# EVALUATION OF EARLY SUMMER SPLITS ON *VARROA* MITE REDUCTION AND COLONY PRODUCTIVITY

by

Kathleen Ciola Evans

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Entomology

Spring 2015

**EVALUATION OF EARLY SUMMER SPLITS ON *VARROA* MITE REDUCTION**

**AND COLONY PRODUCTIVITY**

by

Kathleen Ciola Evans

Approved: ______________________________
Deborah A. Delaney, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: ______________________________
Jacob L. Bowman, Ph.D.
Chair of the Department of Entomology and Wildlife Ecology

Approved: ______________________________
Mark W. Rieger, Ph.D.
Dean of the College of Entomology and Wildlife Ecology

Approved: ______________________________
James G. Richards, Ph.D.
Vice Provost for Graduate and Professional Education

## ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my academic advisor Dr. Deborah A. Delaney, who has been an amazing advisor and mentor throughout my graduate studies. I appreciate her patient guidance over the course of the project and her enthusiastic encouragement.

Thank you to Elizabeth Hill for her help in developing the infrastructure to support the citizen science field project, Bugonia, and to all the Bugonia participants who participated in the study and dedicated their time and energy into the project. Thank you to my committee members for their support and constructive revisions throughout the course of the project, Judy Hough-Goldstein and John McDonald. Thank you to the Environmental Protection Agency and the National Honey Board for their interest in the project and their financial support. In particular thank you to John Butler, Regional Pesticide Expert, for his support throughout the project.

Thank you to my family members (parents and sisters) who are my biggest supporters. They have encouraged me throughout my academic career to accomplish the goals that I have set for myself. Thank you to all in the Department of Entomology and Wildlife Ecology at UDEL for providing continued support while pursuing a degree. Specifically I thank the BEE-autiful Bee Folk of Dr. Deborah Delaney's bee lab, David Gardner, Grace Savoy-Burke, James Wolfin and Owen Cass for their continued support throughout my degree. A special thanks to my friend Jaclyn Smolinsky for her help.

## TABLE OF CONTENTS

LIST OF TABLES ........................................ vi
LIST OF FIGURES........................................vii
ABSTRACT ........................................viii

Chapter

1 EVALUATION OF EARLY SUMMER SPLITS ON *VARROA* MITE REDUCTION AND COLONY PRODUCTIVI........................................1

1.1 Introduction ........................................1

1.1.1 *Varroa* mite biology ........................................2
1.1.2 Virus ........................................4
1.1.3 Apiary Management & control tactics ........................................5

1.1.3.1 Pest control strategies ........................................5

1.1.3.1.1 Cultural & mechanical control ........................................6
1.1.3.1.2 Genetics ........................................7
1.1.3.1.3 Behavior ........................................8

1.1.3.2 Biological control ........................................9
1.1.3.3 Chemical control ........................................9

1.1.4 Hypothesis ........................................10

1.2 Methods ........................................11

1.2.1 Apiary design ........................................11
1.2.2 Colony assessments ........................................13

1.2.2.1 Monthly assessments ........................................13
1.2.2.2 *Varroa* mite monitoring ........................................14
1.2.2.3 *Nosema*, viral, pesticide, temperature........................................14

1.2.3 Statistics ....................................................................................15

1.3 Results ....................................................................................16

1.3.1 2012 field season ....................................................................................16
1.3.2 2013 field season ....................................................................................18

1.3.2.1 *Varroa* mite to bee ratio ....................................................................................22
1.3.2.2 Pollen, nectar & honey ....................................................................................23
1.3.2.3 Drone comb removal ....................................................................................25
1.3.2.4 Virus ....................................................................................26
1.3.2.5 *Nosema* spore & hive temperature ....................................................................................26
1.3.2.6 Brood patterns ....................................................................................27

1.3.3 2014 field season ....................................................................................27
1.3.4 Pesticide analyses ....................................................................................28
1.3.5 Apiary one ....................................................................................29

1.4 Discussion ....................................................................................31
1.5 Citizen science field study 2014 ....................................................................................35

1.5.1 Methods ....................................................................................35

1.5.1.1 Protocol ....................................................................................36
1.5.1.2 Survey ....................................................................................36

1.5.1.2.1 Survey-1 ....................................................................................37
1.5.1.2.2 Survey-1 ....................................................................................38

1.5.2 Conclusion ....................................................................................38

REFERENCES....................................................................................40

Appendix

A COLONY SPLIT PROTOCOL....................................................................................44
B DATA ENTRY SHEET....................................................................................45
C SURVEY-1 2013....................................................................................46
D SURVEY-2 2014....................................................................................52
E CITISEN SCIENCE FIELD PROTOCOL....................................................................................54

LIST OF TABLES

Table 1: Average monthly values of bee population, brood area and *varroa* mite fall 2012 ........17

Table 2: Average seasonal values of bee population, brood area and *varroa* mite fall 2012 ........18

Table 3: Average monthly values of pollen, nectar and honey 2012 ........18

Table 4: Average monthly values of bee population 2013 ........21

Table 5: Average monthly values of brood area 2013 ........21

Table 6: Average monthly values of *varroa* mite fall 2013 ........22

Table 7: Average seasonal values of bee population, brood area and *varroa* mite fall 2013 ........22

Table 8: Average monthly values of pollen, nectar and honey 2013 ........25

Table 9: Average seasonal values of pollen, nectar and honey 2013 ........25

Table 10: *Varroa* mite count form drone comb removals ........26

Table 11: Average monthly values of bee population, brood area and *varroa* mite fall 2014 ........28

Table 12: Pesticide analysis results ........29

Table 13: Average monthly values of bee population, brood area, *varroa* mite fall, pollen, nectar and honey 2013 ........31

## LIST OF FIGURES

Figure 1: Apiary two study design ……………………………………………………………..…..12

Figure 2: Average monthly values of mite-to-adult bee ratios 2013 ……….…….23

## ABSTRACT

Beekeeping is continuing to gain popularity across the country, but since 2006 beekeepers have suffered a 20 - 33% annual bee colony loss. Colony decline is not linked to one factor, but evidence shows that the *varroa* mite, *Varroa destructor*, is one of the primary causes behind the decline. The apiary community has explored a variety of pest management practices to reduce *varroa* mite infestations, but few practices have yielded long term success as stand-alone treatments. We conducted a study to test the efficacy that splitting a colony into two (parent colony and daughter colony) will have on *varroa* mite reductions and the long term survival of a colony. Our results showed that the timing of a split during the season, early summer through fall, is critical to long term reductions in *varroa* mites and increased colony survival. Based upon the strong correlations we found between bee populations, brood area and *varroa* mite numbers in mid-late summer, we predict that late summer splits will produce the best results. Based upon data collected from 2012 and 2013, a citizen science field study was launched in summer 2014 to test the managerial efficacy of splitting colonies among hobbyist and small scale beekeepers including the costs and time required to split a colony.

# Chapter 1

## EVALUATION OF EARLY SUMMER SPLITS ON *VARROA* MITE REDUCTION AND COLONY PRODUCTIVITY

### 1.1 Introduction

Honey bees have a close-knit relationship with people. The practice of beekeeping (humans managing bee populations) has been traced back to ancient Egyptian times and the honey bee, *Apis mellifera* L., is the most commonly managed bee in the world. It is native to Europe, western Asia and Africa; however, within the previous 400 years its range has expanded to cover the majority of the globe, quickly becoming one of the most economically valuable pollinators in modern agriculture.

Beekeepers range from commercial and sideliners to small scale and hobbyists and these different beekeeping designations dictate what management practices will be used. Common commodities produced from bees include honey, wax, royal jelly and pollen, and common services that are provided by bees include pollination and queen rearing. Commercial beekeepers manage colonies as a primary income source (typically 300 or more colonies); small scale beekeepers manage colonies on a part-time basis (typically 50-300 colonies); hobbyist beekeepers maintain colonies for enjoyment purposes (typically fewer than 25 colonies). Hobbyist beekeepers make up the majority of beekeepers across the country. This range in beekeepers requires that different types of management strategies be developed that match the time and resources that beekeepers can invest into their apiaries. The focus of this project was on small scale and hobbyist beekeepers.

During the 20th century the beekeeping industry flourished, until the mid to late 1980's when a suite of insect pests and viruses were introduced to North America. These pests and viruses have had a devastating impact on the beekeeping community. Severe colony decline was not realized until ~2006 when colonies began to disappear at an alarming rate. The media and experts in the field coined the term "colony collapse disorder" or "mad bee disease" in the U.S. and Europe respectively. Since this early diagnosis honey bee decline has been attributed to an accumulation of multiple factors including chemicals, limited nutrition and environmental conditions, small hive beetles, tracheal mites, *varroa* mites, viruses, fungi and bacterial diseases. It is now widely accepted that an accumulation of these stress factors weakens a colony and makes it more vulnerable to pests and pathogens, particularly the *varroa* mite, *Varroa destructor* Anderson and Truman.

The *varroa* mite is an ectoparasitic brood mite that causes physical damage to immature honey bees and also vectors many viruses that provoke a colony wide stress response.  Studies have shown that *varroa* mites can vector up to 20 different viruses. Many treatments are available for *varroa* mite control but few have yielded long term success (Elzen et al. 1999). Current methods have focused on the commercial beekeeping sector and primarily involve the use of synthetic acaricides, but they are becoming less effective as *varroa* mites are building a tolerance. There is a need to explore alternative beekeeping management strategies targeted for small scale beekeepers.

### 1.1.1 *Varroa* mite biology

The *varroa* mite is currently global in its distribution (except Australia). It was originally confined to Southeast Asia, but over the last 100 years this tiny mite (1.05-1.09 mm long by 1.5-1.7 mm wide) has quickly spread along with the movement of *Apis mellfera*. *Varroa* mites were discovered throughout Europe, northern Africa, and South America in the 1970's and first discovered in the U.S. in 1987 (De Jong 1997). The *varroa* mite is the foremost pest for *Apis mellifera*, the European honey bee. Generally, a colony left untreated for more than two years will suffer a *varroa* mite population explosion and succumb to a slow decline from vectored viruses. *Apis cerana*, the Asian honey bee, is the native host for *varroa* and has evolved more aggressive hygienic behavior and mite detection abilities than *A. mellifera*. Also minor differences in the developmental timing of *A. cerana* and *A. mellifera* allow for an increase in *varroa* reproduction within an *A. mellifera* colony (Martin 1998).

There are two distinct phases in the life cycle of a female *varroa* mite; phoretic and reproductive. During the phoretic phase, female *varroa* mites feed attached to an adult bee before entering the reproductive phase inside a brood cell. The phoretic phase lasts 4-5 days and occurs in between reproductive cycles. The female mite is attracted by volatiles released from the bee larva and enters a brood cell approximately 1 day prior to capping. Oviposition begins 60 hours after the cell is capped. The first egg laid will hatch into a haploid male followed by 2 to 5 diploid females laid at 30 hours intervals (Donze 1996). Males mature in 5 to 6 days. They mate with each emerging sister and die shortly after the emergence of the adult bee, completing its life cycle without exiting the cell (De Jong 1997).

*Varroa* mite fecundity is differentially affected by the sex of the developing bee it is parasitizing. The duration of a *varroa* mite's reproductive cycle and the number of daughter mites that are born is limited by the length of the capped brood stage of the bee. Drone brood is in the capped stage for 14 days, typically 2.5 to 3.5 days (depending on *Apis* species) longer than worker brood. This results in the production of more female offspring in drone brood (Donze et al. 1996) than worker brood, and studies have shown that up to twice as many female offspring are produced in drone cells than in worker cells (Calderone

2005). Reproduction of the *varroa* mite is restricted to drone brood in *A. cerana*. Worker brood is in the capped stage for 12 days in *A. mellifera* and 11 days in *A. cerana*. This one day difference prevents *varroa* mites from reproducing in *A. cerana* worker brood.

