa jacobsoni transferred from *A. cerana*, its original host, to the western honey bee, *A. mellifera*, at least twice in the last 100 years (Oldroyd 1999). The first infestation of *Apis mellifera* took place in Russia.

Apis mellifera became infested with *V. jacobsoni* shortly after the construction of the Trans-Siberian Railroad in 1905. Colonies of *A. mellifera* were moved from west to east, to locations where they contacted populations of *A. cerana* infested with *V. jacobsoni*. Later on, when commercial beekeeping operations returned *A. mellifera* into western Russia, the infestation spread rapidly (Boot, *et al.* 1997). Mites were detected on *A. mellifera* in Eastern Russia in 1952 (Oldroyd 1999). The second infestation of *Apis mellifera* with varroa mites occurred in Japan. *Apis mellifera* was introduced in Japan as early as 1877 and *V. jacobsoni* transferred to it, probably in 1957 from bees introduced from Indonesia (Oldroyd 1999). The species of bee was not indicated.

Since *A. mellifera* colonies became infested in Russia and Japan, *V. jacobsoni* has successfully spread around the world. In the early 1960's, this parasite was detected in *A. mellifera* colonies in Vietnam (Nguyen *et al.* 1997). *Varroa jacobsoni* began to spread to the Mediterranean area in the 1970s (Colin *et al.* 1997), and by 1981 it had reached Northern Italy (Eischen 1995). In 1982, *V. jacobsoni* reached Germany and The Netherlands (De Ruijter and Eijnde 1982). The first reports of the presence of *V. jacobsoni* in Denmark are from 1984 (Brødsgaard and Brødsgaard 1998). The existence of the mite was reported in the U.K. in 1992 (Martin 1997). The mite entered South America on bees imported from Japan in 1972 (Robaux 1986). *Varroa Jacobsoni* reached the United States in 1987 (Ellis *et al.* 1997) ([Appendix 2](#) and [Appendix 3](#)).

¹In this paper, biogeography is understood as distribution of organisms, which involves evolution, extinction and dispersal. Evolution is defined as any irreversible change in the genetic composition of a population (Hengelveld 1992).

1.2.2. The present concept: *Varroa destructor*, a new species

In 1999, a study of mitochondrial DNA (mt-DNA) of *V. jacobsoni* revealed genotypic² variation among populations of the mite (De Guzman and Rinderer 1999). More recently, a second independent mt-DNA study found 18 haplotypes³ that infest *A. cerana*, 6 of which are reproductively isolated (Anderson and Trueman 2000). Different terms are used in each study to refer to the detectable genetic variation among populations of *V. jacobsoni*, but both studies reached the same conclusion: genetic variation can be correlated with mite virulence and bee tolerance to the parasite (De Guzman and Rinderer 1999, Anderson and Trueman 2000).

De Guzman and Rinderer (1999) demonstrated that there are several genotypes of the mite: Russian, Japanese, and Papua New Guinean. Each genotype has a particular distribution, bee host and differences in reproductive performance on *A. cerana* and *A. mellifera*. The Papua New Guinean genotype was originally described by Oudemans and is only found in Asia. Thus, it never spread worldwide as was originally supposed. The Japanese genotype is found in Asia, North and South America. The Russian genotype is the most widely distributed and is found in Europe, Russia, South and North America, Africa, and Asia. The Papua New Guinean genotype reproduces in *A. cerana* drone brood, but it does not reproduce in *A. mellifera* brood. On the contrary, the Russian genotype is capable of reproducing in both *A. mellifera* and *A. cerana* brood. The Japanese genotype is less virulent. It is found in Brazil and Puerto Rico, where *A. mellifera* colonies have survived infestations for over 12 years without any treatment (De Guzman and Rinderer 1999).

There is evidence of at least two independent introductions of *V. jacobsoni* into the United States. The Russian genotype is only found in five states: Wisconsin, Louisiana, Maryland, Minnesota, and Oregon. The Japanese genotype is found in Puerto Rico. Both Russian and Japanese genotypes are found in Arizona, Connecticut, Florida, Georgia, Iowa, Nebraska, Texas, and Virginia. The Russian genotype is the most abundant in the United States and it may have first entered this country from Europe (De Guzman *et al.* 1999).

More recently, Anderson and Trueman (2000) published a new classification for *V. jacobsoni*, based on mt-DNA *CoI* gene sequences of varroa mites collected in 32 countries. These authors described 18 different haplotypes of this mite. Nine occur in the Malaysia-Indonesian region infesting *A. cerana* and include *V. jacobsoni* first described by Oudemans in 1904. Three other haplotypes of varroa occur in the Philippines infesting *A. cerana*, but their taxonomic position is not yet established. Six haplotypes occur in mainland Asia parasitizing *A. cerana* and are reproductively isolated from specimens of varroa found in the Malaysia-Indonesian region. Thus, they are considered a new species named *Varroa destructor*. Two haplotypes belonging to *V. destructor* transferred from *A.*

²

Genotype is understood as “those mites having common DNA variations within the native range of *V. jacobsoni*” (De Guzman and Rinderer 1999).

³

A haplotype is defined as a set made up of one allele of each gene comprising the genotype (Rojas personal communication)

cerana to *A. mellifera*. The Korean⁴ haplotype is the most widely distributed, affecting *A. mellifera* in Europe, the Middle East, Africa, Asia and the Americas. The Japan-Thailand⁵ haplotype is less common and is found in Japan, Thailand and the Americas ([Appendix 4](#)). According to these discoveries, differences in mite haplotypes may explain bee tolerance to the parasite. It is likely that past studies on *V. jacobsoni* are applicable to *V. destructor* (Anderson and Trueman 2000).

1.3 Honey bee defense mechanisms.

Varroa mites and the Eastern honey bee, *Apis cerana*, have a long history of coevolution (Akcratanakul 1990). *Apis cerana* evolved several behavioral mechanisms for resisting the mites and for keeping mite populations within tolerable limits (Sammataro 1996, Spivak 1996). *Apis cerana* bees have a shorter post-capping period, which reduces mite populations by decreasing the mite's available development time (Sammataro 1996). *Apis cerana* displays hygienic behavior as well. The Eastern honey bee has the ability of recognizing, tearing apart, and removing mites from the hive (Spivak 1996). Hygienic behavior is triggered by foreign odors within the hive and it is generally described as a defense mechanism against disease (Rothenbuhler 1964). Responses can be specific to the odor of diseases, varroa-infested, or dead pupae. Honey bees that display hygienic behavior uncap and remove abnormal pupae. The most important components of the behavior are detection and uncapping whereas the removal component is relatively easy and can be done by many bees. The hygienic behavior has little to do with cleanliness, per se (Spivak, personal communication). However, bees also detect and remove from the hive any material that does not have the characteristic smell of the colony (Amrine, personal communication). Adult bees of the Eastern honey bee also display strong grooming behavior toward varroa mites. Some *A. mellifera* colonies remove mites present in uncapped worker brood cells and uncap cells to remove infested larvae (Boeking *et al.* 1993). But, *A. mellifera* remove relatively few infested worker-brood, whereas *A. cerana* remove infested worker-brood and do not uncap cells of drone-brood that have died as a result of mite infestation, which traps the mites within the cells (Fries *et al.* 1994).

The final strategy of Eastern honey bees to escape the attack of varroa mites is absconding; adult bees leave the hive, abandoning infested brood (Akcratanakul 1990).

1.4 Varroa mite morphology.

Several morphological features allow the mites to attach and feed on honey bees. Their body shape helps them enter between the abdominal sternites of adult bees, and extensions of the peritremal tubes regulate the respiration of the mites (Grobov 1977). The empodium of each tarsus is transformed into a lobed sucker, and the mouthparts of the female are adapted for piercing and sucking (Anonymous 1995).

⁴De Guzman *et al.* (1999) refer to the Korean haplotype as the Russian genotype (Andersen and Trueman 2000).

⁵

De Guzman *et al.* (1999) refer to the Japan-Thailand haplotype as the Japanese genotype (Andersen and Trueman 2000).

The body of the female varroa mite is reddish brown to dark reddish brown, and is heavily sclerotized. It is flattened dorso-ventrally, with the dorsal portion slightly convex. Its shape is transverse oval. The body size is approximately 1.0 - 1.8 mm long x 1.5 - 1.9 mm wide. Males are round, about 0.7 mm by 0.7 mm and yellow to grayish white in color (Anonymous 1995). The dorsal scutum covers the idiosoma completely. The stigmata are found between the femora of leg pairs III and IV. The peritremal tube adheres to the cuticle for one third of its length, leaving the rest freely mobile. The inside surface of the peritreme is covered with chitoid formations that increase the respiratory surface (Grobov 1977). Peritrimers are capable of contracting or expanding in response to environmental conditions such as temperature, humidity and atmospheric pressure (Robaux 1986).

The bee louse, *Braula coeca*, is a dipteran parasite of the honey bee, and is often mistaken for varroa mite. It is similar in shape and color, but the bee louse is a wingless fly with six legs and antennae, while the varroa mite has eight legs and lacks antennae. The bee louse prefers queens or worker bees, especially nurse bees, whereas varroa prefer drones and drone brood. The bee louse generally is found on the dorsal surface at the junction of the bee's thorax and abdomen, while varroa is frequently found in between the abdominal segments. The damage caused by the bee louse to a bee colony is minimal; honey yield is not affected, and larval tunnels do only cosmetic damage to honey comb sections (Frazier 1999). *Braula coeca* used to occur in the eastern panhandle of West Virginia, but may be extinct by now (Amrine, personal communication).

1.5 Varroa mite biology.

Honey bee and mite life cycles are closely related. The adult female mites enter the brood cells just before they are capped. A female mite will only lay eggs if it began feeding on its host before the cocoon is spun. After 60 hours of entering the cell, the adult female lays its first egg, which usually becomes a male, and subsequently lays 1 to 5 female eggs, one at a time, at approximately 30 hour intervals (Fries, *et al.* 1994, Steiner *et al.* 1994) ([Appendix 5](#)).

Development from egg to adult takes about 6 days for males and 7 days for females in worker cells (Martin 1995). In drone cells development is one day shorter (Amrine, personal communication). After 15 to 20 hours of embryogenesis, the chorion appears, and a thick layer of epithelium surrounds the oocyte, which ends the vitellogenesis phase. The blastoderm is visible 30 to 35 hours after embryogenesis starts. Segmentation is apparent 40 to 45 hours after embryogenesis. About 40 to 45 hours after embryogenesis, the chelicera and the legs are visible. Between 60 to 65 hours after embryogenesis, the protonymph is formed, which then leaves the egg. This is followed by development to the deutonymph, which then becomes an adult (Steiner 1992, Steiner *et al.* 1994).

Female mites display parental care. A female mite invests up to 60 minutes opening a wound on the bee pupa that will be used as a communal feeding site for her and her offspring (Donzé and Guerin 1994). Protonymphs are incapable of piercing the bee integument; thus they rely on their mother to survive. The mother mite also prepares a rendezvous place for her offspring to mate. This site consists of a mound of her feces, generally on the upper, central wall of the cell. This is thought to be an evolutionary adaptation to its original host *Apis cerana*, which may have prevented detection of the offspring when the workers uncapped the cells (Donzé and Guerin 1994). Female offspring

must mate within their natal cells prior to the emergence of the bee in order to be inseminated. Males die after mating. Gravid and juvenile adult female mites leave the cell when the adult bees emerge, attach to passing bees, and enter new brood cells (Spivak 1996). All immature female mites die when the host bee removes the cell capping and leaves the cell as an adult (Harris and Harbo 1999). Adult female mites enter a new brood cell 4 to 13 days after emerging with the bee and repeat the cycle. Each female passes through several cycles (Martin and Kemp 1997). The total life span of a female mite depends on the season. Mites will live about two months during the summer and from five to six months in the winter (Büchler 1994).