There are also stark behavioral and physiological differences between *A. mellifera* and *A. cerana* hosts that effect *varroa* fecundity. Because drone brood is seasonal, mite populations oscillate in accordance with drone brood production, peaking during spring and summer months. In *A. cerana* colonies *varroa* mites can only reproduce in drone brood and the rearing of drone brood ceases during fall and winter months resulting in decreased mite populations, leaving *A*. *cerana* colonies with relatively no mites. However, in *A. mellifera* the *varroa* mites have the ability to reproduce in both worker and drone brood allowing phoretic mites to overwinter on adult worker bees until the decline of the colony (Donze et al. 1996).

### 1.1.2 Virus

*Varroa* mite populations continue to rise in the U.S. along with *varroa*-vectored viruses. There is strong evidence that the spread of *varroa* mites has facilitated the spread of viruses (Chen & Siede 2007). *Varroa* mites are prominent vectors of 18 known viruses, and among these 10 have been found in the U.S. Six of these viruses are of greatest concern because of their prevalence among bee colonies worldwide, including Deformed wing virus (DWV), Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Sacbrood virus (SBV) and Chronic bee paralysis virus (CBPV). The most important virus in the U.S. is DWV, which can shorten the life of a colony to less than a single season (Ribière, Ball and Aubert 2008; de Miranda 2010). Viruses are difficult to identify based on field observations because of a lack of overt signs, and many times hives are infected with multiple viruses making it difficult to differentiate multiple viral infections (Chen et al. 2007). Molecular tools greatly assist in rapid identification of bee viruses, for example QT-PCR. In summary, the control of viruses vectored by *varroa* mites is dependent on *varroa* mite control (Ribière et al. 2002).

### 1.1.3 Apiary management & control tactics

#### 1.1.3.1 Pest control strategies

Many methods have been developed to reduce or suppress *varroa* mite infestations, but few have yielded long term success as a stand-alone treatment (Elzen et al. 1999; Wantuch et al. 2009). Currently chemicals (specifically miticides) are the most heavily relied upon control tactic for beekeepers, but in the early 2000's, after *varroa* mites began showing resistance to miticides, an alternative approach to *varroa* mite control was needed. Integrated pest management (IPM) is a widely known concept throughout agriculture and is commonly used for pest control in urban environments. IPM is a multifaceted approach to pest management that combines cultural, mechanical, biological and chemical tools into one program that will ultimately reduce or replace the use of synthetic pesticides (Caron 1999). It can be visualized as a pyramid with cultural tools comprising the base of the pyramid, followed by mechanical and biological tools, and the tip of the pyramid being chemicals. Pest monitoring and economic thresholds are at the heart of IPM. An economic threshold signifies the appropriate population levels at which to treat a colony and what method of control to use, whereas monitoring a population will identify when to treat. It is only in recent years that IPM has received great attention as a non-chemical or reduced chemical method of *varroa* mite control (Caron 1999; Delapane 2005; Hapke 2008).

Population monitoring is a vital component of IPM. Beekeepers must track *varroa* mite populations to determine when *varroa* mites have achieved threshold levels that warrant chemical treatment. Common monitoring techniques include sticky boards, sugar shakes and brood uncapping. Sticky boards and sugar shakes are the more common monitoring tools. Sticky boards provide an average mite drop per day, sugar shakes provide a mite per bee average and brood uncapping provides a mites per brood average (Ostiguy et al. 2000). A reliable threshold for *varroa* mites has not yet been determined and is highly variable depending upon geographic location, weather, bee stock, colony strength and the specific monitoring tool.

#### 1.1.3.1.1 Cultural & mechanical control

Cultural and mechanical control methods both constitute the foundation of IPM. Cultural control and mechanical control have similar management tactics. Cultural control is the manipulation of the environment or disruption of the relationship between the host and pest to prevent or suppress infestation, while mechanical control involves physical methods to eliminate pest infestations, but the overall goal of both is to prevent the establishment of a pest. In beekeeping, cultural and mechanical control tactics are familiar to beekeepers and include such tactics as using pest-resistant stock (Hapke 2008; Rinderer et al. 2004; Rinderer 2010), interrupting the brood cycle (De Jong 1997; Fries, Imdorf et al. 2006; Conte 2007; Seeley 2007), screen bottom boards (Pettis et al. 1999), apiary location (Rinderer et al. 2004) and drone comb removal (Delaplane 2005). Screened bottom boards lace the floor of a bee hive with mesh. *Varroa* mites will fall from the colony through the screen which prevents their return to the brood nest. This technique has shown to significantly reduce *varroa* mite populations and is also credited with increasing circulation throughout the hive (Pettis et al. 1999). Drone comb removal is not as commonly used as screen bottom boards, but is familiar to beekeepers. Drone comb removal exploits the *varroa* mites' preference for drone brood parasitism. When the hive is provided with a frame of appropriate size comb the queen lays drone eggs, which are eventually entered by the female mite. After the drone cells are capped the frame is removed and the mites are killed, typically by freezing. This technique has limited efficacy because drone rearing is seasonal and is impractical for beekeepers because of the time required for implementation (Calderone 2005; Wantuch et al. 2009). Dusting a hive with powdered sugar to prevent host attachment is a common and simple control tactic that is also commonly used by beekeepers.

#### 1.1.3.1.2 Genetics

Genetics is another popular cultural control strategy in IPM. For many decades beekeepers have been selecting for natural resistance among bee populations toward common pests and diseases, including: *varroa* mites, tracheal mites and American Foulbrood. In recent years, strong efforts have been focused on the propagation of bee stock that is naturally more resistant to *varroa* mites, such as the Russian honey bee and the *Varroa*-Sensitive-Hygienic (VSH) bee. Both stocks have better hygienic characteristics and more proficient grooming behavior than the average honey bee. They are able to better detect, uncap and remove diseased brood and pupae from the brood nest and injure or kill a mite while removing it from the colony (Rinderer 2003; Hapke 2008; Rinderer 2010).

Interbreeding of honey bee populations has shown to be successful in increasing *varroa* resistance, although it has led to a decrease in other desirable traits such as honey production and brood viability (Ibrahm et al. 2007). It can also be a tedious process because it requires beekeepers that use *varroa* resistant queens to continue to monitor their hives (Tarpy et al. 2007; Rosenkranz et al. 2010).

#### 1.1.3.1.3 Behavior

Another common cultural control strategy is host behavior. Research around the world has shown strong correlations between the natural swarming behaviors of honey bees and an increase in honey bee survival in accordance with pests or diseases (De Jong 1997; Fries, Imdorf et al. 2006; Conte et al. 2007; Seeley 2007). It has been well documented that managed and feral populations of African honey bees have built a stable host-parasite relationship with *varroa* mites, but this relationship has not been widely shown in European honey bees, particularly managed colonies (Conte et al. 2007). Dividing a colony, either naturally through swarming or by splitting, causes an interruption in the brood cycle of both the original and daughter colony. After the swarm leaves (with the existing queen and more than 50% of the adult workers) they must locate a new nest cavity and comb must be drawn out before the queen can begin laying eggs. There is also a brood delay in the original colony because the new queen must be reared and mated prior to laying eggs (Wilde et al. 2005). The interruption in brood rearing leads to a delay in *varroa* mite reproduction and ultimately a reduction in *varroa* mite populations (Fries 2006).

Preventing swarming among managed colonies is a common practice among beekeepers because swarming is believed to be detrimental to honey production and pollination (Fries 2006; Ferrari 2008). Common management techniques used to prevent swarming include transferring combs between colonies, removing queen cells, and providing more space for egg laying and honey production (Fries et al. 2003). Research supports that European honey bees and *varroa* mites can co-exist and this has been demonstrated in feral populations in the U.S. and Europe.

#### 1.1.3.2 Biological control

Biological control is a pest control method using natural enemies. It is used in many agricultural systems and is a small but growing field in apiculture. Natural enemies of *varroa* mites have been identified, although they have yet to be developed into an effective management tool. They include the entomopathogenic fungi *Metarhizium anisopliae* and *Hirsutella thompsonii* which caused *varroa* mite mortality in a laboratory setting without affecting bee health, but they have not had as much success in the field. Mixtures of wax powder and *Beauveria bassiana* were shown to increase *varroa* mite fall and *Bacillus thuringiensis* is thought to have great potential (Chandler 2001; Dietemann 2012). Pseudoscorpions (*Ellingsenius indicus*) were thought to be a potential biocontrol agent of *varroa* mites because they naturally coexist in hives with honey bees in Africa; however, instead of feeding on *varroa* mites, they fed on dead honey bee larvae (Thapa et al. 2013).

#### 1.1.3.3 Chemical control

Chemical application is a last resort in IPM, comprising the tip of the pyramid. Chemicals were not heavily used among beekeepers until the arrival of multiple pests in the 1980's, particularly *varroa* mites. By the late 1990s miticides became a regular component of apiary management. Two synthetic pesticides, fluvalinate (a pyrethroid) and coumaphos (an organophosphate), were the first widely available and more commonly used miticides, but within 10 years of use resistance to both of these chemicals was observed in mite populations across the U.S. (Elzen et al. 1999; Thompson et al. 2002). Additional approved miticides include Mite AwayII® (formic acid), ApiLife Var® (thymol, eucalyptol and L-menthol), Apiguard® (thymol) and essential oils, but their use is limited because they are only effective at certain temperatures, and if not used properly they can negatively affect queen and drone development and have sub-lethal effects on the anatomy and behavior of the bee (Johnson et al. 2010). Residues of these chemicals have been found in wax, pollen, propolis and honey; therefore, the long term health risk to both bees and people is of great concern (Bogdanov 2006; Johnson et al. 2010).

#### 1.1.4 Hypothesis

The control tactics stated above have shown little success in *varroa* reductions over extended periods of time. Splitting of colonies has not been widely researched as a tool to reduce and control *varroa* mites and this project tested the effects that splitting has on *varroa* mite populations and colony survival. In addition, we hosted a citizen science field study to test the managerial efficacy of splitting colonies among hobbyist and small scale beekeepers. Our project aimed to address these hypotheses:

1. Spring or early summer splitting of a honey bee colony (disrupting the brood cycle) will reduce *varroa* mite numbers.
2. Spring or early summer splitting of a honey bee colony will not reduce seasonal honey productivity or affect pollen collection.
3. The splitting of colonies will have a significant effect on viral presence.
4. Combining drone brood removal and brood cycle interruptions (splitting) will complement each other and overall have a greater impact on mite reduction in a colony than either method used independently.

## 1.2 Methods

This project focused on the development of an IPM program for small-scale beekeepers and tested its effectiveness in reducing *varroa* mites and viruses while increasing colony survival in the mid-Atlantic. The program incorporates the splitting and natural swarming of colonies with the addition of popular *varroa* mite control methods used in the beekeeping community, including drone comb removal, screen bottom boards, and popular mite monitoring techniques including powdered sugar shaking and sticky boards. There was a preliminary season in 2012; the project officially began spring 2013 with 2 apiaries of similar design and concluded in fall 2014. Similar data was collected each season. Additional data including *Nosema* and viral presence among colonies was collected in 2013.

### 1.2.1 Apiary design

All colonies, control colonies and treatment colonies, were housed in a Langstroth hive with screen bottom boards. Control colonies during all seasons were maintained through swarm preventative tactics including removing queen cells, adding increasing space for egg laying, and pollen and honey storage. Treatment colonies consisted of colonies that were split into two, a daughter colony and parent colony (apiary two). An additional apiary in 2013 consisted of treatment colonies that were allowed to swarm (apiary one).