1.6 Varroa mite population dynamics model

Three factors are important in a varroa mite population model: entrance of the female mites into the brood cells, reproduction, and mortality (Calis *et al.* 1999b).

1.6.1. Entrance of mites into brood cells

The entrance rate into brood cells depends on the season and availability of brood. The time of the year has an effect on mite numbers and stage of the bees parasitized (Anonymous 1995). In general, varroa mites prefer drone brood during the spring and summer. In late fall and winter when brood is scarce or absent, mites are found more frequently on adult bees, which increases their chance for dispersal. This is called the phoretic phase (Fries *et al.* 1994).

The ratio of the number of mites entering drone cells to the number of mites entering worker cells is called the preference factor (Fries *et al.* 1994). Shultz (1984) calculated a preference factor of 8.6 to 1 in *Apis mellifera ligustica*. Fuchs (1990) reported a preference factor of 12 to 1 in *Apis mellifera carnica*. There are five main reasons for mite preference for drone cells: longer pre-capping periods, longer post-capping periods, larger cells, higher protein content of drone hemolymph, and differences in the chemical composition of the drone cuticle (Fries *et al.* 1994, Ifantidis 1988, Calis *et al.* 1999b, Rosenkranz 1996, Rosenkranz *et al.* 1993, Le Conte *et al.* 1989).

Drone cells are left uncapped for a longer period than worker cells. After entering a brood cell, mites are "trapped" in the larval food. This promotes the parasite's reproduction because the mite is immobile and cannot be removed by nurse bees. The maximum time of immobilization is 15 h in worker cells and 45 h in drone cells (Fries *et al.* 1994, Ifantidis 1988).

The length of the brood postcapping period affects the number of mite offspring. Drone and worker postcapping periods are 14 and 13 days, respectively (Amrine, personal communication). Selective breeding of bees with short postcapping times has been attempted to control varroa mites. Büchler (1994) found that a reduction of one hour in the worker postcapping time resulted in a 8.7% reduction in mite population growth. According to Calis *et al.* (1999b), a one-day reduction of the postcapping time results in 50% reduction in the number of mite offspring in subsequent brood cycles. A short postcapping period decreases the average number of viable fertilized female offspring (Trouiller and Milani 1999), and unmated young females may either produce only males by parthenogenesis or do not produce any offspring (Calis *et al.* 1999b).

Mite reproductive success is also influenced by the ratio of worker-to-drone brood. The number of mite offspring depends on the type of cells the female has entered (Salvy *et al.* 1999). A female mite is capable of successfully producing 1.6 to 1.8 offspring in a worker cell, whereas it produces 2.9 to 3.2 offspring in a typical drone cell (Shulz 1984). Robaux (1986) found that a female mite is capable of producing 1.5 to 2.0 offspring in a typical worker cell and 2.5 up to 3.0 offspring in a typical drone cell. Martin (1995) found that a single mother mite infesting *A. mellifera* will produce about 1.1 to 2.5 mated female offspring in drone cells and 0.83 to 1.3 in worker cells.

The mites ability to discriminate between drone and worker cells appears to be based on chemical attractants secreted by the larvae and present on the cuticle (Rosenkranz *et al.* 1993). Le Conte *et al.* (1989) found ten straight-chain fatty acid esters in an extract of drone bee larvae that attracted varroa females.

Varroa mites do not restrict invasion to drone cells in *A. mellifera* colonies. Boot *et al.* (1995a) developed a mathematical model to explain selective forces that induce reproduction in worker cells. According to this model, mites that invade *A. mellifera* colonies have several strategies to maximize their reproductive effort. Mites may invade only drone cells or invade both types of cells. The type of cell chosen depends on the time a mite has to spend searching for a drone cell; mites may die during the phoretic phase on adult bees while waiting for a drone cell. The model predicts that varroa mites can wait for a drone cell for seven days before invading worker cells. Invasion of worker cells also occurs when mite population growth is negative and invasion of worker cells is better than staying on adult bees without reproducing. This may occur when drone brood cells are absent or scarce (Boot *et al.* 1995a).

Researchers have measured the rate of invasion of mites into worker and drone cells in the field. Boot *et al.* (1995b) related the invasion rate per day to the ratio of available cells and the number of adult bees:

$$\text{Invasion rate} = \# \text{ phoretic mites} \cdot \left(1 - e^{-(\text{Drone rate} + \text{worker rate})}\right)$$

Where:

$$\text{Drone rate} = \frac{\text{number of drone cells available}}{\text{weight in grams of adult bees in the colony}}$$

$$\text{Worker rate} = \frac{\text{number of worker cells available}}{\text{weight in grams of adult bees in the colony}}$$

According to Calis *et al.* (1999b) the invasion rate equation proved to be important in establishing the efficacy of biomechanical control methods that trap mites in worker brood or in drone brood cells. The value of the equation lies on the dynamic relationship between the number of brood cells available and the number of bees on which the mites are phoretic.

1.6.2. Reproduction.

In a study of mite fecundity, Harris and Harbo (1999) define a reproductive mite as one that can produce at least one reproductive daughter before the host bee emerges from its cell. The same study shows that three situations can prevent mites from reproducing: mites that are alive in the cell but do not lay eggs, mites with progeny too young to mature and mate before the host emerges from its cell, and mites that die in the cell before laying eggs. Accordingly these authors proposed the following equation to describe the growth of a mite population:

$$\text{Final mite population} = \text{Initial mite population} \cdot (rs)^{\text{Average number of reproductive cycles per mite}}$$

Where:

$$r = \frac{\text{mature daughters} + \text{surviving foundresses that exit the cell}}{\text{\# of foundresses that entered the cells}}$$

s = the probability that an adult mite will survive outside of the brood

According to De Ruijter (1987), female mites are physiologically capable of reproducing several times. His study shows that mites are able to reproduce up to seven times and produce up to 30 eggs in artificial conditions. However, other authors indicate 1.5 to 3 reproductive cycles are typical in natural conditions (Martin and Kemp. 1997, Fries and Rosenkranz 1996). Not all female mites that enter brood cells reproduce. Shulz (1984) reported that 92% of mites reproduced once and only 8% will enter cells for a second time and one third of these mites will actually reproduce. Ifantidis (1984) reported that 1.9% of mites reproduced four times. According to Fries and Rosenkranz (1993), 13% of mites reproduced three times.

In the opinion of Harris and Harbo (1999) the ability to produce progeny is related to fertilization of the female mite. These authors reported that mites that did not lay any eggs had significantly fewer spermatozoa in their seminal receptacle than normally reproductive mites.

In the opinion of Fuchs and Lagenbach (1989), varroa mite reproductive success is density dependent. Maximum fruition occurs when no more than three females reproduce in the same cell. Higher infestation rates negatively affect mite fitness and increases offspring mortality as a result of food competition (Salvy *et al.* 1999). Eguaras and Marcangeli (1994) reported an inversely proportional relationship between the number of female mites and the number of eggs deposited by each female. These authors suggested that crowded conditions might produce a delay in the time of the normal oviposition sequence and in the development of immature stages of the mite. In their opinion, host-immune responses could be responsible for the reduced reproductive potential under crowded situations. Erickson *et al.* (1997) reported the existence of opaque white nodules in the gaster of worker honey bees infested with *Varroa destructor*. The study of the nodules revealed that their principal component is tyrosine, an important compound for wound healing and immunological reactions in insects. This suggests the existence of physiological defense mechanisms in bees

parasitized with varroa mites. Traditionally, it had been accepted that bee resistance to pathogens can be achieved by behavioral, rather than physiological mechanisms (Kraus and Page 1998).

In a varroa mite population model, it is also important to consider the race, and geographical location of bees, and the genotype of the mites. Differences in mite fertility appear to be related to honey bee race and mite genotype (Trouiller and Milani 1999, De Guzman and Rinderer 1999, Anderson and Trueman 2000).

Researchers found that environmental conditions may have an impact on tolerance and resistance to *V. destructor*. According to De Jong and Soares (1997) Italian bees are able to tolerate varroa mite infestations under tropical conditions. Some tropical environmental conditions may affect mite reproduction. Kraus and Velthuis (1997) found that relative humidity (RH) has a tremendous impact on the proportion of varroa mite female offspring that reach adulthood. Their results show that 53% of the mites reproduced within the range of 59-68% RH. Only 4% of the mites will reproduce if RH is within the range of 79 - 85%. Varroa mites never reproduced at levels higher than 80% RH. High temperatures and high relative humidity levels are common in some tropical areas. When RH is high, bees are frequently unable to control temperature and RH inside of the hive. When this occurs, bees evacuate the nest and cluster at the hive entrance. In some tropical areas, hive RH levels are frequently above 80%, which negatively affect mite population growth (Kraus and Velthuis 1997).

1.6.3. Mite mortality.

Estimations have shown that the mortality of *V. destructor* in the wintering cluster may be as high as 50%. During the summer it is difficult to calculate the mortality of phoretic mites on adult bees (Fries *et al.* 1994). Female mites may fall from bees or foraging bees with phoretic females may die and not return to the hive. Male mortality in sealed brood cells also appears to have a great impact on mite reproductive success because non-fertilized females fail to produce viable female offspring (Calis *et al.* 1999b).

1.7 Varroa mite control measures.

Due to the reproductive capacity of *V. destructor*, the extensive damage that high populations of this mite can produce on honey bee colonies, and the inability of western honey bees to resist the mites, control measures are necessary. Biomechanical techniques, selective breeding, chemical miticides, organic acids, and botanical extracts are commonly used to regulate mite populations in bee hives. Biological control is currently under study.

1.7.1. Biomechanical control.

1.7.1.1. Drone comb traps.

Exploiting varroa preference for drone brood can reduce their numbers. Drone comb traps, and the destruction of brood after the honey harvest season, are techniques currently used in

Vietnamese apiaries. However, there is no available data about the efficacy of this method (Nguyen *et al.* 1997). Drone manipulation is widely used in Europe as well (De Ruijter 1999). Several European studies report 20 to 30% decreases in varroa mite population when drone brood is removed in the spring (De Ruijter 1999). In the opinion of Delaplane (1997) such manipulations cannot completely replace chemical treatments, but may reduce the amount needed. Drone removal may also increase selection pressure for mites that prefer worker brood. Overall, biomechanical varroa control is impractical for large-scale beekeeping (Delaplane 1997).

1.7.1.2. Worker comb traps.

Mites also can be trapped inside worker brood frames. These frames then are treated either with high temperature or formic acid outside of the colony, which selectively kills the mites without destroying the brood. Mite trapping within worker brood has several advantages over mite trapping within drone brood. First, an unrestricted amount of brood can be used to trap the mites. Second, it can be performed throughout the season, whereas mite trapping within drone brood only is possible during the drone brood production period (Calis *et al.* 1999a).

1.7.1.3. Bottom board inserts.

In the opinion of Pettis and Shimanuki (1999), screened bottom boards act as a physical barrier between mites and bees. Mites that fall through the screen to the bottom board cannot climb back on the bees and re-infest brood cells. These authors have demonstrated that screened bottom boards slow the population growth of varroa, but the boards by themselves are incapable of controlling the mite.