The design for apiary one mimicked a colony's natural behavior in that they were allowed to swarm and the colonies that did were included in the study, both parent and daughter (caught swarm). Apiary one was scanned on a regular basis for swarms. Two swarms were caught in June; the last swarm was caught in July. Therefore a total of 3 parent and 3 daughter colonies were used in the study. The 2014 field season began with two parent colonies and three daughter colonies. One parent colony had died during the 2013 field season; it and the daughter swarm were removed from the data analysis. Colony origins of parent colonies were from local swarms or mid-Atlantic queen breeders.

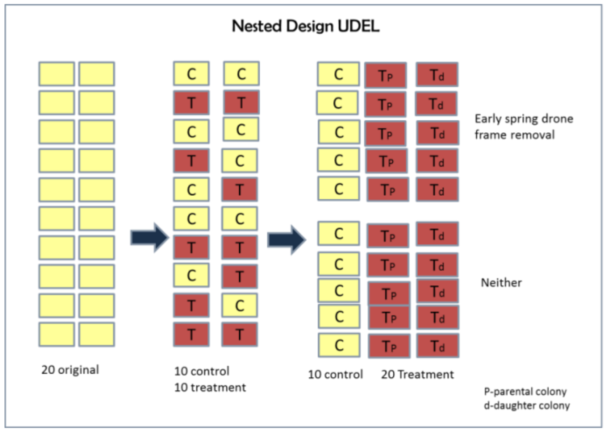



Figure 1. Apiary two study design

When the treatment colonies in apiary two were split the natural behavior of a swarming colony was mimicked, including behavior and timing. When splitting a colony two requirements were met: (1) the parent queen was given to the daughter colony (the split) and the parent colony was left to rear a new queen; (2) the splits mimicked the natural timing and behavior of a swarming colony. When splitting the colonies, approximately 1/3 of the brood frames were given to the daughter colony and 2/3 were left in the parent colony, replacing the frames in the parent colony with drawn comb. The honey and nectar frames were split between both colonies. Refer to APPENDIX-A for an example of a split. The colonies were split primarily during swarming season in early June when capped queen cells were present. Drone frames were randomly given to 10 of the original 20 colonies in apiary two (Fig. 1). They were removed from the hive when a minimum of ¼ of the cells on the frame were capped. They were stored in a -80 °C freezer and assessed for parasitism later in the season. In 2012, twenty colonies were purchased from a queen breeder in GA, while in 2013 twenty colonies were purchased from a queen breeder in West Virginia.

### 1.2.2 Colony Assessments

Data collection occurred during the summer of 2012, and spring and summer of 2013 and 2014. Hive origins of 2012 colonies were from a queen breeder in GA; hive origins of 2013 colonies were from a local queen breeder in PA. The purpose of hive assessments was to assess the health of the colonies. In 2012 hive assessments began in June and concluded in August; in 2013 and 2014 hive assessments began in May and continued every month through October. Assessments were made at approximately 30 day intervals. They ceased during winter months; however, *varroa* mite counts were taken in all the colonies throughout winter. The same monthly assessments were used for both apiaries.

#### 1.2.2.1 Monthly assessments

Monthly assessments included an evaluation of the strength and productivity of the colony and *varroa* mite counts (figure 2). The strength of each colony was based upon 6 variables: adult bee and brood population, brood pattern quality, and the amount of pollen, nectar and honey in each colony. Adult bee and brood population and the amount of pollen, nectar and honey were measured by estimating its frame coverage to the nearest quarter, (0, ¼, ½, ¾ and 1). The quality of the colony's brood pattern was based upon a 1-3 scale, 3 being the strongest and 1 being the weakest. Additional data recorded for each hive included hive temperament, number of queen cells, queen status, *varroa* mite populations, symptoms of disease, and the presence of chalkbrood or American foulbrood. Colony temperament was estimated on a 1-3 scale, 1 being docile and 3 being aggressive. Common queen status' included (1) present and laying, (2) no queen or (3) virgin queen. We also assessed the status of the queen cells as: (1) queen cell(s), (2) charged with egg or larvae, or (3) capped queen cell. Refer to APPENDIX –B for a copy of the data entry sheet. The data collected each month from colony assessments was ultimately correlated with overall colony health.

#### 1.2.2.2 *Varroa* mite monitoring

During each field season two methods for estimating (monitoring) *varroa* mite populations were performed during the assessment period. Method 1 estimated phoretic *varroa* mites using the sugar shake method from a sample of 300 bees removed from the brood frames. Method 2 estimated the natural fall of *varroa* mites to the bottom of a hive via sticky boards. The boards were covered with vegetable oil and inserted sticky side up below the screen bottom board or in the hive entrance. The sticky boards were removed 72 hours later and *varroa* mites were tallied.

#### 1.2.2.3 *Nosema*, viral, pesticide, temperature

Additional data was collected from colonies in each apiary including *Nosema* spore counts and viral presence in every colony, pesticides and hive temperature.

Samples of ~ 50 adult worker honey bees were collected in 50ml falcon tubes from each colony during every assessment period for *Nosema* analysis during 2013 and 2014. *Nosema* samples were stored in 70% ethanol and spore loads of *Nosema* spp. (*N. ceranae* and *N. apis*) were calculated from 25 foraging bees and tallied with a hemocytometer (Reuter et al.). Samples of ~ 50 adult worker honey bees were collected in falcon tubes from each colony during every assessment period for viral analysis during 2013. Viral samples were stored in -80 °C freezer and sent to Dr. David Tarpy at North Carolina State University's Honey Bee Genetics Lab, Raleigh, NC.

Pesticide analysis was performed on bee colony samples collected throughout the season in 2012 and 2014. Samples were stored in -80 °C freezer and sent to Dr. Da Chen at Southern Illinois University, Carbondale, IL and were tested for 7 neonicitinoids (Acetamiprid, Clothianidin, Dinotefuran, Imidachlorprid, Nitenpyram, Thiacloprid, Thiamethoxam).

The hive temperature and humidity of the colonies was taken in apiary two. The temperature and humidity of 10 hives, 5 control and 5 parent colonies, were collected from HOBO® Data Loggers (Onset, Bourne, MA) at half hour intervals throughout the study.

### 1.2.3 Statistics

Data were analyzed using JMP® (Version *10*. SAS Institute Inc., Cary, NC, 1989-2012) statistical programming software. Variables tested included: brood area ($cm^2$), pollen area ($cm^2$), nectar area ($cm^2$) and honey area ($cm^2$), bee population, natural daily mite fall (sticky boards) and phoretic mite count (sugar shake), viral prevalence, and *Nosema* spore count. The effects that splitting (brood disruption) had on the dynamics of a colony was measured by analysis of variance (ANOVA) on data collected in 2012, 2013 and 2014. The distributions of raw data collected in in 2012 and 2013 did not meet normal distribution assumptions. Therefore, a log transformation was performed to normalize the data sets, but the data are presented un-transformed. When a statistical significance between data sets was detected, a Tukey-Kramer test was conducted to compare the individual means between months and colonies. The average *varroa* mite counts for each monitoring method was measured by ANOVA to determine the similarity in averages. A relationship was considered strong when r > 0.5. Separate data analysis was conducted for each year on a month-to-month basis. Results were considered significant at the $\alpha \leq 0.05$ level.

## 1.3 Results

### 1.3.1 2012 field season

There were no statistically significant differences among colonies at the onset of the study in June with regards to bee population, brood area, *varroa* mites, pollen, nectar and honey. Daughter colonies had significantly fewer *varroa* mites than both control and parent colonies in July ($F = 12.34$, $df = 2, 20$; $P = 0.003$) and August ($F = 18.36$, $df = 2, 20$; $P < 0.0001$) (Table 1). There was a significant increase in *varroa* mites among control colonies in July ($F = 33.6$; $df = 2, 36$; $P < 0.0001$) and August ($F = 33.6$; $df = 2, 36$; $P < 0.0001$) (Table 1). In August there was a significant increase in brood area ($F =8.14$; $df = 1,8$; $P = 0.0214$) and bee population ($F =5.38$; $df = 1,8$; $P = 0.049$) among daughter colonies (Table 1). In July there was a significant difference in bee population between control and daughter colonies ($F = 6.33$; $df = 2, 20$; $P = 0.0074$) (Table 1). No significant differences in bee populations, brood area or *varroa* mites were observed among parent colonies; however, after they were split there was a noticeable decrease in brood area and *varroa* mites (Table 1). Overall, there were no significant differences in brood area between control, parent or daughter colonies; daughter colonies maintained a significantly lower bee population ($F = 6.5$, $df = 2, 61$; $P = 0.0028$) and *varroa* mite average ($F = 13.33$, $df = 2, 66$; $P < 0.0001$) than control and parent colonies (Table 2). A strong correlation existed between bee population and brood area in June ($r = 0.8$; $P = 0.016$) and between brood area and *varroa* mites in July ($r = 0.5$; $P = 0.05$).

Data for pollen and honey was collected from control and parent colonies during June and July (Table 3), but was only available from daughter colonies in

July. Pollen and honey quantities did not significantly differ between parent and control colonies in June or July. Nectar data was not collected. Strong correlations existed between bee population and pollen in June ($r = 0.5$; $P = 0.038$) and July ($r = 0.5$; $P = 0.022$) and between pollen and honey in June ($r = 0.5$; $P = 0.004$).

There were no significant differences in brood patterns between control, parent or daughter colonies throughout the season. The majority of the colonies maintained a healthy brood pattern.

One control colony died for unknown reasons during the sampling season in 2012 and was removed from data analyses. Only one colony survived (7A1) through winter and the remaining colonies died in mid-late winter.

**Table 1.** Average monthly values of bee population, brood area ($cm^2$) and varroa mite fall (recovered on a 72-hour mite monitoring sticky sheet) for control (13), parent (5) and daughter (5) colonies for every sampling month (June-August) in 2012. Data retrieved during monthly assessments from colonies.

| | **Colony** | **N** | **June** | **July** | **Aug** |
|---|---|---|---|---|---|
| Bee population | Control | 13 | 29558 | 31823a | 39492 |
| | Parent | 5 | 38701 | 21274ab | 34305 |
| | Daughter | 5 | - | 14994b | 20489 |
| | P-value | | 0.136 | **0.0074** | 0.087 |
| Brood area ($cm^2$) | Control | 13 | 5461 | 4504 | 3978 |
| | Parent | 5 | 6848 | 1901 | 4383 |
| | Daughter | 5 | - | 3075 | 4752 |
| | P-value | | 0.146 | 0.347 | 0.617 |
| *Varroa* Mite Fall | Control | 13 | 6 | 25a | 104a |
| | Parent | 5 | 5 | 17a | 21a |
| | Daughter | 5 | 3 | 2b | 5b |
| | P-value | | 0.26 | **0.003** | **< 0.0001** |

**Table 2.** Average seasonal values (± s.e.) of bee population, brood area (cm$^2$) and varroa mite fall (recovered on a 72-hour mite monitoring sticky sheet) for control, daughter and parent colonies throughout the sampling season (June-August) in 2012. Data retrieved during monthly assessments from colonies.

| Colony | N | Bee population | Brood area (cm$^2$) | *Varroa* mite fall |
|---|---|---|---|---|
| Control | 39 | 33624 ± 2136 a | 4648 ± 335 | 45 ± 10 a |
| Daughter | 10 | 17741 ± 1483 b | 3913 ± 396 | 3.1 ± 1 b |
| Parent | 15 | 31426 ± 3176 a | 4377 ± 711 | 14.4 ± 5 a |
| P-value | | **0.0028** | 0.415 | **< 0.0001** |

**Table 3.** Average monthly values of pollen (cm$^2$), nectar (cm$^2$) and honey (cm$^2$) for control (n = 13), parent (n = 5) and daughter (n = 5) colonies for every sampling month (June-August) in 2012. Data retrieved during monthly assessments from colonies.

| | Colony | N | June | July |
|---|---|---|---|---|
| Pollen (cm$^2$) | Control | 13 | 3397 | 2021 |
| | Parent | 5 | 1901 | 2683 |
| | Daughter | 5 | - | 671 |
| | P-value | | 0.671 | 0.42 |
| Honey (cm$^2$) | Control | 13 | 2838 | 475 |
| | Parent | 5 | 3466 | 2512 |
| | Daughter | 5 | - | 783 |
| | P-value | | 0.208 | 0.076 |

### 1.3.2 2013 field season

The 2013 season began with 21 colonies. In late June, after splitting there were 32 colonies total. One control colony was diagnosed with European foulbrood early in the season and removed from the study; seven colonies died throughout the sampling season and were removed from data analyses. Four colonies survived through winter 2013/2014 (1 control, 1 daughter, 2 parent) and the remaining 20 colonies died during mid-late winter. The causes of death are unknown, although environmental stresses including a cooler and longer winter are thought to have played a significant role.

Across a 6-month period the colonies followed the same biological pattern as would be expected naturally. Bee populations, brood area and *varroa* mites peaked in mid-late summer (July-August) and brood area began to decline in early fall (September) as bees prepared for winter. There were no statistically significant differences among colonies at the onset of the study in May with regards to bee population, brood area, *varroa* mites, pollen, nectar or honey. Immediately after colonies were split in June there was a significant difference in bee populations between control and daughter colonies ($F = 5.34$; $df = 2,23$; $P = 0.0124$) (Table 4). In control colonies, bee populations peaked in July and were significantly higher than both parent and daughter colonies ($F = 5.48$; $df = 2,23$; $P = 0.0113$) (Table 4). Parent colonies peaked in August; daughter colonies peaked in September. On average control colonies had significantly higher bee populations than daughter and parent colonies ($F = 6.75$; $df = 2,144$; $P = 0.0016$) (Table 7). During each month no significant differences were detected in brood area ($F = 1.75$; $df = 2,144$; $P = 0.177$) among control, daughter or parent colonies (Table 5). On average, control colonies had greater brood area than both parent and daughter colonies (Table 7). There was a noticeable decrease in brood area among parent and daughter colonies after splitting in June.

After May, *varroa* mites numerically decreased in parent colonies and for the remainder of the season both parent and daughter colonies had fewer *varroa* mites than control colonies (Table 6). However, no significant differences in *varroa* mites were detected among control, daughter or parent colonies (Table 7).

A separate ANOVA was run on parent, control and daughter colonies and each group had significantly fewer *varroa* mites in June compared to other months; control ($F = 7.6$; $df = 5,36$; $P < 0.0001$), daughter ($F = 4.49$; $df = 4,40$; $P = 0.0043$), parent ($F = 2.99$; $df = 5,54$; $P = 0.0187$).

The strongest correlations ($r > 0.5$) between bee population, brood area and *varroa* mite were seen in June, July, August and September. The months of June, July and August are the prime bee breeding and swarming seasons in the mid-Atlantic. The weakest correlations ($r < 0.5$) were seen in May and October. The bee-to-brood correlation was strongest in June ($r = 0.64$, $P = 0.004$), July ($r = 0.62$; $P = 0.002$), August ($r = 0.64$; $P < 0.0001$) and September ($r = 0.73$; $P < 0.0001$) and was weakest in May ($r = 0.18$; $P = 0.262$) and October ($r = 0.09$; $P = 0.736$). The bee-to-mite correlation steadily rose in May ($r = -0.12$; $P = 0.63$), June ($r = 0.27$; $P = 0.051$) and July ($r = 0.34$; $P = 0.005$), was the strongest in August ($r = 0.51$; $P = 0.0003$) and declined in September ($r = 0.43$; $P = 0.019$) and October ($r = 0.34$; $P = 0.074$). The mite-to-brood correlation followed a similar pattern, the correlation steadily rose in May ($r = 0.02$; $P = 1.0$) and June ($r = 0.2$; $P = 0.253$), was strongest in July ($r = 0.51$; $P = 0.024$) and August ($r = 0.6$; $P = 0.0001$) and declined in September ($r = 0.34$; $P = 0.012$) and October ($r = 0.33$; $P = 0.143$).

**Table 4.** Average monthly values of bee population for control (n = 7), parent (n = 10) and daughter (n = 9) colonies for every sampling month (May-October) in 2013. Data retrieved during monthly assessments from colonies. Outliers were excluded from the following averages in October: control (17281 ± 3057), parent (11478 ± 2558) and daughter (10054 ± 2860).

| | | Bee population | | | | | |
|---|---|---|---|---|---|---|---|
| **Colony** | **N** | **May** | **June** | **July** | **August** | **September** | **October** |
| Control | 7 | 14667±2056 | 28645±3293a | 39965±4969a | 26286±5010 | 27421±4572 | 17281±3294 |
| Parent | 10 | 16349±1720 | 20596±2755ab | 12083±4157b | 23913±4192 | 23170±3825 | 11478±2756 |
| Daughter | 9 | - | 12362±2904b | 14842±4382b | 22714±4419 | 23869±4032 | 12121±2905 |
| P-value | | 0.423 | **0.0124** | **0.0113** | 0.682 | 0.495 | 0.643 |

**Table 5.** Average monthly values of brood area (cm²) for control (n = 7), parent (n = 10) and daughter (n = 9) colonies for every sampling month (May-October) in 2013. Data retrieved during monthly assessments from colonies. Outliers were excluded from the following averages in October: control (346 ± 265), parent (603 ± 234) and daughter (695 ± 2340).

| | | Brood area (cm²) | | | | | |
|---|---|---|---|---|---|---|---|
| **Colony** | **N** | **May** | **June** | **July** | **August** | **September** | **October** |
| Control | 7 | 3406±599 | 4398±635 | 3697±929 | 4336±782 | 4314±807.6 | 346±357 |
| Parent | 10 | 2775±501 | 1341±531 | 2884±777 | 2198±654 | 3731±2447 | 931±299 |
| Daughter | 9 | - | 1593±560 | 3205±819 | 2880±689 | 3375±2580 | 695±315 |
| P-value | | 0.257 | 0.441 | 0.423 | 0.065 | 0.705 | 0.567 |

**Table 6.** Average monthly values of varroa mite fall (recovered on a 72-hour mite monitoring sticky sheet) for control (n = 7), parent (n = 10) and daughter (n = 9) colonies for every sampling month (May-October) in 2013. Data retrieved during monthly assessments from colonies. Outliers were excluded from the following monthly averages: June (parent (1 ± 0.9) and daughter (1.7 ± 0.9)); July (daughter (4.8 ± 3)).

| | | *Varroa* mite fall | | | | | |
|---|---|---|---|---|---|---|---|
| **Colony** | **N** | **May** | **June** | **July** | **August** | **September** | **October** |
| Control | 7 | 6±3 | 3±2 | 13±4 | 30±8 | 48±12 | 25±7 |
| Parent | 10 | 9±2 | 3±1 | 8±3 | 14±7 | 20±10 | 18±6 |
| Daughter | 9 | - | 3±2 | 7±3 | 16±7 | 28±11 | 20±6 |
| P-value | | 0.2110 | 0.864 | 0.475 | 0.143 | 0.194 | 0.628 |

**Table 7.** Average seasonal values (± s.e.) of bee population, brood area ($cm^2$) and *varroa* mite fall (recovered on a 72-hour mite monitoring sticky sheet) for control, daughter and parent colonies throughout the sampling season (May-October) in 2013. Data retrieved during monthly assessments from colonies. Outliers were excluded from the following varroa mite seasonal averages: control (12 ± 1.7), parent (8 ± 1.4) and daughter (9 ± 1.6).

| | **N** | **Bee population** | **Brood area ($cm^2$)** | ***Varroa* mite fall** |
|---|---|---|---|---|
| Control | 42 | 25711 ± 2101a | 3416 ± 329 | 21 ± 4 |
| Daughter | 45 | 17160 ± 1763 b | 2350 ± 301 | 15 ± 3 |
| Parent | 60 | 17932 ± 1414 b | 2310 ± 252 | 12 ± 2 |
| P-value | | **0.0016** | 0.177 | 0.14 |

### 1.3.2.1 *Varroa* mite-to-bee ratio

Over the course of the season mite-to-bee ratios increased, regardless of colony status (Figure 2). Mites were measured on a basis of phoretic mites per 300 bees. There was a significant difference between control and daughter colonies in June, shortly after the colonies were split (F = 6.02; df = 2,22; P = 0.008).

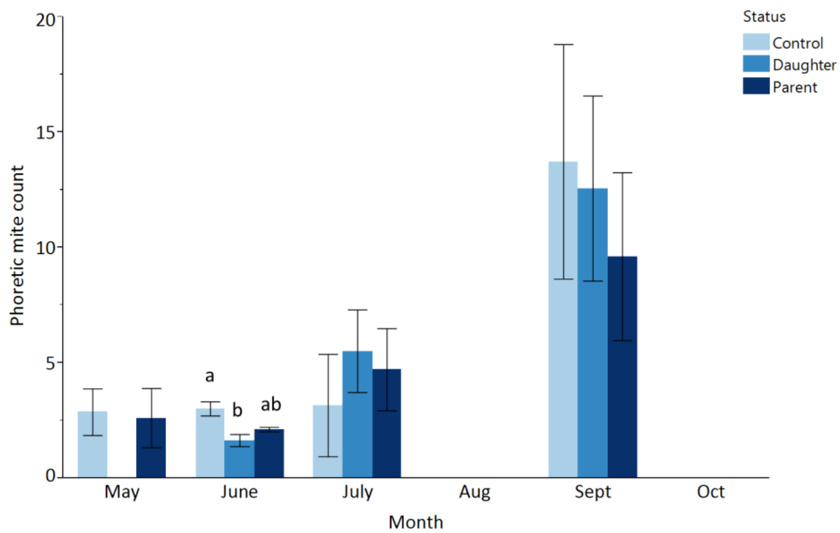



**Figure 2.** Average phoretic mite-to-300 adult bee ratios for control (n = 7), parent (n = 10) and daughter (n = 8) colonies for every sampling month in 2013. Data was retrieved from the sugar shake mite monitoring method during monthly assessments from colonies; no data collected during August and October. Letters denote significant differences between colony status; error bars indicate 95% confidence intervals; P-value < 0.5 is significant.

### 1.3.2.2 Pollen, nectar & honey

The strongest correlations were seen in the months of June, July and September and between the variables pollen, honey, nectar and bee population. Pollen-to-bee correlation was strongest in June ($r = 0.67$; $P < 0.0001$), July ($r = 0.72$; $P < 0.0001$) and September ($r = 0.57$; $P = 0.006$); nectar-to-bee correlation was strongest in June ($r = 0.5$; $P = 0.044$) and July ($r = 0.71$; $P = 0.012$); honey-to-bee correlation was strong in July ($r = 0.6$; $P = 0.025$). Weak correlations existed between pollen, nectar, honey and *varroa* mites ($r < 0.5$) in all months except September between pollen-to-*varroa* mite ($r = 0.57$; $P = 0.003$). The only strong correlation that existed with brood area was with pollen (June ($r = 0.56$; $P = 0.039$)).