1.7.2. Chemical control.

Over 140 chemical compounds have been tested for their efficacy against *V. destructor*. Fluvalinate, flumethrin, and bromopropylate are commonly used in several countries (Delaplane 1997). In the United States, fluvalinate (Apistan®) is registered for use in controlling Varroa on honey bees (Ellis *et al.* 1997). Recently, in some states where fluvalinate resistance has been detected, the Environmental Protection Agency allowed treatment of hives with coumaphos, (Checkmite®), by issuing an emergency exemption under provisions of section 18 of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). This emergency exemption will expire January 18, 2001 (Steinhauer 1999, Sandford 2000).

1.7.2.1. Effects of pesticides on bees.

Chemical acaricides are easy to use and can keep varroa populations partially under control, but their use in beehives is controversial (Le Conte *et al.* 1989). Misuse of acaricides has resulted in the development of resistance and contamination of honey and other honey bee products. Reports from Japan have shown that the mite has become resistant to phenothiazine, tetradifon and chlorodifon after five years of application (Eischen 1995). There are also reports of decreasing efficacy of Apistan in Sicily, other parts of Italy, and the United States due to mite resistance (Delaplane 1997, Colin *et al.* 1997, Eischen 1995, Lodesani *et al.* 1995, Steinhauer 1999).

Fluvalinate, the active pyrethroid ingredient of Apistan®, is the most widely used varroa miticide in the United States. The efficacy of this product has been well demonstrated. A mite mortality of 98% can be achieved if used for 3 capping periods, about 6 weeks (Colin *et al.* 1997). However, bees from colonies treated with Apistan® are more vulnerable to pesticides. Studies conducted to determine synergism between pesticides used on crops and in beehives have shown that honey bees treated with Apistan® are more susceptible to bifenthrin (Ellis *et al.* 1997). Pyrethroid insecticides can also synergize the toxicity of fungicides to honey bees because the pyrethroid blocks the bee's detoxification mechanism for some fungicides (Pilling *et al.* 1995).

1.7.2.2. Effects of pesticides on bee products.

It has been observed that varroacides with a low tendency to migrate, accumulate very easily in beeswax, and leave residues in honey (Wallner 1995). Some researchers considered that the residues of varroacides in honey bee products have negative implications, especially regarding the use of honey and beeswax in the cosmetics and pharmaceutical industries. Additionally, the presence of active ingredients in wax at sub-lethal concentrations for long periods may induce resistant mite strains (Lodesani *et al.* 1992).

1.7.3. Organic acids.

Treatments with organic acids are considered good tools for fighting the mite in several countries. Studies in Germany have shown that a 98% mite mortality can be achieved by placing 1-2 absorbent pads containing formic acid on the bottom of the hive (Wachendörfer *et al.* 1985). The pads must be in place for 4 days and the treatment repeated three times (Delaplane 1997). Other methods for the application of formic acid are the "Illertisser mite plate"⁶, soaked cheesecloth, and containers with wicks. Porous polyethylene bags containing a 65% formic acid gel are popular in Canada. This kind of formulation reduces the number of applications since the gel acts as a slow release agent (Feldlaufer *et al.* 1997).

The first organic acid legally available to U.S. beekeepers was formic acid. Apicure® is 65% formic acid in a gel form, packed in a special pouch, made of three layers of anticorrosive plastic. It has a general use (Section 3) label. This product must be used in combination with biomechanical measures or as part of an Integrated Pest Management Program (Sanford 2000, Ellis 2000).

Although the efficacy of formic acid has been demonstrated, there are some concerns about the bee's health. Formic acid treatments coincide with deleterious effects on the brood nest (Eischen 1998). Bee death has also been related to formic acid treatments at the recommended rates (Nelson *et al.* 1994). In addition, formic acid is caustic and there is justified concern for beekeeper safety (Delaplane 1997).

Radetzki (1994) demonstrated that aerosol treatments with 3% oxalic acid on bees infested with adult mites produced 97.3% mite mortality. However, it has been suggested that oxalic acid

⁶ Cardboard like material soaked in the acid and placed in the hive (Feldlaufer *et al.* 1997)

treatments may damage tissues of the digestive system of bees (Brødsgaard *et al.* 1999b). Oral ingestion of oxalic acid is also harmful, and may be deadly to humans. It causes damage to the skin by dermal contact and damage to mucous membranes by inhalation (Radetzki 1994). Oxalic acid treatments must only be applied during late fall and winter, which will avoid residues in the honey. Oxalic acid is not liposoluble; therefore residues will not build up in the wax (Brødsgaard *et al.* 1999b).

1.7.4. Essential oils.

Essential oils are highly volatile terpenes and phenolic compounds, which have an intense aroma (Imdorf *et al.* 1999). Essential oils are byproducts of the secondary metabolism of certain plants. Plants have evolved essential oils, to serve as defense or dispersal mechanisms (Rohloff 1999). Terpenes are usually predominant in plants and are unsaturated cyclic hydrocarbons. Terpenes may contain 10, 15 and 20 carbons and are classified as monoterpenes, sesquiterpenes, and diterpenes, respectively. Monoterpenes are highly volatile and are usually found in combination with alcohols, phenols, aldehydes, ketones, esters and oxide derivatives (Imdorf *et al.* 1999). Phenolic compounds are aromatic hydrocarbon derivatives of benzene. Essential oils are extracted by simple vapor distillation, cold pressing or chemical extraction (Lewinsohn *et al.* 1998).

Varieties of one plant species may have different essential oil compositions. The composition of each variety is called the chemotype (Imdorf *et al.* 1999). The chemical composition of a particular essential oil may also vary according to the extraction process, climatic conditions and cultivation of the plants (Lewinsohn *et al.* 1998).

The uses of essential oils are innumerable. They have been found to be useful for the control of bacteria, fungi, and Triatominae (Hemiptera: Reduviidae). They also act as insect repellents and insecticides (Laurent *et al.* 1997, Franzios *et al.* 1997, Ngoh *et al.* 1998, Amvan *et al.* 1998, Shahi *et al.* 1999). The use of essential oils as acaricides has been documented since the 1930's as control agents of the honey bee tracheal mite, *Acarapis woodi* (Angelloz 1930).

The potential use of essential oils to control varroa mites was reported by several authors (Mautz 1982, Rittter and Hoppe 1986, Colin 1990, Imdorf 1995, Amrine *et al.* 1996, Calderone *et al.* 1997, Hoppe and Ritter 1997, Sammataro *et al.* 1998, Bogdanov *et al.* 1998, Murillo - Yepes 1998).

Treatments with essential oils represent a potentially superior control for varroa mites. Because of their origin and mode of action, it is possible that such compounds are more easily degraded, more specific, and less susceptible to the production of resistance than synthetic pesticides currently used (Gerard *et al.* 1997, Imdorf *et al.* 1999).

More than 150 essential oils and their components have been screened for their potential use as varroa mite controls (Imdorf *et al.* 1999). Kraus *et al.* (1994), in an effort to determine the mode of action of several essential oils on mites, ran a series of laboratory tests. The experiments were designed to prove attractiveness, repellence, toxicity or masking effects of bee or brood pheromones

by essential oils on varroa mites. The results of the study show that citronella, marjoram, and lavender oils affected the olfactory orientation of mites while producing a repellent effect. Marjoram oil appears to impede brood recognition. Cinnamon, clove, and wintergreen oils were attractants. The attractiveness of wintergreen appears to decrease with high mite infestations. The authors concluded that the capacity of the mite to differentiate between drones and workers was not affected by these oils, but the capacity to distinguish between nurse bees and foragers was impaired.

Hoppe (1990), cited in Imdorf *et al.* (1999), studied the acaricidal effect and bee compatibility of 55 different essential oils. In laboratory trials, mites and bees were exposed to various concentrations of different essential oils. Treatments were delivered by topical application or by evaporation inside bee hives. Twenty-four essential oils produced mite mortality greater than 90% after 48 hours of evaporation. Three oils produced the same effect by topical application. Only nine of the 55 oils produced bee mortality less than 10%. The results of the study showed that the most effective substances for controlling *V. destructor* were clove and wintergreen oils. Volatilization of wintergreen at doses of 3µl / liter of air resulted in 7% bee mortality and 30µl/liter of air produced 33% bee mortality. These results demonstrated that bee mortality increases with higher doses of wintergreen oil (Imdorf, personal communication)

Field evaluations of wintergreen were made after intensive laboratory studies. Passive evaporation of this oil either failed to control the mites or produced behavioral changes and bee mortality in the treated colonies. Passive evaporation of 5 ml of wintergreen essential oil per hive produced no effect, whereas evaporation of 10 ml of wintergreen oil resulted in 72% mite mortality and 2% bee mortality. Evaporation of 15 ml of the same oil resulted in 95% mite mortality and 7% bee mortality. Evaporation of both 10 ml and 15 ml produced behavioral disturbances in bees (Hoppe 1990 cited in Imdorf *et al.* 1999).

In the opinion of Noel and Amrine (1996), varroa mites are very susceptible to wintergreen during the late fall and early winter due to the lack of brood, and the formation of the cluster. Preliminary studies showed that the number of gravid females was reduced in comparison with untreated hives. Inspections of the sealed brood revealed that mite progeny was reduced as well. Wintergreen also had an effect on mite behavior; treated mites tended to move faster than untreated mites and appeared unable to find the brood (Amrine *et al.* 1996). However, Bunsen (1991) cited in Imdorf *et al.* (1999) demonstrated the attractant effect of wintergreen oil to mites in field trials. One comb from a hive that was sprayed with 20 ml acetone-wintergreen solution (9:1 vol/vol) became highly infested with mites.

Skinner *et al.* (1997) evaluated the efficacy of several essential oil treatments to control varroa mites, and compared various application methods to deliver the oils within the hives. The first treatment was a mixture of thymol, eucalyptus, camphor and menthol absorbed into a sponge and placed above the brood cluster. The second treatment was spearmint syrup prepared by mixing 1 ml of spearmint with one pound of sugar in a quart of water, which was fed to the bees. The third treatment consisted of peppermint syrup prepared by mixing 1 ml of peppermint oil with one pound of sugar in a quart of water, which was fed to the bees. The fourth treatment consisted of 8 oz. of shortening patties made by mixing two parts of sugar, one part of shortening. Patties were placed

above the brood chamber. The fifth treatment consisted of peppermint patties prepared by mixing 0.8 ml of peppermint oil with 2 parts of sugar and 1 part of shortening. Mite populations were assessed by using sticky boards. Assessments were made at 0, 30, and 60 days after applying the treatments. The results of the study showed that all essential oil treatments failed to control varroa mites. The mite population in hives treated with the spearmint syrup increased 258% after treatment while the control rose 100%. However, in this study, initial mite populations were not standardized for all treatments. Similar studies to test the efficacy of wintergreen used grease patties (5 cc of wintergreen in a 227 g patty made with 2 parts of granulated sugar in one part of Crisco ®) and menthol grease boards (22 X 28 cm corrugated board smeared with medicated Chest Rub® (Equate, Wal-Mart Corp.) containing camphor, menthol, eucalyptus, thymol plus botanical and mineral oils) showed an average mite mortality of 16% and 70%, respectively (Baxter *et al.* 2000).

On the contrary, 96.8% mite mortality can be achieved using the essential oil thymol. Apilife VAR®, a product containing 76% thymol, accomplishes 97% mite mortality and is currently used in Italy (Delaplane 1997). Despite positive results, it is necessary to be prudent in recommending the use of Apilife VAR® for controlling varroa. Some studies have shown that colonies have difficulties taking supplementary feeding during treatment periods with this product (Imdorf *et al.* 1995). Negative effects on colony development, stimulation of robbing, and an increase in aggressiveness have also been observed after Apilife VAR® treatments (Imdorf *et al.* 1999). Mautz (1982), observed bee mortality following treatment with Apilife VAR® and proved that thymol is a contact and respiratory poison for bees. But his conclusions need to be confirmed under natural conditions.

1.7.5. Biological control.

There is growing interest in finding and using varroa mite natural antagonists. Kleespies *et al.* (2000) reported a putative iridovirus that appears to affect varroa mites. The symptoms included black colored spots of the gut and fat body of the mite. Under a transmission electron microscope, symptomatic varroa mites show spherical virus-like particles in the nuclei of the fat body and muscle tissue. The particles often destroy the membrane of the nucleus when propagation advances. The research on the virus-like particles that affect varroa mites is in progress, but preliminary results show that there is low fertility in black-colored mites and that the virus does not affect bees.

The fungus *Metharrizium anisopliae* has been isolated from varroa mites and studies on its pathogenicity against the mite are in progress (Shaw 2000).

1.7.6. Selective breeding.

A different approach for controlling varroa mites is to select bees with traits that allow them to tolerate or suppress mites. Colonies of *A. mellifera* tolerant to varroa have been identified in Europe, South America and Mexico (Büchler 1994, Medina 1998, Moretto *et al.* 1991). In the United States, several authors report the existence of bee colonies capable of suppressing mite reproduction (Spivak 1996, Harris and Harbo 1999, Harbo and Harris 1999). Tolerance or suppression of mites appears to be related to heritable and environmental factors. Honey bee traits important for selecting breeding are: short developmental time, grooming and hygienic behaviors, and

uncapped drone cells (Erickson *et al.* 1997). Additionally, since mites require bee juvenile hormones for their reproduction, and hormone titers may vary among bee larvae from different races, Harbo and Harris (1999) considered mite fertility as a factor that depends on the bees.

Heritability⁷ (h^2) can be measured. A characteristic must have an h^2 value higher than 0.25 for selective breeding in honey bees to be successful (Harbo and Harris 1999). Heritability of different characteristics has been measured in honey bees. Moritz (1995) estimated an $h^2 = 0.8$ for the duration of the capped period. According to Harbo and Harris (1999) four characteristics have an $h^2 > 0.25$. They are: mite reproduction, proportion of mites in brood, hygienic behavior, and duration of the capped period.

1.8 Mite population assessment methods.

Several methods have been developed to estimate mite populations. Mite detection techniques are useful for establishing treatment efficacy, and are necessary to determine thresholds for reducing the frequency of chemical use (Delaplane 1997, Brødsgaard and Brødsgaard 1998).

A common mite population assessing method is the ether roll technique. This method requires capturing 300 - 500 adult bees in a glass jar, spraying the bees with ether, shaking the jar, and examining it for mites. This method covers a small fraction of the mite population, and does not predict the mite population accurately (Delaplane 1997).

The heating method is an alternative to the ether roll method. Bees are shaken into a wire cage, which is then placed into an oven over a white paper. Bees are heated for approximately 10 minutes at 46°C, and the white paper examined for the presence of mites (Frazier 1999). In the opinion of Amrine (personal communication) this method is not accurate.

Usually, detection of mites in bee colonies requires the use of Apistan® or tobacco smoke in conjunction with a bottom board insert (De Ruijter and Eijnde 1982). Counting mites on the bottom boards of the hives can only be used as a rough estimation of mite mortality. This method does not take into account mites that are concealed within brood cells and mites that die attached to adult bees (Fries *et al.* 1994). In the opinion of Calderon and Turcotte (1998), there is a weak correlation between daily mite fall on bottom board inserts and the total number of mites per adult bee. Since mite population size may be underestimated when calculated from daily mite mortality, Brødsgaard and Brødsgaard (1998) developed a mathematical model to avoid underestimation. According to them, by counting naturally dead varroa mites that fall on hive inserts over a period of 1 to 3 weeks, it is possible to estimate the absolute number of mites. There is a linear relationship between the absolute number of mites and the number of dead mites collected on the inserts. The confidence of this model will increase if it is applied to colonies with capped brood cells. According to these

⁷Heritability is defined by Harbo and Harris (1999) as “the proportion of the observed variance, among a group of bee colonies, for which differences in heredity are responsible”.

authors, the treatment threshold for varroa mites in a temperate climate is approximately 1000 mites/colony in the spring.

According to Witherel and Bruce (1990), counting mites by randomly opening at least 100 brood cells, using a capping's scraper or entomological tweezers, accurately estimates the mite population in a honey bee colony. Larvae and pupae can be directly inspected for the presence of mites, or put onto a wire mesh and washed with alcohol to dislodge the mites. It is important to wash the empty brood cells by using a pipette with alcohol, and recover mites that do not fall out with the brood. The bottom and wall of the cells must be carefully inspected for the presence of immature mites and mite feces. Colonies with 5% or fewer infected brood cells are considered lightly infested, whereas those with more than 25% infected brood cells are severely infested and in danger of suffering collapse.

1.9 Hive beetle.

The small hive beetle, *Aethina tumida* Murray, has spread rapidly since its introduction into Florida in 1998. There are reports of the presence of the beetle in Georgia, North and South Carolina, Ohio, New Jersey, and Pennsylvania. The ability of the beetle to overwinter in Minnesota has been reported, and suggests its capability of adapting to cold. Due to the complex life cycle of the beetle, no control measures have proven to be effective (Eischen and Baxter 1998, Eischen 1999, Taber 1999).

Adult female beetles lay large masses of eggs on beeswax combs. The larval stage is completed within 10 to 16 days. Hive beetles pupate in the soil around hives, and adults emerge in approximately 3-4 weeks. Females are capable of reproducing one week after emerging. *Aethina tumida* is a good flyer and beetles can disperse rapidly to new colonies, where they start new generations (MAAREC 1999).

A small number of beetles can produce large masses of larvae, which consume the resources of the honey bee colony and defecate in the honey causing it to ferment. For this reason, it is important to find the beetles in early stages of infestation. Varroa mite sticky boards are ineffective for detecting adult beetles (Sanford 1999, MAAREC 1999). Eischen and Baxter (1998) have experimented on beetle detection by placing various materials in the hive, but were unable to achieve positive results.

1.10 Life cycle of the honey bee, *Apis mellifera*.

The queen deposits a single egg at the bottom of each comb cell. Workers and drones develop from fertilized and unfertilized eggs, respectively. Worker eggs hatch after three days and first-instar larvae are fed regularly by nurse bees. After successive stages of growth and molts, larvae completely cover the floor of their cells, and stretch out along the depth of the cells. Fully-grown worker larvae are not fed and house bees cap their cells with a thin layer of wax. This stage is known as "sealed brood"; then larvae defecate, spin a cocoon and begin to pupate. Worker pupae grow

gradually darker in color and transform into adults that slowly chew their way out of the cell. Complete metamorphosis from newly laid egg to emerging adult worker requires a total of 21 days: three as an egg, seven as a larva, nine as a pupa and one as an unemerged adult. Worker emerge the 21st day. Worker cells are capped on day 8. The development of drones requires 24 days: three as an egg, 10 as a larva, 8.5 as a pupa, and 1.5 as an unemerged adult. Drone emerge the on the 23th or 24th day. Drone cells are capped on day 10. Emerging drones are fed honey and royal jelly until they are about a week old. Drones start flying when they are from 6 to 8 days old, but they are sexually mature only after 12 to 14 days (Akratanakul 1990, Winston 1987).

Queens are produced to prepare for swarming or to replace old or accidentally lost queens. The development period of a queen is 16 days from egg to adult: three as an egg, 6 as a larva, 5 as a pupa, and 1 as an unemerged adult. Queens emerge the 16th day (Winston 1987).

1.11 Some aspects of the behavior of the honey bee, *Apis mellifera*.

1.11 .1. Water collection.

Water is needed to maintain the osmotic balance of adult bees, prepare liquid food for the brood, and to cool the hive, which prevents irreparable damage to the brood. High ambient temperatures increase the consumption of water because intensive evaporative cooling is necessary (Seeley 1985). According to Kühnholz and Seely (1997), water collector bees are able to determine the colony's water need by perceiving how easily water is received. When the colony is in need of water there are a greater number of bees receiving water. This suggests a cross-inhibition between water collection and nectar collection. An increase in the number of water receivers results in a decrease in the number of nectar receivers.

2. Materials and Methods

The essential oil experiment had three main phases: preliminary preparations, essential oil and Apistan testing, and analysis of data. Preliminary preparations included: managing surviving colonies from the previous season, installing 40 honey bee packages at bee yards (three at the WVU Agronomy farm, two at the WVU Horticulture farm and one at the greenhouse on the West Virginia University Evansdale campus), artificially infesting hives with varroa mites, practicing standard beekeeping management of 51 colonies, and assessing initial mite population levels. The essential oil-Apistan® testing phase included standardization of mite populations, essential oil and Apistan® testing, and gathering of data. Standardization was done by selecting colonies with more than 8% mite infestation and randomly assigning them to bee yards where essential oils will be tested. There were 10 bee yards in total, of which seven were used for the treatments. Out of fifty-one colonies, thirty-five were used for the treatments. Data was gathered in different ways: observations on bee behavior made during treatments and mite population assessment after 29 days of treatment.

2.1 Preliminary preparations.

2.1.1 Establishing colonies.

Bee yards were sites where sets of honey bee colonies were maintained. Bee yards used at the beginning of the 1999 season were established in 1998 with the following criteria: a barrier of trees nearby, high position to avoid flooding, close to a water source, and easy access. Bee yards were at three different locations: the West Virginia University Agronomy Farm, the West Virginia University Horticulture farm, and at the greenhouse on the West Virginia University Evansdale campus. There were three, two, and one bee yards at each location, respectively; each had five hives. Spring management of the surviving colonies started on the third week of April 1999. Strong colonies were divided in half. Two frames of eggs were carefully added to the queenless halves in order to ensure all hives had a queen. Weak colonies were combined with strong colonies or were placed in the position of a colony with a strong foraging force, thus it would be strengthened. The strong colony was moved to the position of the weak colony. Honey supers were provided as needed. After the divisions, 10 colonies were obtained ([Appendix 6](#)).

The position of each hive was carefully chosen. All hives faced South, Southeast or Southwest, and were near trees to protect them from wind. Hives were placed on cinder blocks or bricks; a level was used to assure that hives inclined toward the front so water would not pool in the back of the hive. Most of the equipment consisted of wooden Illinois supers with ten Rite-cell ® foundation frames each, wooden bottom boards, and wooden top covers ([Figure 1](#)).

Figure 1 - Experimental bee yard with five hives facing South and with a nearby barrier of trees

In the last week of April, forty bee packages were purchased from York's Bees, Jesup, Georgia and transported to Morgantown by truck. After arriving in Morgantown, packages were kept overnight in a dark room and hand fed with sugar syrup using a paintbrush. On the following day, bees were fed again and transported to the bee yards. Bee packages were installed on May third, after four o'clock. Each package was placed into two Illinois supers with two frames of honey in the upper super. Hives were arranged in horizontal rows, two meters apart from each other. In order to allow nurse bees to release the queen from its cage, a small hole was made through the candy plug and the queen cage was hung inside the hive from a central frame of the top super. The following day, a number was assigned to each colony, including old and new colonies. Numbers were painted on the top cover and the front of each hive; there were 50 colonies in total.