Pollen, nectar and honey monthly averages are seen in Table 8 and seasonal averages are seen in Table 9. Control colonies had consistently higher averages than parent and daughter colonies for pollen, nectar and honey throughout the season. In June, pollen averages for control colonies were significantly higher than both daughter and parent colonies ($F = 6.72$; $df = 2,23$; $P = 0.005$) (Table 8). Control colonies did show a significant increase ($F = 7.81$; $df = 5,36$; $P < 0.0001$) in pollen in June. Control colonies had significantly more nectar than both daughter and parent colonies throughout the season ($F = 3.86$; $df = 2,144$; $P = 0.0233$) (Table 9), specifically in June ($F = 5.15$; $df = 2,23$; $P = 0.0142$) and July ($F = 4.96$; $df = 2,23$; $P = 0.0162$) (Table 8). There was an evident increase in nectar in control colonies from May to June that approached significance ($F = 2.38$; $df = 5,36$; $P = 0.058$). Control colonies had significantly more honey than daughter and parent colonies ($F = 17.1$; $df = 2,144$; $P < 0.0001$) (Table 9), specifically in June ($F = 16.07$, $df = 2,23$; $P < 0.001$) July ($F = 26.54$; $df = 2,23$; $P < 0.001$), Aug ($F = 7.82$; $df = 2,23$; $P = 0.0026$) and Sept ($F = 4.86$; $df = 2,23$; $P = 0.0174$) (Table 8).

**Table 8** Average values (± s.e.) of pollen (cm²), nectar (cm²) and honey (cm²) for control (n = 7), parent (n = 10) and daughter (n = 9) colonies for every sampling month (May-October) in 2013. Data retrieved during monthly assessments from colonies.

| | | N | May | June | July | August | September | October |
|---|---|---|---|---|---|---|---|---|
| Pollen (cm²) | Control | 7 | 290± 159 | 1527±188a | 2075±431 | 1302±320 | 1963±349 | 835±250 |
| | Parent | 10 | 298±133 | 654±158b | 734±360 | 504±268 | 735±292 | 223±210 |
| | Daughter | 9 | - | 325±166b | 657±380 | 978±283 | 1249±308 | 511±221 |
| | P-value | | 0.491 | **0.005** | 0.114 | 0.078 | 0.228 | 0.971 |
| Nectar (cm²) | Control | 7 | 794±353 | 4798±886a | 4005±663a | 2790±460 | 4553±474 | 3821±823 |
| | Parent | 10 | 974±295 | 2734±741b | 1475±554b | 890±385 | 1332±397 | 2190±689 |
| | Daughter | 9 | - | 1511±782b | 942±584b | 744±405 | 1689±418 | 2387±726 |
| | P-value | | 0.695 | **0.014** | **0.016** | 0.079 | 0.052 | 0.91 |
| Honey (cm²) | Control | 7 | 344±577 | 3061±493a | 4661±588a | 4520±756a | 3938±860a | 3643±636 |
| | Parent | 10 | 869±483 | 2428±413b | 1634±492b | 716±632b | 288±719b | 2181±532 |
| | Daughter | 9 | - | 262±435b | 395±519b | 227±667b | 190±758b | 1005±561 |
| | P-value | | 0.754 | **< 0.001** | **< 0.001** | **0.0026** | **0.0174** | 0.383 |

**Table 9.** Average seasonal values (± s.e.) and sampling size (n) of pollen (cm²), nectar (cm²) and honey (cm²) for control, daughter and parent colonies throughout the sampling season (May-October) in2013. Data retrieved during monthly assessments from colonies.

| | N | Pollen (cm²) | Nectar (cm²) | Honey (cm²) |
|---|---|---|---|---|
| Control | 42 | 1332 ± 193 | 3460 ± 423a | 3360 ± 469a |
| Parent | 45 | 525 ± 77 | 1599 ± 175b | 1353 ± 192b |
| Daughter | 60 | 744 ± 108 | 1455 ± 215b | 416 ± 115b |
| P-value | | 0.113 | **0.0233** | **< 0.0001** |

### 1.3.2.3 Drone comb removal

Analyses showed that drone comb removal did not have a significant effect on *varroa* mite number or colony survival. There was an average of 300 capped drone brood cells per drone frame when removed and an average 39 *varroa* mites per frame. *Varroa* mite averages for the colonies are seen in Table 10.

**Table 10**. *Varroa* mite count (recovered from a 72-hr sticky board) comparing mite averages between control (n = 5) and parent (n = 6) colonies that drone comb was removed and control (n = 4) and parent (n = 1) colonies that drone comb was not removed.

| | | May | June | July | August | September | October |
|---|---|---|---|---|---|---|---|
| No drone comb removal | Control | 10 | 0 | 10 | 8 | 28 | 8 |
| | Parent | 6 | 1 | 7 | 5 | 9 | 20 |
| drone comb removal | Control | 5 | 4 | 15 | 39 | 60 | 28 |
| | Parent | 11 | 5 | 9 | 20 | 27 | 17 |

### 1.3.2.4 Virus

Apiary two was positive for all seven viruses. There was not a noticeable trend between colony status and viral prevalence. A total of 129 samples were run for viral prevalence: Black queen cell virus (BQCV), acute bee paralysis virus (ABPV), deformed wing virus (DWV), Kashmir bee virus (KBV), Israeli acute bee paralysis virus (IAPV), sacbrood virus (SBV) and chronic bee paralysis virus (CBPV). Every colony was infected with multiple viruses, while every colony appeared to be asymptomatic. Of the 25 colonies sampled the most prevalent viruses were BQCV, DWV and SBV, in order of prevalence.

### 1.3.2.5 *Nosema* spores & hive temperature

The temperature and relative humidity of 10 randomly selected colonies in apiary 2 were recorded in June, July and August of 2013. The temperature for each colony remained at a consistent 98°F, but the humidity for each colony did not remain constant and varied (4 – 30 gm/M3) over the testing period. We believe due to the excessive dampness of the apiary site it was more difficult for the colonies to maintain a consistent relative humidity within the colony. A total of 127 samples were collected for *Nosema* analyses; 83 tested positive for *Nosema* spores. There was not a relationship between *Nosema* and colony status.

#### 1.3.2.6 Brood patterns

There were no significant differences in brood patterns between control, parent or daughter colonies. Between May and September the majority of colonies maintained a healthy brood pattern, but in October the colonies showed very little brood because of winter preparation and the quality of brood pattern was no longer a predictor of colony health.

### 1.3.3 2014 field season

The 2014 season began with four surviving colonies from 2013: one control, two parents and one daughter. All surviving colonies in 2013 entered winter with less brood area and *varroa* mites than sister colonies which could have contributed to their survival. Bee population, pollen, nectar and honey averages were similar to the 2013 averages.

In 2014 bee population, brood area and *varroa* mite averages did not significantly differ from 2013, although control colony 7A1 and daughter colony S8B1 had greater *varroa* mites in July and August than in the previous year (Table 11). Colonies in 2014 were split later in the season; colony 7A1 was split in early July and daughter colony S8B1 was split twice mid-way through the season. All colonies survived the 2014/2015 winter.

**Table 11**. Average monthly values of bee population, brood area ($cm^2$) and *varroa* mite fall (recovered on a 72-hour mite monitoring sticky sheet) for each colony in 2014 (May, July, August). Data retrieved during monthly assessments from colonies.

| | **Colony** | **May** | **July** | **Aug** |
|---|---|---|---|---|
| Bee population | 18B1 | 10598 | 23550 | 32803 |
| | 7A1 | 15280 | 24093 | 64373 |
| | S7A1 | N/A | 21878 | 57438 |
| | 8B1 | 23953 | 8020 | 7140 |
| | S8B1 | N/A | 8785 | 56328 |
| Brood Area ($cm^2$) | 18B1 | 8052 | 4053 | 8170 |
| | 7A1 | 0 | 5343 | 5644 |
| | 8B1 | 5053 | 0 | 0 |
| | S7A1 | N/A | 7235 | 0 |
| | S8B1 | N/A | 5300 | 2236 |
| *Varroa* Mite Fall | 18B1 | 8 | 2 | N/A |
| | 7A1 | 11 | 14 | 135 |
| | 8B1 | 2 | 0 | 0 |
| | S7A1 | N/A | 7 | N/A |
| | S8B1 | N/A | 13 | N/A |

### 1.3.4 Pesticide analysis

Pesticide analysis was performed on bee samples from the apiary in 2012 and 2014. Bee samples were collected during the prime nectar flow in July and assessed in Dr. Da Chen at Southern Illinois University, Carbondale, IL. Samples were tested for 7 neonicitinoids (Acetamiprid, Clothianidin, Dinotefuran, Imidachlorprid, Nitenpyram, Thiacloprid, Thiamethoxam) and nine additional chemicals commonly used in agriculture and also found in bee colonies: Atrazine, Chloropyrifos, Chloropyrifos oxon, Chlorothalonil, Coumaphos, Coumaphos oxon, Flucythrinate I, Flucythrinate II and Sulfentrazone. Trace amounts of five chemicals were detected in both years (Table 12).

**Table 12.** Pesticide analysis results from samples collected in 2012 (25 colonies) and 2014 (5 colonies); analyzed January 5, 2015.
Method limit of quantitation (MLOQ)
Not detectable (ND)
Analyte Concentration: ng/g bee

| **Pesticides** | **UD Apiary** 2014 | **UD Apiary** 2012 |
|---|---|---|
| Acetamiprid | <MLOQ | 1.85 |
| Clothianidin | ND | ND |
| Dinotefuran | ND | ND |
| Imidachlorprid | 6.99 | <MLOQ |
| Nitenpyram | ND | ND |
| Thiacloprid | 0.66 | 3.05 |
| Thiamethoxam | ND | ND |
| Atrazine | ND | 0.29 |
| Chloropyrifos | <MLOQ | <MLOQ |
| Chloropyrifos oxon | ND | ND |
| Chlorothalonil | 22.7 | 7.2 |
| Coumaphos | ND | ND |
| Coumaphos oxon | ND | ND |
| Flucythrinate I | ND | ND |
| Flucythrinate II | ND | ND |
| Sulfentrazone | ND | ND |

### 1.3.5 Apiary one

Apiary one mimicked a colony's natural swarming behavior. The apiary was regularly scanned for swarms (S); 3 total swarms were caught. The parent (P) colonies were found based upon smaller brood area, smaller bee populations, fewer or no eggs and queen cells present. Data was collected and assessed from six colonies in apiary one during the 2013 field season, May-July and September-October. One parent colony died from unknown causes; it and the daughter swarm were removed from data analysis. All colonies were survivors from 2012. Two swarms were captured mid-May. Throughout the course of the season the parents and swarms followed similar patterns as those in apiary two; bee populations peaked in mid-summer while brood productivity and mite number peaked shortly after in early fall (Table 13). The seasonal bee population averages for each parent colony are drastically different; 1P with 46,000 and 2P with 16,000. All four colonies had similar brood area and *varroa* mite averages throughout the season. Significant differences were detected between parent and daughter colonies in pollen ($F = 6.66$; $df = 1, 18$; $P < 0.0188$), nectar ($F = 5.2$; $df = 1, 18$; $P < 0.039$) and honey ($F = 6.23$; $df = 1, 18$; $P < 0.0225$).

There were no significant differences in brood patterns between parent or daughter colonies throughout the season. All colonies maintained a healthy brood pattern.