One week after the new colonies were installed, they were checked for the presence of the queens or eggs. Colonies 8, 6, 34, 42, 28, and 46 became queenless, and a frame of eggs was added to each one. Four days later, they were inspected for the presence of queen cells at the bottom of the frames. Colonies 31, 42, 23 and 36 had the tendency to produce laying workers. They were managed according to Bob Noel's⁸ method: bees were dumped out 15 yards away from their hive, a bee brush

⁸ Bob Noel is a beekeeper from Cumberland, Maryland.

was used to remove all bees from each frame. Frames were reinstalled, a frame of eggs and a frame of sealed brood were provided⁹.

Once colonies were well established, a routine check was made twice a month for the remainder of the season. Old and new colonies were inspected for disease or overpopulation and it was confirmed that all had a good laying queen. Brood was monitored periodically and inspected for varroa mites.

Bees were fed with a 50:50 solution of sucrose and water every other day from July 15th until October 25th. Quart Mason jars, with perforated lids, containing the solution were placed on Boardman feeders to deliver the syrup to the bees. Dividing strong colonies and feeding the bees reduced the likelihood of swarming. Two swarms were captured and managed. After divisions were made there were sixty-eight colonies in total.

2.1.2 Hive infestation.

Infestation started on June 2nd and continued until July 15th. Fifty-one beehives were artificially infested with mites: 10 old colonies, 40 new colonies, and one swarm. Colonies from the previous season were checked for the presence of mites from May 12th to May 28th. Mites were detected in colonies 2 and 6. Colonies 2, 6 and six colonies provided by local beekeepers¹⁰ were used as a source of mite infestation. Hives were infested with varroa mites in different ways: shaking frames with infested adult bees in front of the hives, inserting infested drone comb, inserting infested brood frames, and a combination of the last two methods ([Appendix 7](#)). For the drone comb insertion method, medium deep frames were put into a highly infested deep super, which enabled the production of drone brood on the bottom of the frames. The drone brood comb on that frame became infested, was cut into 10 x 5 cm. pieces and placed into the hives. For the infested brood frame method, colonies were treated as follows: one deep frame infested with mites was put into the medium depth top super of an uninfested hive, so it would hang down into the lower super. Infested drone comb was produced in the space between the bottom board and each deep frame bottom.

Three field surveys were performed on all hives to establish the presence of mites. One frame of each hive was randomly chosen and examined in the field. Twenty to 100 randomly selected, sealed brood cells were opened using tweezers. Larvae, pupae or adults about to emerge were carefully removed and examined with the naked eye for the presence of varroa mites. The empty cells were then carefully examined for the presence of immature mites or mite feces. The presence or absence of mites was registered, and mites and frames were returned to the hives. After each survey, infestation was attempted again on the hives that were not infested ([Appendix 8](#)).

⁹ Bob Noel's method improves the chance of colonies' survival by removing all laying workers. The newly inserted frame of eggs guarantees that a queen will be produced.

¹⁰ Donna Maleck, Myrna Huffman, and Bob Noel.

A detailed survey was done four weeks after the last attempt of infestation. The following method was used: one randomly chosen frame of each hive was replaced by an empty frame and brought to the laboratory for inspection. Using tweezers, one hundred brood cells were opened, one by one, on each frame; fifty cells were examined on each side of the frame. Larvae, pupae or adults about to emerge were carefully examined with the naked eye, and with the aid of a fiber optic lamp. The bottom and walls of the cells were examined for the presence of immature mites and mite feces. Bees with mites were put in vials containing 70% alcohol, and labeled with the hive number. The number of cells with mites was registered discriminating between bee stages ([Appendix 9](#)).

2.1.3 Hive beetle survey.

All hives were checked for the presence of hive beetles, *A. tumida*, because bees were purchased in Georgia (known to be infested). Detector boards for hive beetles were installed on June 10th, with the cooperation of the West Virginia State bee inspectors. Three different detector boards of equal dimensions, 10x15 cm, were tested: cardboard covered with epoxy, cardboard covered with beeswax, and cardboard covered with adhesive tape. One type of board was placed on the bottom board of each hive. Twenty-five hives had cardboard coated with epoxy, eleven hives had cardboard covered with adhesive tape and fourteen hives had cardboard coated with beeswax. The corrugated part of the cardboard faced down to provide hiding places for hive beetles. Cardboard detectors were checked eleven days after placement. The best board was the type least damaged by the bees. The percentage of hives containing chewed or intact detector boards was calculated.

2.2 Essential oil and Apistan testing.

2.2.1. Essential oil testing.

Hives with a good laying queen and strong foraging force, free of any other disease and with 8% or more mite infestation, were used to test the efficacy of plant essential oils to control varroa mites. Hives that met those requirements were randomly assigned to experimental bee yards (Appendices [10](#), [11](#), [12](#), and [13](#)). The remaining hives were moved to non-experimental bee yards ([Appendix 14](#)). There were 10 bee yards in total, of which 6 were used to test the efficacy of essential oils. Five hives were placed in each bee yard. Only one treatment was administered in a particular yard. Treatment yards were separated as much as possible to avoid bias from bee drift. Because of the lack of space, some bee yards were only 400 meters apart. There were three treatments with their respective controls. Treatments were: wintergreen in mineral oil delivered with a wick system, sucrose syrup containing lemongrass and spearmint, and a combination of wintergreen patties and lemongrass soaked paper towels. All treatments and controls started on August 19th, and were administered for 29 days, the wintergreen-mineral oil treatment and control were discontinued on September 7, 19 days after the treatments started. All hives were checked periodically and managed as needed.

2.2.1.1. Wintergreen-mineral oil treatment (Wick system).

The wintergreen-mineral oil treatment contained 8% (by volume) wintergreen oil (Tony Delia, from Ungerer & Co., Lincoln Park NJ) and 92% mineral oil (Avatar Corp., Univ. Park IL, 60466, "citation 55". Lot # 9-109-02.) Both oils were measured in a graduate cylinder and mixed in a plastic container, which had a nozzle for dispensing the mixture in the field. This treatment was administered using a modified hive (Figures 2, and 3), which had an external oil reservoir (tin can with foil cover) welded to a copper pipe, which was attached to a PVC pipe connected to a bottom board with three openings to insert wicks. Wicks were positioned vertically between the central frames of the brood chamber and were made of 1/2" x 1/8" wood strips to which half-inch wide blotter stock material was stapled (Knowlton specialty papers, NY). Wicks were soaked with the oils two hours prior to assembling the modified hives in the bee yards. Oil evaporated from the wicks and nurse bees also came in contact with the wicks containing the oil. Colonies were managed with two brood chambers. A queen excluder was placed between the brood chambers and the honey supers. Mineral oil hives were assembled in the same way as the wintergreen mineral oil treatment hives, in a different bee yard, but only mineral oil was administered (Figure 4). Colonies were fed syrup every other day. Oil reservoirs were checked while syrup jars were administered. Reservoirs were refilled as needed and the amount of oil used per hive was recorded. Modified bottom boards were checked at the same time and replaced if leaking.

The wintergreen-mineral oil treatment was suspended after two weeks and five days. The application of treatment was stopped on September 3rd, after observing abnormal behavior of the queen in all five colonies. Observations of the queens' behavior continued until September 5th. Hives were switched to regular bottom boards on September 7th.

Figure 2- Modified bottom board with three vertical wicks and an oil reservoir

2.2.1.2. Lemongrass-Spearmint treatment (Sucrose syrup).

Tony Delia (Anpack Associates, Connecticut) provided the essential oil concentrates to make the lemongrass and spearmint sugar syrup treatment. The concentrate is a proprietary formula (now called Honey - B- Healthy), consisting of lecithin, sodium lauryl sulfate, water, and a 2:1 mixture of spearmint to lemongrass essential oils. An 8 oz. Bottle contains 156 grams spearmint and 78.1 grams lemongrass. Boardman feeders were used to administer this treatment ([Figure 5](#)). Quart Mason jars with perforated lids were placed onto the feeders ([Figure 6](#)). Two teaspoons of concentrate were placed in each jar containing syrup, which provided one cc of total essential oil per quart. Control colonies were fed syrup, which contained no essential oils. A quart jar of syrup was administered to each bee hive every other day for one month. When the bees did not completely consume these syrups, the jars were replaced with jars containing fresh syrup. A record was maintained to show when the syrup was not completely consumed. Colonies were managed with two Illinois depth brood chambers. A queen excluder was placed between the brood chambers and the honey supers.

Figure 5- Boardman feeder.

Figure 6- Feeders on hives.

2.2.1.3. Lemongrass (Paper towels)Wintergreen treatment (Grease patties).

The lemongrass and wintergreen treatment was administered with a combination of two methods: paper towels, and grease patties ([Figure 7](#)). Both methods were applied simultaneously. For the paper towel method, thirty regular kitchen paper towels were placed in a zip-lock plastic bag and saturated with one cup of canola oil containing 5 cc of wintergreen and 5 cc of lemongrass essential oil. These towels were used for three applications of the treatment. Two applications were made with paper towels saturated with one cup of canola oil containing 10 cc. of lemongrass essential oil. [Table 1](#) shows the treatment applied and application dates. The saturated paper towels were put on top of the top bars of the upper super. The control consisted of paper towels saturated with plain canola oil. Paper towels saturated with the oils were removed and replaced with fresh towels every week.

Table 1. Paper towel application dates.

Hive number	Date	Treatment	Number of towels
1, 2,38,51,17	08-19-99	1	2
11,15,32,33,46	08-19-99	0	2
1, 2,38,51,17	08-25-99	1	2
11,15,32,33,46	08-25-99	0	2
1, 2,38,51,17	08-31-99	1	2
11,15,32,33,46	09-1-99	0	2
1, 2,38,51,17	09-8-99	2	2
11,15,32,33,46	09-8-99	0	2
1, 2,38,51,17	09-13-99	2	2
11,15,32,33,46	09-13-99	0	2
	09-16-99	End of treatments	

0= Control. Plain canola oil.

1= 5 cc. of wintergreen and 5 cc. of lemongrass oil.

2= 10 cc. of lemongrass oil.

Grease patties were prepared by mixing 4 pounds of granulated sucrose, 1.5 pounds of shortening (hydrogenated vegetable oil), 0.5 pounds of honey and 45 cc. of wintergreen oil. Two 2-3 oz (56-84 g). grease patties were placed on top of the queen excluder, which was between the brood chambers and the honey supers. The control consisted of grease patties without wintergreen. Grease patties were removed and replaced with fresh ones every 13 days. [Table 2](#) shows grease patty application dates. Both grease patties and paper towels were administered for 29 days. Colonies were managed with two Illinois depth brood chambers. A queen excluder was placed between the brood chambers and the honey supers. Colonies were fed with sugar syrup every other day.

Table 2. Grease patty application dates.

Hive number	Date	Treatment	Number of patties
1, 2,38,51,17	08-19-99	1	2
11,15,32,33,46	08-19-99	0	2
1, 2,38,51,17	08-31-99	1	2
11,15,32,33,46	09-1-99	0	2
1, 2,38,51,17	09-13-99	1	2
11,15,32,33,46	09-13-99	0	2
	09-16-99	End of treatments	
0= control		1=treatment

Figure 7- Hive with grease patties and paper towels indicated.

2.2.2. Apistan® treatment.

The Apistan® treatment started on August 19th. Mite populations were assessed 29 days after treatment started. Five colonies were treated with Apistan® in a bee yard at the Agronomy farm. Following the manufacturer's instructions, two Apistan® strips were placed in the upper brood chamber of five hives (Hives 45,22,48,49, and 53). Colonies were fed with syrup every other day during the experiments. Colonies were managed with two Illinois depth brood chambers. A queen excluder was placed between the brood chambers and the honey supers.

2.2.3 Gathering of data

After treatments, mite populations were estimated by randomly selecting a brood frame from each hive. All frames were collected the same day, numbered and kept in a hive in the laboratory. The entrance of the hive was covered with wire mesh to prevent emerging bees from escaping. The sampling process in the laboratory took three days, which ensured that brood remained alive and that mites found dead were likely killed by the treatments. Bees emerging from the frames were examined for the presence of mites or symptoms of Parasitic Mite Syndrome. They were not counted because it was impossible to determine from which frame they emerged. One hundred brood cells were opened in each frame and carefully examined for the presence of mites. Larvae, pupae or adults about to emerge were carefully inspected with the naked eye, and with the aid of a fiber optic lamp. The bottom and walls of the cells were examined for immature mites, dead adult mites and mite feces. Bees with mites were put in vials containing 70% alcohol. The number of brood cells with mites was recorded, discriminating between bee stages. Observations of adult mite mortality were made.

2.3 Statistical treatment of data.

A one way completely randomized Analysis of Variance with unequal sample size was made. The ANOVA determined differences between essential oil treatments, their controls and Apistan®. A Duncan test was performed to establish significant differences between essential oil treatments, their controls and Apistan®. The wintergreen-mineral oil treatment and its control were not included in the ANOVA because this experiment was stopped due to loss of queens.

3. Results

3.1 Hive infestation.

The results for the different infestation methods are based on observations on 51 honey bee colonies; infestation methods had no apparent influence on the behavior of the bee colonies. Shaking infested adult bees in front of the hives did not produce measurable infestation of the bees 10 and 24 days after initial attempt to infest. The drone comb insertion method was the easiest and least labor-intensive method of infesting hives with varroa mites. The detection and removal components of the bee hygienic behavior were observed in all colonies that were infested using the brood comb insertion method. Bees detected and removed pieces of the inserted brood. However, mites that were freed from the infested comb during this process were not removed.

On June 28th, six and twenty-eight hives were infested with the frame insertion method and comb insertion method, respectively. The survey performed 20 days after this infestation attempt showed that 100 and 28.57 percent infestation were achieved with the frame insertion method and drone comb insertion method, respectively. [Table 3](#) shows the results of the survey taken after the final infestation attempt. Mites were found in the scarce drone cells present, worker cells, and queen cells. The percentage of infested cells ranged from 0 to 56 with an average of 16.4 percent. [Figure 8](#) shows a sample of mites found in 100 uncapped cells during the last infestation survey.

Table 3. Number of infested cells per hive on August 12-1999

Hive Number	Location	Larvae	Pupae	Adult	% of infested cells per hive
1	GH	2	23	1	26
2	GH	13	11	5	29
3	GH	5	20	0	25
4	GH	3	1	25	29
5	GH	0	5	2	7
6	GH	7	3	0	10
7	GH	1	17	1	19
8	GH	12	21	1	34 + I Q
9	GH	13	3	0	16
10	GH	1	8	15	24
11	HF1	6	15	0	21
12	HF1	4	6	0	10
13	HF1	5	5	3	13
14	HF1	5	2	0	7
15	HF1	10	5	5	20
16	HF1	3	11	1	15
17	HF1	4	26	0	30
18	HF1	0	5	1	6
19	HF1	22/70	0	0	31.4
20	HF1	4	8	0	12
21	HF1	0	5	29	34
22	HF1	0	7	12	19
23	HF1	0	0	0	***
24	HF2	0	1	0	1
25	HF2	10	0	1	11
26	HF2	0	1	8	9
27	HF2	17	0	0	17
28	HF2	0	0	0	***
29	HF4	0	0	0	0
30	HF2	0	12	0	12
31	HF2	Combined			
32	HF2	12	10	0	22
33	AF1	3	22	1	26
34	HF4	0	0	0	***
35	AF1	0	0	0	***
36	HF4	0	0	0	***
37	HF4	3	2	3	8
38	AF2	8	0	0	8
39	AF2	0	0	3	3
40	AF2	5	5	0	10
41	AF2	4	2	0	6
42	AF2	0	1	13	14
43	AF2	0	3	3	6
44	AF3	0	6	14	20
45	AF3	5	31	20	56
46	AF3	7	25	0	32
47	AF3	5	0	0	5
48	AF3	20	1	0	21
49	GH	8	16	5	29
50	HF1	0	27	7	34
51	HF1	0	4	4	8
52	HF1	18	2	0	20
53	HF2	6	0	8	14
54	AF3	0	50	2	52
55	JWA	1	0	0	1
56	AF3	4	53	7	64
57	AF3	62	3	3	68
58	HF2	1	35	0	36
59	HF2	0	10	2	12
60	GH				***

*** No capped cells IQ= infested queen cell

GH = Greenhouse HF = Horticulture Farm AF=Agromony Farm

Figure 8- Sample of bees with mites found in 100 capped cells.

3.2 Hive beetle survey.

No hive beetles were found. However, bees detected and disposed of most detector boards. The comparison between the percentage of detector boards remaining in the hive after eleven days is shown in [Figure 9](#). Bees chewed and tore apart boards coated with epoxy and chewed the boards covered with adhesive tape, leaving only the tape. Boards coated with beeswax remained intact.

Figure 9- Percentage of hives with detector board after eleven days.

3.3 Essential oil treatments.

[Table 4](#) shows the number of infested cells found per hive after application of treatments. The Anova for the number of infested cells in colonies treated with essential oils, their respective controls and Apistan® is shown in [Table 5](#). There is evidence of differences among essential oil treatments, their controls, and Apistan ([Appendix 15](#)).

Table 4. Number of mites found in one hundred cells on September 20- 1999

Treatment	Hive	Larval	Pupal	Adult	% of infested cells	% of change
Wintergreen-Lemongrass	1	36	17	0	53	+27
Wintergreen-Lemongrass	3	24	14	0	38	+13
Wintergreen-Lemongrass	17	18	38	0	56	+26
Wintergreen-Lemongrass	37	24	15	0	39	+31
Wintergreen-Lemongrass	51	6	14	0	20	+12
Wintergreen-Lemongrass ctl.	11	Broodless			Broodless	Broodless
Wintergreen-Lemongrass ctl.	15	0	37	13	50	+30
Wintergreen-Lemongrass ctl.	32	0	17	5	22/82	+5
Wintergreen-Lemongrass ctl.	33	1	36	0	37	+11
Wintergreen-Lemongrass ctl.	46	5	33	0	38	+6
Spearmint lemongrass	7	0	37	0	37	+18
Spearmint lemongrass	8	61	3	0	64	+29
Spearmint lemongrass	19	19	1	0	20	-11.4
Spearmint lemongrass	57	10	44	1	55	-13
Spearmint lemongrass	59	17	8	2	27	-15
Spearmint lemongrass ctl.	10	50	25	0	75	+51
Spearmint lemongrass ctl.	20	12	16	15	43	+37
Spearmint lemongrass ctl.	16	0	39	0	39	+24
Spearmint lemongrass ctl.	44	0	15	2	17	-3
Spearmint lemongrass ctl.	58	0	26	9	35	-1
Apistan®	48	0	0	0	0	-21
Apistan®	53	0	0	0	0	-14
Apistan®	49	0	0	0	0	-29
Apistan®	22	0	0	0	0	-19
Apistan®	45	0	0	0	0	-56

Table 5. Analysis of variance for the essential oils and Apistan® treatments and controls

Source	df	SS	MS	F	F critic
Among groups	4	6525.0	1631.2	7.27	5.811
Within groups	19	4261.6	224.29		
Total	23				

$\alpha = 0.05$

[Table 6](#) shows the Duncan test for the essential oil treatments, their controls and Apistan®. The Apistan® treatment is significantly different from all essential oil treatments and controls. There are no significant differences between the essential oil treatments, nor between the essential oil treatments and their respective controls. According to these results, the use of essential oils against varroaosis, in the concentrations, blends, with the application methods, and on the dates of this

experiment did not reduce mite populations. There is significant evidence that Apistan® reduces mite populations after 29 days. The means for the mite populations after 29 days of treatment can be observed in [Figure 10](#). Figures [11](#) and [12](#) show mites on larvae and adults, respectively. This was commonly observed throughout the experiments.

Table 6. Duncan test for essential oil treatments, their controls and Apistan®.

Treatment	Mean of number of cells with mites.
Apistan®	0 ^a
Wintergreen + Lemongrass control	37.75 ^b
Lemongrass + Spearmint	40.6 ^b
Wintergreen + Lemongrass	41.2 ^b
Lemongrass + Spearmint control	41.8 ^b

Treatments with different letters are significantly different
 $\alpha = 0.05$

Figure 11- Varroa mites on honey bee larvae.

Figure 12- Varroa mites on adult honey bee.

3.3.1. Wintergreen - Mineral oil treatment.

The wintergreen-mineral oil treatment was stopped 19 days after it was started. The total amount of oil used per hive is recorded in [Table 7](#). But, this data is not accurate. Some of the modified bottom boards leaked and were replaced on four occasions. Because of this, it is difficult to determine the amount of oil lost due to volatilization and the amount of oil lost due to leakage.

Table 7. Amount of oil used in cc.

Hive	Date									TOTAL
	08-19	08-20	08-22	08-24	08-26	08-28	08-30	09-1	09-3	
2 T	300	0	0	0	0	75	0	10	0	385
4 T	275	0	50	0	0	255*	0	0	0	530
21 T	245	0	0	0	230*	24	0	0	0	499
54 T	255	0	25	0	0	120	20	0	0	420
52 T	265	0	0	250*	0	20	0	20	20	575
9 C	285	0	20	0	45	50	0	0	0	400
30 C	250	0	0	0	25	125	0	0	0	400
42 C	275	0	20	0	0	100	0	50	0	445
50 C	220	0	100	0	0	95	200*	0	25	640
56 C	270	0	0	0	0	200	20	0	0	490

T= treatment C= Control * Board replaced.

It was observed that bees behaved differently after the wintergreen mineral oil treatment was delivered into the hives. Queens stopped laying eggs or supercedure occurred in four of the five treated colonies. The queen in the fifth treated colony (colony 52) was laying abnormally in the outer frames. Control colonies did not exhibit a change in behavior.

3.3.2. Spearmint - Lemongrass treatment.

Bees from treatment and control colonies were fed a quart jar (0.95 liters) of syrup on dates seen in table 8. Normally, the syrup was consumed the same day the treatment was delivered to the hives. However, all jars were not completely emptied on seven occasions. It appears that there might be a relationship between syrup consumption and temperature. [Table 8](#) shows maximum daily temperature and qualitative observations of syrup consumption for the treatment period.

Table 8. Syrup consumption and temperature.

Date	Temp(°F)	Treatment Consumption	Control Consumption
08-19-99	85	Not observed	Not observed
08-20-99	79	Incomplete	Two hives did not consume
08-22-99	80	One hive did not consume	Jars not completely empty
08-24-99	88	Jars not completely empty	Jars not completely empty
08-26-99	79	1 quart/hive	1 quart/hive
08-28-99	85	Not observed	Not observed
08-30-99	74	1 quart/hive	1 quart/hive
09-1-99	86	Jars not completely empty	Jars not completely empty
09-3-99	92	Jars not completely empty	Jars not completely empty
09-5-99	78	Jars not completely empty	Jars not completely empty
09-7-99	84	1 quart/hive	1 quart/hive
09-9-99	76	Two hives did not consume	Jars not completely empty
09-10-99	76	1 quart/hive	1 quart/hive
09-13-99	83	Jars not completely empty	Jars not completely empty
09-15-99	71	1 quart/hive	1 quart/hive
09-16-99	60	End of treatments	

Temperatures measured by the National Oceanic and Atmospheric Administration (NOAA) at Hart field, Morgantown.