**Table 13.** Average monthly values of bee population, brood area (cm²) and *varroa* mite fall (recovered on a 72-hour mite monitoring sticky sheet) for each colony, parents (1P, 2P) and daughters (1S, 2S), for every sampling month (May-October); apiary one, 2013. Data was retrieved during monthly colony assessments.

| | Colony | May | June | July | Aug | Sept | Oct |
|---|---|---|---|---|---|---|---|
| Bee Population | 1P | 28055 | 55945 | 54843 | - | 38505 | 55348 |
| | 1S | 5393 | 7065 | 20075 | - | 20570 | 26468 |
| | 2p | 15598 | 12645 | 29150 | - | 18365 | 6430 |
| | 2S | 7898 | 15795 | 24300 | - | 26010 | 15758 |
| Brood Area (cm²) | 1P | 28055 | 55945 | 54843 | - | 38505 | 55348 |
| | 1S | 5393 | 7065 | 20075 | - | 20570 | 26468 |
| | 2p | 15598 | 12645 | 29150 | - | 18365 | 6430 |
| | 2S | 7898 | 15795 | 24300 | - | 26010 | 15758 |
| *Varroa* mite Fall | 1P | 1 | 0 | - | - | 19 | 21 |
| | 1S | - | 0 | 0 | - | 9 | 18 |
| | 2p | - | 2 | 7 | - | 17 | 13 |
| | 2S | - | 0 | 11 | - | 1 | 4 |
| Pollen (cm²) | 1P | - | 4289 | 3161 | - | 3161 | 1355 |
| | 1S | - | 0 | 505 | - | 1548 | 677 |
| | 2p | - | 2258 | 677 | - | 1860 | 1355 |
| | 2S | - | 452 | 903 | - | 452 | 677 |
| Nectar (cm²) | 1P | - | 9159 | 8998 | - | 8858 | 11137 |
| | 1S | - | 698.75 | 1043 | - | 3419 | 2720 |
| | 2p | - | 4687 | 2709 | - | 2440 | 2666 |
| | 2S | - | 0 | 1129 | - | 0 | 2129 |
| Honey (cm²) | 1P | - | 11632 | 9191 | - | 6020 | 8439 |
| | 1S | - | 4472 | 4225 | - | 871 | 3386 |
| | 2p | - | 4451 | 6106 | - | 4698 | 3118 |
| | 2S | - | 0 | 226 | - | 0 | 1011 |

## 1.4 Discussion

In this study we found a temporary reduction in *varroa* mite populations after the colonies were split. The colonies were split in early summer, at the beginning of the swarming season in the mid-Atlantic, when colonies began to show signs of swarming. Immediately after the colonies were split (parents and daughters) there was a noticeable decrease in *varroa* mites; however, toward the end of the season (early fall) the *varroa* mite averages for all colonies were about equal. Bee population and brood area also decreased in split colonies temporally after they were split but by the end of the season colony populations were all similar and brood area eventually ceased. Control colonies were not split and continued to grow without interruption, which led to a significant increase in bees, almost double that of splits in July, and mites during mid-summer.

Results from this study suggest that timing of splits is important. Colonies were split during early summer when correlations among bee, brood and *varroa* mites were weak ($r > 0.5$) and before the colonies had time to increase in bee populations and brood area. The strong correlations found between bee, brood and *varroa* mites during mid- summer suggests that splitting during this time would have a greater impact on *varroa* mite populations than early summer splitting. Splitting a colony in mid to late summer would reduce *varroa* mite levels during the same time that the overwintering worker population is developing. High *varroa* mite levels during late summer or early fall has shown to be detrimental to colony health and overwintering survival (Fries et al. 2001). The colonies that survived through the 2013/2014 winter had the lowest average mites per day (4.3) throughout the season; the majority of colonies that died had an average of 17.5 mites per day. Higher mite infestation levels have shown to decrease colony life span (DeGrandi-Hoffman et al. 2004; Dooremalen 2012) as well as the individual life span of a bee. The higher *varroa* infestation averages among the colonies that died could have played a significant role in the late winter death of the majority of colonies during both seasons.

The results did not support our hypothesis that spring splitting will not affect seasonal honey productivity. Over the course of the season there were noticeable decreases in honey productivity among split colonies, while control colonies had consistently higher averages of pollen, nectar and honey. However, other factors such as geographic location, weather, colony age or timing of the split could have attributed to the reduced amounts of pollen, nectar and honey productivity. The swarming colonies in apiary one had higher averages of honey than the control and split colonies in apiary two, suggesting that colony age could play a significant role in honey productivity because the swarming colonies were a minimum of one year old. A primary concern of beekeepers is that splitting colonies will reduce honey harvest, but if honey productivity significantly increases in a colony's second year of survival, managerial splitting could have profound effects on the apiculture industry.

The averages of bee, brood, mite in the swarming colonies were similar to the control and split colonies. There were only four swarming colonies and high variability among them; therefore, it difficult to estimate a representative average of bee population, brood area and *varroa* mites.

In both seasons, colonies with the VSH trait (2013) and without the VSH trait (2012) showed similar trends in bee population, brood area and *varroa* number although colonies with the VSH trait overwintered more successfully and had fewer mites than colonies in 2012. Our results did not support our hypotheses that spring splitting will affect viral presence or that by combining drone comb removal with splitting that it will have a greater impact on *varroa* mite populations than either method alone. Viral prevalence did not correlate with mite load, month or colony origin and all colonies, regardless of status or origin, had a minimum of four viruses. Drone comb removal did not appear to have long term effects on mite numbers or colony survival which is supported by other literature (Wantuch et al. 2009).

Regulating swarming and not splitting is a common management practice among beekeepers because both are thought to be detrimental to honey production. Our data did show a significant impact on honey production, but our colonies were newly established and first year colonies are not expected to have a high honey yield. Splitting colonies in late summer or fall will allow colonies to build up bee populations to take advantage of the spring nectar flow (the most predominant nectar flow in the mid-Atlantic) and thereby increase honey production.

Our results support international research that has shown that swarming colonies have successfully developed a balanced *varroa*-bee relationship (Seeley 2007). Splitting of a colony does affect *varroa* mite populations as predicted by our hypothesis; however, timing of the split is critical. Early spring splitting allows sufficient time for the mites to rebound and reduces the productivity in the parent and daughter colonies during important nectar flows. Because there are stronger relationships between bees, brood and *varroa* mites during summer, we predict that a late-summer splits will have a greater impact on the reduction of *varroa* mites and increase winter survival of the colonies. Late-summer splits will also allow full colonies to reap the benefits of early summer nectar flows, and increase the number of colonies going into winter leading to the maintenance of sustainable colony numbers within an apiary.

## 1.5 Citizen science field study 2014

In spring 2014 there was a new approach to apiary science, an approach to beekeeping from a beekeeper's perspective. In recent years the apiary community has explored a variety of pest management practices to reduce *varroa* mite infestations, but few practices have yielded long term success as a stand-alone treatment (Elzen et al. 1999, Wantuch et al. 2009). Over the previous two years, based upon the data collected during the 2012 and 2013 field seasons, we have begun to develop a new managerial approach to beekeeping based upon colony splitting. In spring 2014 we launched a citizen science field study in the mid-Atlantic to test the effects that splitting has on *varroa* mite populations and the managerial efficacy of the program that we developed, "Testing the Efficacy of Integrated Pest Management (IPM) Programs for Controlling *Varroa destructor*".

### 1.5.1 Methods

The field study was launched June 1, 2014; registration opened in spring 2014 to participants. The field study was launched through the Bugonia website, http://bugonia.com/. Bugonia was the website in which beekeeper recruitment and registration was accomplished and it allowed beekeepers to upload data and keep track of others' data. A total of 159 beekeepers registered for the field study that ranged from small scale to hobbyist beekeepers throughout the mid-Atlantic. Due to website complications the data collected during the 2014 citizen science field study was deleted.

#### 1.5.1.1 Protocol

The program (Testing the Efficacy of Integrated Pest Management (IPM) Programs for Controlling *Varroa destructor*) was developed from the data collected during the 2012 and 2013 field seasons and the protocols given to participants were similar to the methods used during the 2012 and 2013 field seasons. Refer to APPENDIX -E for a copy of the protocol. Each participant chose to be in one of two groups: the control group or the treatment group. The colonies in each group were treated the same as in the previous field seasons. Control colonies at both apiaries were maintained through swarm preventative tactics, while treatment colonies were split into two, a daughter colony and parent colony. Monthly colony assessments were performed at 30 day intervals from June 2014 through October 2014. Colony health was based upon adult bee populations, brood area, brood pattern quality, hive aggression, foulbrood, queen presence, number of queen cells, *varroa* mite populations, the presence of chalkbrood infection, and the quantities of honey, pollen and nectar.

The goal was to maintain *varroa* mite populations at reduced levels using non-chemical tactics; however, if the *varroa* mite threshold was reached then chemical control methods were permitted. There are two styles of sticky boards, each with different thresholds: checkered sticky board with a 60 mite threshold and a full white sticky board with a 120 mite threshold.

#### 1.5.1.2 Surveys

Two surveys were conducted over the course of the project; survey-1 was launched in early 2013 and survey-2 was launched in mid-2014. Both surveys provided us with knowledge of the common management methods that are used among beekeepers to reduce *varroa* mites and beekeepers' awareness of non-chemical management methods, specifically splitting colonies. Due to website complications the data from survey-2 was deleted

#### 1.5.1.2.1 Survey-1

Survey-1 was conducted through Qualtrics (qualtrics.com), a research software company that provides online platforms for surveys. It was launched in May 2013 and was closed in October 2013. Refer to APPENDIX -C for a copy of the survey and results. A total of 307 surveys were completed which represented beekeepers in 5 states: VA, DE, MD, PA, NJ. The majority (84%) of respondents were hobbyist beekeepers with 1-10 years of experience (83%). The majority of respondents attained their colonies from queen breeders (52%); 62% attained their colonies from southern beekeepers and 36% attained their colonies from local breeders in the mid-Atlantic region. About 30% of respondents attained their colonies from a swarm capture or a split. Deaths were linked mostly to unknown causes (31%), weak colonies (18%) and “other” causes (18%), for example weather and bears. Viral symptoms were not evident in many of the respondent’s colonies.

This survey was useful in gathering data regarding beekeepers attitudes and viewpoints on non-chemical management techniques. The majority of respondents use non-chemical methods to control *varroa* populations in their hives: screen bottom boards (80%), drone comb removal (32%), mite tolerant or hygienic bee stock (23%) inert dusts (4%), “other” (9%) and “none” (14%). Eight respondents used splitting colonies as a method of *varroa* mite control. Many respondents were familiar with the term Integrated Pest Management (IPM), but only few were aware of the different components (cultural, mechanical, biological and chemical). Respondents were asked to give a personal definition of IPM; of 267 total responses 98 did not know, 50 said it was a non-chemical method to control pests, and 40 said it was a combination of chemical and non-chemical methods to control pests.

#### 1.5.1.2.2 Survey-2

Survey-2 was disseminated through Bugonia to beekeepers participating in the field study. A total of 45 participants from 12 states completed the Bugonia survey. Survey-2 was issued to participants when they registered on Bugonia. Refer to APPENDIX -D for a copy of the survey. The most common pests encountered were small hive beetles, *varroa* mites and ants; the most common control methods were Formic acid (e.g., MiteAway quick strips), Thymol (e.g., Api Life Var) and powdered sugar; on average beekeepers spent 4 hours per month in the apiary. The goal of many of the participants was to manage healthy colonies that will survive the winter season.