3.3.3. Wintergreen - Lemongrass treatment.

No behavioral changes were observed when grease patties were applied together with lemongrass-soaked paper towels or when applied together with lemongrass-wintergreen soaked paper towels. The intended mechanism for oil delivery with grease patties was physical contact, which occurred when bees consumed grease patties.

Colonies tore apart paper towels impregnated with the wintergreen - lemongrass blend. However, when lemongrass was applied by itself, three out of five colonies did not display the detection and removal parts of the hygienic behavior of bees. One week after the lemongrass was administered by itself, paper towels were found intact in the treatment hives. Paper towels were always removed in control colonies.

3.4 Mortality.

All hives treated with essential oils contained adult mites alive and actively moving. It was not determined if the immature mites found were dead or alive. No immature mites nor adult mites were found in hives treated with Apistan®.

4. Discussion

Numerous factors adversely influenced the final outcome of the essential oil experiment. The lack of colonies naturally infested with varroa mites at WVU apiaries at the beginning of the 1999 season delayed the beginning of the experiment. The basis for the experiments was preliminary field work with local beekeepers, which provided valuable information for selecting oils and delivery systems. But, there were no previous laboratory tests to establish appropriate doses regarding mite mortality and honey bee compatibility. Despite negative results, this work contributes toward understanding bee behavior when bees are exposed to essential oil treatments, and reveals the importance of an adequate infestation method for research purposes.

The beekeeping equipment used, particularly the foundation could explain why hive infestation was difficult to accomplish. Hives had two brood chambers with ten frames each. Out of 20 frames, 19 were built with Rite-cell ® foundation, which consisted of worker-sized cells. Only one wax frame suitable for producing drones was provided. The scarcity of drone brood in the experimental hives decreased the number of mite offspring, making the artificial infestation difficult to achieve. The results of the infestation attempts are consistent with this. Shaking infested bees in front of the hives did not infest the bees whereas introducing infested drone comb pieces and wax frames with infested drone brood resulted in 28%, 57% and 100% of infestation, respectively. Mites infested the worker-sized brood cells, but infestation of hives with adequate numbers of varroa mites took ten weeks. This occurred probably because the size of the cell has an effect on mite reproduction. Shulz (1984) calculated the number of mite offspring produced in worker and drone cells. He found that a typical female will produce 1.82 offspring in a worker cell, whereas it will produce 2.69 offspring in a drone cell. Several factors explain why mites prefer drone-sized cells over worker-sized cells for reproduction. The smaller size of the worker cell is detrimental for mite reproduction because crowded conditions result in competition for food, which limits the development of immature stages of the mite (Fuchs and Lagenbach 1989, Eguaras and Marcangeli 1994). Longer drone post-capping time provides more opportunity for mites to be fertilized and reproduce in subsequent brood cycles (Fries *et al.* 1994). Martin and Kemp (1997) simulated Varroa population dynamic models in order to determine which factors have the greatest impact on mite population growth. Host development time influenced the number of reproductive cycles achieved by each mite and the number of fertile offspring produced per cell. Drone cells have a postcapping period that is one day longer than that of worker cells which has a tremendous effect on the number of females that are fertilized within the cells. Büchler (1994) found that a one hour reduction of the postcapping time resulted in an 8.7% reduction of mite population growth. A one-day reduction can result in a 50% reduction of mite offspring in subsequent cycles (Calis *et al.* 1999b).

The results of the infestation attempts clearly show that Rite-cell ® foundation is capable of slowing down mite population growth at initial stages of infestation. Thus, this type of foundation may be a good tool to be included in IPM programs for varroa mites. Hives built with wax foundation are generally easier to infest and would be more suitable for varroa research. However, in continued studies in 2000, the foundation seems to have little effect on build up of mites (Amrine personal communication).

Another cause of the difficulties in infesting the hives could be the weather. Some studies indicate that honey bee colonies alter brood production in response to climate (Janmaant *et al.* 2000). Mite population growth depends upon brood availability, which depends upon the colony's ability to collect pollen and nectar and convert these resources into brood (Janmaant *et al.* 2000). Bee packages were installed during the spring, when the temperature was cool. This may have reduced brood production and mite reproduction at the beginning of the season (Amrine, personal communication).

Hive infestation may have been hindered by a drought that occurred in West Virginia during 1999. According to the National Drought Mitigation center and the National Oceanic and Atmospheric Administration (NOAA), precipitation levels during the infestation period were significantly below¹¹ normal in the North-central part of West Virginia. According to the Palmer¹² Drought Index for July and August, North-central West Virginia was experiencing a “severe drought” ([Appendix 16](#)). A drought during the experiment may have reduced the availability of pollen and nectar and may have had a negative impact on brood production. Some colonies appeared weak. It has also been demonstrated that a frequent reaction to food shortage in honey bee colonies is brood cannibalism (Schulz *et al.* 1998).

Treatments started in mid-August after several attempts of infestation. The experiments were stopped in mid-September to avoid bias from the mite population assessment method. The cell-uncapping assessment method can only be used when brood is available and may lead to biased results during the fall, when brood production decreases and mites tend to concentrate in the shrinking number of brood cells available. Brood is scarce or absent during late fall and winter because colonies respond to changes in resource availability (Pankiw *et al.* 1998). One month of treatment may have not been sufficient to detect any difference in mite control.

Hives were checked for the presence of hive beetles because bee packages for the experiments were imported from Georgia, where this pest is present. A simple method to detect the small hive beetle is to take advantage of its tendency to hide in crevices. Pieces of corrugated cardboard peeled on one side and placed facing down on the bottom board of the hive provides an excellent refuge for the beetles. Nevertheless, the hygienic behavior of the bees represented difficulties. The results of the experiment with different detector boards indicated that the bees were able to destroy cardboard covered with foreign materials such as epoxy and adhesive tape, but usually did not damage cardboard inserts coated with beeswax. Bees destroyed the detector boards coated with epoxy and tape possibly because unfamiliar odors in the hive triggered the removal part of the hygienic behavior in bees. It has been demonstrated that olfactory signals from diseased and parasitized brood activate hygienic

¹¹ NOAA reports 1.61" at Hart Field, Morgantown in August whereas the historical average is 4.01".

¹²The Palmer Drought Index (PDI) is a meteorological index that assesses the severity of dry and wet periods. The PDI is calculated according to the water balance equation, which includes all inflows and outflows of water. This index incorporates information on temperature, precipitation, moisture, evapotranspiration, soil recharge, run off, available water content of the soil, and moisture loss by the soil surface layer. The scale of the the PDI varies from 6.0 to - 6.0. ([Appendix 17](#)) (Hayes 1999).

behavior in bees (Spivak and Guillian 1998). On the contrary, bees did not destroy detector boards coated with beeswax possibly because they recognized these boards as part of the colony.

The chemical composition of the essential oils, the doses and delivery systems were designed to target the mites without affecting the bees. However, the results obtained suggest that doses and application methods should be reviewed. None of the essential oil treatments were effective in controlling varroa mite populations under the conditions of the experiment. It is necessary to develop laboratory-screening tests for the essential oil mixtures used at WVU. Other researchers have screened wintergreen and spearmint and obtained good results, regarding mite control and bee health (Hoppe 1990). But, experiments with different chemotypes of the same oil may produce a different outcome. The emulsifier used in the WVU essential oil formulation, lecithin, should also be considered as a potential cause of variation. Lecithin enhances insect reproduction (Chapman 1998) and it may have the same effect on mites.

In regard to the toxicity of the essential oils that were tested, the results clearly indicated that high concentrations of wintergreen oil (8% by volume) evaporated within the brood chamber altered the normal functioning of the colony. Queens were superceded, stopped laying eggs, or were laying in the outer frames far from where the oil was released. Control colonies, for this treatment, did not show any change in behavior. It is believed that the observed changes occurred as a consequence of the essential oil treatment and not as consequence of the prevalent environmental conditions, the wicks themselves or any other variable. Kraus (1994) and Hoppe (1990) found behavioral disturbances in bee colonies treated with wintergreen. Essential oils that are toxic when evaporated should never be applied within the brood chamber (Imdorf, personal communication). Similar experiments evaporating other toxic essential oils revealed that high concentrations of essential oils within the brood chamber are capable of altering bee behavior. It was found that when thymol oil was applied as wafers between supers, the brood was destroyed and queens and adult bees left the colonies. However, bees tolerated thymol when it was applied in combination with eucaliptol, menthol, and camphor via a porous ceramic carrier, which released the essential oil slowly (Imdorf, personal communication). This formulation was successfully used in European apiaries (Api Life Var ®) (Imdorf *et al.* 1999). Different delivery methods volatilize essential oils at different rates and there is probably a rate that is optimal for the health of the bees. Different formulations and lower concentrations of wintergreen did not show negative effects that commonly accompany stronger wintergreen treatments. At WVU, no altered bee behavior was observed when wintergreen was delivered with grease patties.

Nevertheless, treating colonies with wintergreen grease patties, wintergreen-lemongrass paper towels and lemongrass paper towels failed to control the mites, possibly because the treatment period was too short and paper towels were prepared inconsistently..The wintergreen oil was mistakenly absent from the paper towels twice so the data from all five hives in this experimental group is questionable. Possibly, a treatment effect may have been seen if the paper towels were prepared with unchanged amounts of essential oils. Also, the intention was for bees to come in contact with the patties while attempting to remove the foreign material from the hive and for the varroa mite to indirectly or directly receive a dosage of the oil. The removal process was observed inside the hive but the transference to the bee body or mite was not verified.

Applying paper towels containing only lemongrass produced an interesting outcome. It was

observed that after one week of applying paper towels soaked with lemongrass, three out of five colonies stopped removing the towels. The fact that lemongrass essential oil may be capable of stopping the removal of foreign objects in a bee hive would have practical applications. The use of lemongrass may be useful for designing a device to detect hive beetles without it being destroyed by bees (Amrine, personal communication). Citral is the main constituent of the lemongrass essential oil. It is a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial and neral (Lewinsohn *et al.* 1998). Lemongrass essential oil also contains small quantities of geraniol, geranylacetate and monoterpene olefins (Lewinsohn *et al.* 1998). Geraniol is also a bee pheromone, produced in the Nasonov gland of the bees and is used as a homing odor (Winston 1987). Small quantities of geraniol present in the lemongrass oil may be sufficient to suppress the hygienic response to any material emitting this compound. Bees may have perceived the paper towels impregnated with lemongrass as part of the colony.

Mite populations were not controlled with the lemongrass-spearmint syrup. Bees did not completely consume the syrups on seven occasions, mostly followed by three-day periods of high temperatures, which indicates that there might be a relationship between temperature and syrup consumption ([Table 8](#)). It is possible that a cross inhibition between water collection and nectar collection occurred. When ambient temperatures are high, honey bee colonies increase consumption of water to prevent damage to the brood. Nectar collection decreases during high temperatures because there are more water receivers than nectar receivers (Kühnholz and Seely 1997). However, to conclude that a relationship between syrup consumption and temperature exists, it would be necessary to measure syrup consumption more precisely over a longer experimental period. In order to determine if syrup consumption stops due to bee behavior rather than unavailability of the syrup, bees must consume syrup out of a reservoir that would never be allowed to deplete. For that purpose, a different syrup delivery system would be necessary. Larger feeders with measuring units may be appropriate. Daily measurements of syrup delivery and syrup consumption would provide data that can be related to temperature and relative humidity.