### 1.5.2 Conclusion

The citizen science field study served as a strong educational infrastructure that informed mid-Atlantic beekeepers of non-chemical methods to reduce *varroa* mites. The project provided us with a large opportunity for extension and outreach to the mid-Atlantic beekeeping community that will be beneficial in future citizen science programs. We received strong support from 78 beekeeping associations throughout the mid-Atlantic and have been invited to speak about the project at local beekeeping meetings. The majority of respondents in survey-1 (95%) showed interest in using non-chemical programs if available to them. There is strong interest among beekeepers in developing non-chemical management tools and supporting future citizen science projects. Using real world data collected from citizen science beekeepers will lead to greater understanding of the relationship between *varroa* mites and honey bees. The data collected from both 2012-2014 field seasons and citizen science field study has provided us with data that support late-summer splits impact on *varroa* mite populations and the infrastructure to develop and promote the adoption of sustainable beekeeping techniques to small scale and hobbyist beekeepers.

## REFERENCES

Bogdanov S., Imdorf A. and Kilchenmann V. (1998). Residues in wax and honey after Apilife VAR® treatment, Apidologie 29 (6): 513-524.

Calderone N. (2005). Evaluation of Drone Brood Removal for Management of *Varroa* destructor (Acari: Varroidae) in Colonies of Apis mellifera (Hymenoptera: Apidae) in the Northeastern United States, Journal of Economic Entomology 98, 645-650.

Caron, D. M. (1999). Honey Bee Biology and Beekeeping. Cheshire, CT, Wicwas Press.

Chandler D., Sunderland K., Ball B. and Davidson G. (2001). Prospective biological control agents of *Varroa destructor* n. sp., an important pest of the European honeybee, *Apis mellifera*, Biocontrol Sci. Technol. 11: 429-448.

Chen Y. and Siede R. (2007). Honey Bee Viruses, Advances in Virus Research 70: 33-80.

Conte Y., Vaublanca G., Crausera D., Jeanneb F., Rousselle J. and Becard J. (2007). Honey bee colonies that have survived *Varroa* destructor, Apidologie 38 (6): 566-572.

DeGrandi-Hoffman, G. and R. Curry. (2004). A mathematical model of *varroa* mite (*Varroa destructor* Anderson and Trueman) and honeybee (*Apis mellifera* L.) population dynamics, International Journal of Acarology 30: 259-274.

De Jong, D. and Morse, A. (1982). Mite pests of honey bees, Annual Review of Entomology 27: 229-252.

De Jong, D. (1997). In Honey bee pests and predators, Morse, Flottum (ed) Medina OH: A.I. Root. 3rd ed: 281-327.

de Miranda J. and Genersch E. (2010). Deformed wing virus, Journal of Invertebrate Pathology 103: S48-S61.

Donze G., Herrmann M., Bachofen B. and Guerin P. (1996). Effect of mating frequency and brood cell infestation rate on the reproductive success of the honeybee parasite *Varroa jacobsoni,* Ecological Entomology 21 (1): 17-26.

Dooremalen, C. V., Gerritsen, L., Cornelissen, B., Steen, J. J. M. V. D., and Langevelde F. V. (2012). Winter Survival of Individual Honey Bees and Honey Bee Colonies Depends on Level of *Varroa* destructor Infestation, PLoS One *7*(4).

Elzen p., Eischen F., Baxter J., Elzen G. and Wilson W. (1999). Detection of resistance in US *Varroa* jacobsoni Oud. (Mesostigmata: Varroidae) to the acaricide fluvalinate, Apidologie 30: 13-17.

Ferrari S., Silvam M., Guarino M. and Berckmans D. (2008). Monitoring of swarming sounds in bee hives for early detection of the swarming period, Computers and Electronics in Agriculture 64(1), 72-77.

Fries I. and Camazine S. (2001). Implications of horizontal and vertical pathogen transmission for honey bee epidemiology, Apidologie 32, 199–214.

Fries I., Hansen H., Imdorf A. and Rosenkranz P. (2003). Swarming in honey bees (Apis mellifera) and *Varroa* destructor population development in Sweden, Apidologie 34, 389-397.

Fries I., Imdorf A. and Rosenkranz P. (2006). Survival of mite infested (*Varroa* destructor) honey bee (Apis mellifera) colonies in a Nordic climate, Apidologie 37, 564-570.

Hapke S. (2008). Integrated Management of *Varroa Destructor* Anderson & Trueman (Acari: Varroidae) in Honey Bees, *Apis Mellifera* L. (Hymenoptera: Apidae), in Western Washington State, USA.

Ibrahm A., Reuter G. and Spivak M. (2007). Field trial of honey bee colonies bred for mechanisms of resistance against *Varroa* destructor, Apidologie 38, 67-76.

Johnson R., Ellis M., Mullin C. and Frazier M. (2010). Pesticides and honey bee toxicity – USA, Apidologie 41, 312-331.

Martin S. (1998). A population model for the ectoparasitic mite *Varroa* jacobsoni in honey bee (Apis mellifera) colonies, Ecological Modeling 109, 267-281.

Ostiguy N. and Sammataro D. (2000). A simplified technique for counting *Varroa* jacobsoni Oud. on sticky boards, Apidologie 31: 707-716.

Pettis, J. S., and H. Shimanuki. 1999. A hive modiÞcation to reduce *varroa* populations. American Bee Journal 139: 471-473.

Ribière M., Triboulot C., Mathieu L., Aurieres C., Faucon J. and Pepin M. (2002). Molecular diagnosis of chronic bee paralysis virus infection, Apidologie 33: 339-351.

Ribière M., Ball B. and Aubert M. (2008). Natural history and geographic distribution of honey bee viruses, Virology and the Honey Bee Aubert, M., et al (ed), European Communities, Luxembourg: 15–84.

Rinderer T., De Guzman L. I., Delatte G. T. and Harper C. (2003). An Evaluation of ARS Russian Honey Bees in Combination with Other Methods for the Control of *Varroa* Mites, American Bee Journal 143 (5): 410-412.

Rinderer T., De Guzman L. I. and Harper C. (2004). The effects of co-mingled Russian and Italian honey bee stocks and sunny or shaded apiaries on *varroa* mite infestation level, worker bee population and honey production. American Bee Journal 144(6): 481–485.

Rinderer T., Harris J., De Guzman L. and Hunt G. (2010). Breeding for resistance to *Varroa* destructor in North America, Apidologie 41 (3): 409-423.

Rosenkranz P., Aumeier P. and Ziegelmann B. (2010). Biology and control of *Varroa* destructor, Invertebrate Pathology 103, 96-119.

Seeley T. (2007). Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa* destructor in the northeastern United States, Apidologie 38, 19-29.
Tarpy, D., Summers J. and Keller J. (2007). Comparison of parasitic mites in Russian-hybrid and Italian honey bee (Hymenoptera: Apidae) colonies across three different locations in north Carolina, Journal of Economic Entomology 100: 258-266.

Thapa R., Wongsiri S., Lee M. and Choi Y. (2013).Predatory behaviour of pseudoscorpions (*Ellingsenius indicus*) associated with Himalayan *Apis cerana,* Journal of Apicultural Research 52 (5): 219-226.

Thompson H., Brown M., Ball R. and Bew M. (2002). First report of *varroa* destructor resistance to pyrethroids in the UK, Apidologie 33, 357-366.

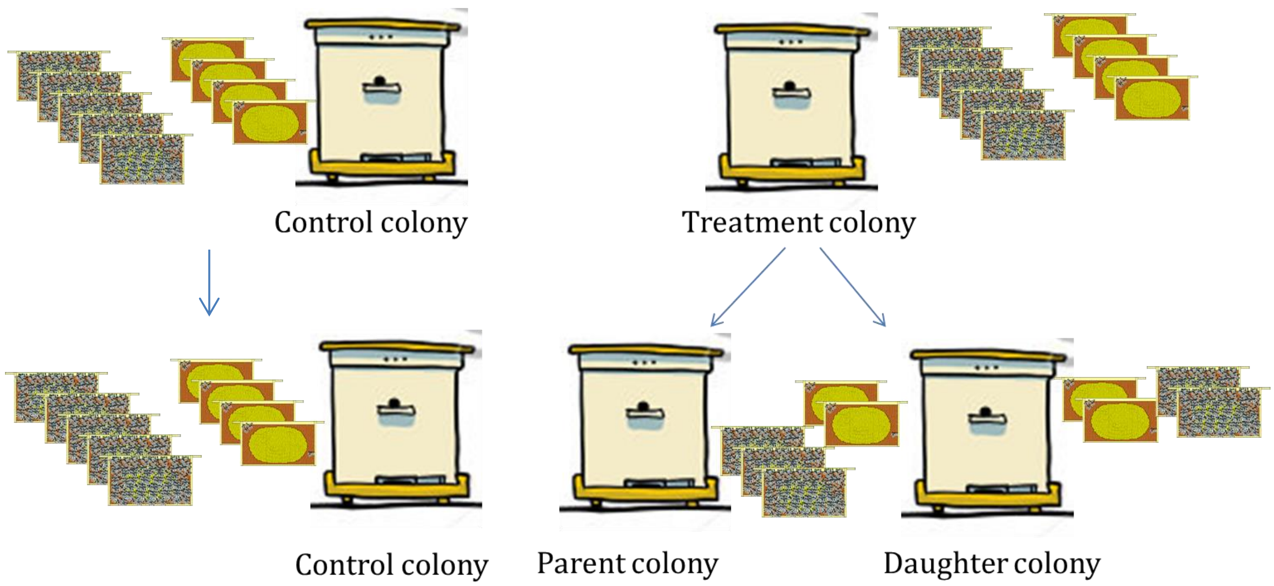
Wantuch H. and Tarpy D. (2009). Removal of Drone Brood From Apis mellifera (Hymenoptera: Apidae) Colonies to Control *Varroa* destructor (Acari: Varroidae) and Retain Adult Drones, Journal of Economic Entomology 102 (6): 2033-2040.

Wilde J., Fuchs S., Bratkowski J. and Siuda M. (2005).Distribution of *Varroa* destructor between swarms and colonies, Journal of Apiculture 44, 190–194.