Skinner *et al.* (1997) treated hives with lemongrass-spearmint syrup and also obtained negative results. Their mite population increased 258% in comparison to their control that increased 100% after feeding colonies with spearmint syrup. It appears that their poor results are attributable to inadequate treatment frequency. Details are vague regarding the total amount of oil delivered over their sixty day experimental period.

In conclusion, varroa infested hives treated with essential oils showed no noticeable reduction in population. Some of the treatments may have been more effective if used over long (>3 month) periods on colonies with lower populations. The overall strategy of the experiment was to assess short-term control of varroa populations. The approach taken was to heavily infest and then to attempt to suppress the population. Perhaps, a long-term, preventative approach is the best way to assess varroa mite controls especially if the nature of bee colonies is such that they cannot be cured after being stricken with high varroa populations.

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APPENDICES

Appendix 1 -Progression of varroatosis

Appendix 2 - Map showing the spread of varroa mite around the world.

Appendix 3 - Map of varroa mite distribution in USA.

Appendix 4 - *Varroa destructor* haplotypes' distribution

Appendix 5 - *Varroa destructor* life cycle.

Picture modified from Purdue University Cooperative Extension Service.

1. Mite enters cell of five day old larva.
2. Mite is trapped in larval food. Fifteen hours in worker cells. Forty-five hours in drone cells (Ifatidis 1988).
3. Worker cell is capped on the 8th day after the queen lays the egg. Drone cell is capped on the 10th day after the queen lays the egg. Mite starts feeding on larvae after cell is capped. First egg is laid 60 hours after. Eggs are laid at 30 hour intervals. 1-6 eggs are laid. Egg development takes about 10 days. A protonymph emerges from the egg. It develops into a deutonymph that becomes an adult. First egg becomes a male.
4. Female offspring mate within the cell before the emergence of the bee. Males die after mating.
5. Worker bees emerge from the cell 21 days after the queen lays the egg. Drones leave the cell 24 days after the queen lays the egg.
6. Gravid and juvenile female mites leave the cell when the adult bee emerges. Undeveloped mite offspring die within the cell.
7. Mites move on to passing bees. Bees infect each other by close contact.
8. Infected nurse bee feeds larvae. Mite enters cell.

Appendix 6 - Chronogram of activities.

Date	Activity
April 12- 23	Prepare equipment Spring management for old colonies starts: divide strong hives, strengthen weak ones, and ensure all hives have a queen
April 26 - 30	Set equipment on bee yards for new colonies.
May 3	Bee packages installed
May 10	Spring management continues for old colonies
May 17- 24	Inspect new colonies for queens or eggs
May 24 -28	Routine check: test for disease, hive beetles and strength of hive
June 1	Infestation starts
June 7 - 11	Routine check
June 14- 18	Quick survey of infestation
June 21- 25	Quick survey of infestation
June 28- July 2	Quick Survey of infestation
July 15	Start syrup feeding, routine check
July 15-19	Re-infestation
July 20- 30	Routine check
August 2- 8	Detailed survey of infestation
August 9-13	Move hives to a yard chosen randomly from ten total yards.
August 16	Begin treatment. Begin control if the hive is in a control yard
August 17- Sept 15	Checking treatments
Sept 16	End of treatments

Appendix 7 - Hive number and infestation method .

Hive number	6/1-6/4	6/28-7/1	7/15-7/17
1		2	
2*			
3		2	
4		2	
5		2	
6*			
7		2	
8		2	
9		3	
10		3	
49		3	
11		3	2
12		3	2
13		3	2
14		3	2
15		3	2
16		3	2
17		3	2
18		3	2
19		3	2
20		3	2
21		3	2
22		3	2
50	2	3	
51		3	2
23	2		
52		3	2
24		3	2
25		3	2
26	2	3	
27		3	2
28	2	3	
29		3	
30	2	3	
31		3	
32	2	3	
53		3	
58*			
59*			
33	1		4
34	1		4
35	1		4
36	1		4
37	1		4
38	1		4
39	1		4
40	1		4
41	1		4
42	1		4
43	1		4
44	1		4
45	1		4
46	1		4
47	1		4
48	1		4
54*			
56*			
57*			
60*			

*Colonies with this symbol were used as a source of infestation.

Method 1= shaking bees in front of hives, 2= Inserting frames, 3= Inserting drone comb, 4= combination of 2 and 3.

Appendix 8- Infestation surveys.

Hive number	06/14-6/18	6/28-7/2	7/15-7/17	8/2-8/8
1	0	0	1	1
2*	*	*	*	*
3	0	0	1	1
4	0	0	1	1
5	0	0	1	1
6*	*	*	*	*
7	0	0	1	1
8	0	0	1	1
9	0	0	1	1
10	0	0	1	1
49	0	0	1	1
11	0	0	0	1
12	0	0	0	1
13	0	0	0	1
14	0	0	0	1
15	0	0	0	1
16	0	0	0	1
17	0	0	0	1
18	0	0	0	1
19	0	0	0	1
20	0	0	0	1
21	0	0	0	1
22	0	0	0	1
50	0	1	1	1
51	0	0	0	1
23	1	1	1	Broodless
52	0	0	0	1
24	0	0	0	1
25	0	0	0	1
26	0	0	1	1
27	0	0	0	1
28	0	0	1	Broodless
29	0	0	0	0
30	0	0	1	1
31	Queenless	Broodless 0	Combined	
32	1	1	1	1
53	0	0	0	1
58*	*	*	*	*
59*	*	*	*	*
33	0	0	0	1
34	0	0	0	Queenless
35	0	0	0	Broodless
36	0	0	0	Broodless
37	0	0	0	1
38	0	0	0	1
39	0	0	0	1
40	0	0	0	1
41	0	0	0	1
42	0	0	0	1
43	0	0	0	1
44	0	0	0	1
45	0	0	0	1
46	0	0	0	1
47	0	0	0	1
48	0	0	0	1
54*	*	*	*	*
56*	*	*	*	*
57*	*	*	*	*
60*	*	*	*	*

*Colonies with this symbol were used as a source of infestation.

0 = No mites present.

1 = Mites present.

Appendix 9 - Example Data sheet.

Date:		
Hive #:		
Bee stage	Number of infested cells	Total
Larvae		
Pupae		
Adult		
Observations		
% of infestation		

Appendix 10 - Overview of bee yard locations.

Appendix 12- Map of location of bee yards at Horticulture Farm

Appendix 13 - Map of bee yard location at greenhouse.

Appendix 14 - List of hives and number of cells with mites

Hive Number	Old location	New location	Treatment	Cells with mites
1	GH	HF1	Paper towel	25
2	GH	AF1	Wick	27
3	GH	HF1	Paper towel	25
4	GH	AF1	Wick	28
5	GH	AF5	None	7
6	GH	AF5	None	10
7	GH	GH	Syrup	19
8	GH	GH	Syrup	32
9	GH	HF3	Wick control	16
10	GH	AF3	Syrup control	25
11	HF1	HF2	P t control 21	
12	HF1	AF5	None	10
13	HF1	HF4	None	13
14	HF1	AF5	None	7
15	HF1	HF2	Pt control	20
16	HF1	AH3	Syrup control	14
17	HF1	HF1	Paper towel	31
18	HF1	AF5	None	6
19	HF1	GH	Syrup	32
20	HF1	AF3	Syrup control	12
21	HF1	AF1	Wick	52
22	HF1	AF4	Apistan	19
23	HF1	HF4	None	***
24	HF2	HF4	None	1
25	HF2	AF5	None	11
26	HF2	AF5	None	8
27	HF2	HF4	None	17**
28	HF2	AF5	None	***
29	HF4	HF4	None	0
30	HF2	HF3	Wick control	12
31	HF2	HF2	None	
32	HF2	HF2	Pt control	22
33	AF1	HF2	Pt control	26
34	HF4	HF4	None	***
35	AF1	HF4	None	***
36	HF4	HF4	None	***
37	HF4	HF4	None	8
38	AF2	HF1	Paper towel	8
39	AF2	AF2	None	3
40	AF2	AF2	None	10
41	AF2	AF2	None	6
42	AF2	HF3	Wick control	12
43	AF2	AF2	None	6
44	AF3	AF3	Syrup control	20
45	AF3	AF4	Apistan	36
46	AF3	HF2	Pt control	32
47	AF3	AF2	None	5
48	AF3	AF4	Apistan	21
49	GH	AF4	Apistan	29
50	HF1	HF3	Wick control	34
51	HF1	HF1	Paper towel	8
52	HF1	AF1	Wick	20
53	HF2	AF4	Apistan	13
54	AF3	AF1	Wick	52
55	JWA	JWA	None	5
56	AF3	HF#	Wick control	64
57	AF3	GH	Syrup	68
58	HF2	AF3	Syrup control	35
59	HF2	GH	Syrup	12
60	GH	HF4	None	***

AF= Agronomy Farm. HF= Horticulture Farm. GH= Greenhouse. JWA= Dr. Amrine house Numbers are yards in each location.

** Laying worker. *** No capped cell

Appendix 15- Means and sums of squares for the ANOVA of essential oil treatments, their controls and Apistan®

j=1						Squares				
j=1	wg-Lg	wg-Lgc	Lg-Sp	Lg-Spc	Apistan					
	20		37	39	0	400		1369	1521	0
	56	26	55	35	0	3136	676	3025	1225	0
	39	38	64	17	0	1521	1444	4096	289	0
	38	50	20	75	0	1444	2500	400	5625	0
	53	37	27	43	5	2809	1369	729	1849	0
n	5	4	5	5	5					
Means	41.2	37.5	40.6	41.6	0					
J^2/n	8487.2	5700.3	8241.8	8736.2	0					
Total j	206	151	203	209	0					

A	31165		Source	df	SS	MS	F	F critic
C F	24640		Treatments	4	6525	1631.25	7.27	5.811
T	35427		Error	19	4261.6	224.29		
			Total	23				

Appendix 16 - The Palmer drought index for North Central West Virginia.

West Virginia-Division 02: 1999 (Monthly Averages)

Drought mitigation center 1999.

Appendix 17 - Palmer Drought Index. Scale.

4.0 or more	Extremely wet
3.0 to 3.99	Very wet
2.0 to 2.99	Moderately wet
1.0 to 1.99	Slightly wet
0.5 to 0.99	Incipient wet spell
0.49 to 0.49	Near normal
-0.5 to -0.99	Incipient dry spell
-1.0 to -1.99	Mild drought
-2.0 to -2.99	Moderate drought
-3.0 to -3.99	Severe drought
-4.0 or less	Extreme drought

VITA

Name: María Mercedes Vargas-Sarmiento

Birthplace: Santafé de Bogotá, Distrito Capital
República de Colombia

Birthdate: November 15, 1966

Education:

Elementary and high school

- Colegio Refous	1972 - 1982	Suba, Distrito Especial. República de Colombia
------------------	-------------	---

- Colegio Santo Domingo	1983 - 1985	Santafé de Bogotá, D.C. República de Colombia
-------------------------	-------------	--

Bachelor Science

- Pontificia Universidad Javeriana. Biology	1986 - 1994	Santafé de Bogotá, D.C. República de Colombia
--	-------------	--

Training

- Naha plant Protection station Plant quarantine and fruit flies disinfestation Techniques	1995	Okinawa, Japan
--	------	----------------

- Curso control biológico de plagas agrícolas	1996 Two month course	Havana, Cuba
--	-----------------------------	--------------