APPENDIX-A

*COLONY SPLIT PROTOCOL*

Colony division (split) protocol used for 2012-2014 and citizen science field seasons applicable to only treatment hives




- Control colonies were not split; treatment colonies were split in early June
- Signs of when to split colonies include: multiple queen cells and multiple frames of brood and honey
- The queen was given to daughter colonies and parent colony rears a new queen
- 1/3 of the brood frames were given to daughter colonies; 2/3 left in parent colony
- Pollen, nectar and honey frames were split between both colonies
- Replace frames in the parent colony with drawn comb

APPENDIX-B

*DATA ENTRY SHEET*

Data entry sheet used for monthly colony assessments in 2012-2014 field seasons

| colony ID | | | | | | | | | | | | temperament | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | frame 1 | frame 2 | frame 3 | frame 4 | frame 5 | frame 6 | frame 7 | frame 8 | frame 9 | frame 10 | brood box type | brood pattern |
| side A | Adult bee | | | | | | | | | | | | |
| side B | | | | | | | | | | | | | |
| side A | Sealed Brood | | | | | | | | | | | Q cups | Q charge |
| side B | | | | | | | | | | | | | |
| side A | pollen | | | | | | | | | | | Q capped | Q status |
| side B | | | | | | | | | | | | | |
| side A | nectar | | | | | | | | | | | | |
| side B | | | | | | | | | | | | | |
| side A | honey | | | | | | | | | | | | |
| side B | | | | | | | | | | | | | |
| | eggs | | | | | | | | | | | | |
| | larvae | | | | | | | | | | | | |
| | Drone brood | | | | | | | | | | | | |
| | notes | | | | | | | | | | | | |

APPENDIX-C

SURVEY-1 2013

Survey-1 launched early 2013

1. What is your primary purpose for having colonies

| | Answer | Response | % |
|---|---|---|---|
| 1 | Hobby | 288 | 84% |
| 2 | primary income source | 3 | 1% |
| 3 | secondary income source (side-liner) | 37 | 11% |
| 4 | Other | 15 | 4% |
| | Total | 343 | 100% |

2. How many years have you been beekeeping

| # | Answer | Response | % |
|---|---|---|---|
| 1 | one-ten | 285 | 83% |
| 2 | eleven-20 | 32 | 9% |
| 3 | 21+ | 28 | 8% |
| | Total | 345 | 100% |

3. Where did the majority of hives in your apiary originate from

| # | Answer | Response | % |
|---|---|---|---|
| 1 | queen breeder or packaged bees, if so provide the location (state) | 152 | 52 |
| 3 | Other | 54 | 18 |
| 4 | splitting of a colony or swarm capture | 89 | 30 |
| | Total | 295 | 10 |

4. What is your personal definition of an IPM (Integrated Pest Management) program

5. In what fashion are you replacing them

| # | Answer | Response | % |
|---|---|---|---|
| 4 | not replacing | 49 | 18% |
| 5 | Other | 7 | 3% |
| 3 | purchasing a | 100 | 37% |

| | | | |
|---|---|---|---|
| 1 | package or nuc splitting of a colony or swarm capture | 113 | 42% |
| | Total | 269 | 100% |

6. How many colonies do you own currently

| # | Answer | Response | % |
|---|---|---|---|
| 1 | 1-10 colony | 274 | 80% |
| 2 | 11-20 colony | 30 | 9% |
| 3 | 21+ colony | 29 | 8% |
| 4 | None | 10 | 3% |
| | Total | 343 | 100% |

7. Would you consider implementing a non-chemical IPM strategy to manage your apiary in the futu

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Yes | 277 | 95% |
| 2 | No | 15 | 5% |
| | Total | 292 | 100% |

8. Were there visible symptoms of a virus or disease, if so please specify

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Yes | 40 | 15% |
| 2 | No | 230 | 85% |
| | Total | 270 | 100% |

9. How many colonies survived over winter 2011/2012

| | |
|---|---|
| Average | 3 |
| Total | 333 |

10 what can their deaths be most directly linked to

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Pests | 29 | 12% |
| 2 | virus or disease | 14 | 6% |
| 3 | pesticides | 4 | 2% |
| 4 | lack of food source | 26 | 10% |
| 5 | unknown | 78 | 31% |
| 6 | other (includes mammals) | 44 | 18% |
| 7 | weak colonies | 45 | 18% |
| 9 | "bad queen" | 8 | 3% |

| | | | |
|---|---|---|---|
| | Total | 248 | 100% |

11. What are your current pest control tactics based upon

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Chemical | 33 | 11% |
| 2 | non-chemical | 229 | 41% |
| 3 | a combination of chemical and non-chemical | 104 | 34% |
| 4 | None | 31 | 11% |
| 5 | Other | 7 | 9% |

12. How many colonies did you have total in 2012

| Colonies | responses | average | mode response |
|---|---|---|---|
| b/t 1-10 | 245 | 3.5 | 2 |
| b/t 11-20 | 29 | 15 | 12 |
| >21 | 25 | 35 | 30 |
| total/most common | 300 | | 2 |

13. In what state do you keep your bees

| | | |
|---|---|---|
| Total | 101 | |
| | | NY (2), DE (1), MD (36), NJ (4), OH (1), PA (35), VA (21), W.DC |

14. how many colonies died over winter 2012/2013

Text Response

| Range | average | responses total | Mode response |
|---|---|---|---|
| 0-72 | 3.259414 | 312 | 0 |

15 Do you use non-chemical *varroa* mite control products, check all used

| | | | |
|---|---|---|---|
| 1 | herbal products | 39 | 10% |
| 2 | powdered sugar | 87 | 31% |
| 3 | mineral oil | 19 | 7% |
| 4 | None | 148 | 52% |
| 5 | Other | 24 | 12% |
| | Total | 317 | 100% |

16. How aware are you of non-chemical pest management techniques

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Very | 79 | 27% |
| 2 | moderately | 192 | 66% |

| | | | |
|---|---|---|---|
| 3 | Not | 22 | 8% |
| | Total | 293 | 100% |

17. Do you implement swarm preventative tactics

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Yes | 207 | 71% |
| 2 | No | 84 | 29% |
| | Total | 291 | 100% |

18. How many colonies do you plan on replacing

| # | Answer | Response | % |
|---|---|---|---|
| 1 | 100% | 162 | 56% |
| 2 | 75% | 8 | 3% |
| 3 | 50% | 21 | 7% |
| 4 | 25% | 13 | 5% |
| 5 | 0 | 84 | 29% |
| | Total | 288 | 100% |

19. What do you feel is the most important component of an IPM program

| # | Answer | Response | % |
|---|---|---|---|
| 1 | problem prevention | 91 | 32% |
| 2 | regular monitoring/record keeping | 60 | 21% |
| 3 | diagnosis | 15 | 5% |
| 4 | threshold development/injury levels | 13 | 5% |
| 5 | management methods | 106 | 37% |
| | Total | 285 | 100% |

20. Do you use a known *varroa* mite control miticide, if so please specify which ones and how often they are used during the year

| # | Answer | Response | % |
|---|---|---|---|
| 1 | ApiLife Var | 25 | 9% |
| 2 | ApiGuard | 63 | 22% |
| 3 | Mite Away II | 47 | 17% |

| | | | |
|---|---|---|---|
| 4 | CheckMite + | 4 | 1% |
| 5 | Apistan | 9 | 3% |
| 6 | Other | 52 | 18% |
| 7 | None | 125 | 44% |

21. Do you regularly use fumagilin (antibodies) to treat for nosema 

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Yes | 38 | 13% |
| 2 | No | 252 | 87% |
| | Total | 290 | 100% |

22. Do you regularly use mit-a-thol (antibody) or grease patties to treat for tracheal mites 

| # | Answer | Response | % |
|---|---|---|---|
| 1 | Yes | 12 | 4% |
| 2 | No | 275 | 96% |
| | Total | 287 | 100% |

23. Do you implement any of the following non-chemical *varroa* mite control tactics, check all that a used

| # | Answer | Response | % |
|---|---|---|---|
| 1 | drone comb removal | 94 | 32% |
| 2 | screen bottom boards | 233 | 80% |
| 3 | mite tolerant stocks | 67 | 23% |
| 4 | inert dusts | 12 | 4% |
| 5 | None | 42 | 14% |
| 6 | Other | 25 | 9% |

24. How much time a month do you dedicate to *varroa* mite control, chemical and/or non-chemical

| # | Answer | Response | % |
|---|---|---|---|
| 1 | 1-5 hrs | 194 | 67% |
| 2 | 5-10 hrs | 16 | 5% |
| 3 | 10-15hrs | 5 | 2% |
| 4 | 15+ hrs | 1 | 0% |
| 5 | None | 75 | 26% |
| | Total | 291 | 100% |

25. On average how much ($) did you spend on *varroa* mite control last year (2012)

| # | Answer | Response | % |
|---|---|---|---|
| 1 | 0 | 78 | 31% |
| 2 | $1-25 | 82 | 33% |
| 3 | $26-50 | 48 | 19% |
| 4 | $51-75 | 16 | 6% |
| 5 | $76-100 | 14 | 6% |
| 6 | $101-150 | 4 | 2% |
| 7 | $151-250 | 8 | 3% |
| 8 | $250+ | 0 | 0% |
| | Total | 250 | 100% |

APPENDIX-D

SURVEY-2

Survey-2 launched in mid-2014

The study group(s) in which you participated (check all that apply)

- ○ Treatment group
  - ○ Colonies were split
  - ○ Colonies swarmed
- ○ Control group (swarm prevention)

**Please rank your level of agreement with each of the following statements based on this scale:**

*I feel better equipped to manage my apiary*

**Strongly Agree  Mildly Agree  Unsure  Mildly Disagree  Strongly Disagree**

*I feel that I can more easily identify the distressors of my colony*

**Strongly Agree  Mildly Agree  Unsure  Mildly Disagree  Strongly Disagree**

*I feel better prepared to deal with destructive problems that could arise in the future while preventing colony decline*

| **Strongly Agree** | **Mildly Agree** | **Unsure** | **Mildly Disagree** | **Strongly Disagree** |
|---|---|---|---|---|
| 84% | 10% | 0% | 5% | 0% |

*I understood the problems that the program identified and the strategies that the program aimed at working through*

**Strongly Agree  Mildly Agree  Unsure  Mildly Disagree  Strongly Disagree**

*I could personally relate to the problems (or experienced the problems myself) that the program focused on managing*

**Strongly Agree  Mildly Agree  Unsure  Mildly Disagree  Strongly Disagree**

*I feel that I have a better understanding of chemical treatments and when to treat my colonies for pests*

**Strongly Agree  Mildly Agree  Unsure  Mildly Disagree  Strongly Disagree**

*I feel that I have built a more sustainable management strategy that is less reliable on chemical use*

**Strongly Agree Mildly Agree Unsure Mildly Disagree Strongly Disagree**

*I feel capable of implementing this program/management strategy into a future management practice that I will use*
**Strongly Agree Mildly Agree Unsure Mildly Disagree Strongly Disagree**

*I have a better understanding of the Integrated Pest Management (IPM) concept*
**Strongly Agree Mildly Agree Unsure Mildly Disagree Strongly Disagree**

*I feel the IPM concept is more applicable to my management style and I foresee it taking a larger role in my management style in the future*
**Strongly Agree Mildly Agree Unsure Mildly Disagree Strongly Disagree**

*I understand the significance of monitoring pest levels and will continue to monitor them next season*
**Strongly Agree Mildly Agree Unsure Mildly Disagree Strongly Disagree**

*I foresee the this program having long term benefits to my colony's health and survival*
**Strongly Agree Mildly Agree Unsure Mildly Disagree Strongly Disagree**

APPENDIX-E

CITIZEN SCIENCE FIELD PROTOCOL

Launched June 2015

# Integrated Pest Management

## Citizen Science Field Study 2014

## Data collection

There are two groups in this study. You must pick one to participate in: the control group or the treatment group. The control group will be preventing the colonies from swarming. The treatment group will be splitting the colonies. You must sign up for one OR the other and specify which swarm preventative tactics you are applying. There is a third group that allows you to have both control colonies and treatment colonies in the same apiary. However, there is a minimum colony number per group so please contact me if you would be interested in participating in the third group. The following data is applicable to BOTH treatment and control hives.

The goal is to maintain *varroa* populations at reduced levels using nonchemical tactics. However, if the *varroa* mite threshold is reached then chemical control methods are acceptable. It is important that if you do use chemicals or medications that they are NOT used until the *varroa* threshold is reached and all information is recorded (the names of the chemicals or medications, how much is being applied and how often).

All personal information you share will be kept confidential. The Bugonia site will be used for recording data collected in this study. This is a versatile honey bee research platform makes data entry easy and provides automatic reminders for research tasks that need to be completed. You are asked to collect and enter data into Bugonia once a month, May through October, which includes hive assessments and *varroa* mite counts.
Uniformity is important for comparing results across samples so please follow the protocol.

## Procedures

Monthly assessments include:

- Mite population estimates via sticky boards
- Symptoms of disease
- Hive productivity based on quantifying the amount of honey, nectar and pollen
- Adult bee and brood estimates
- Temperament and brood pattern quality
- Queen status: present and laying, no queen, virgin ect.
- Queen cells tallied and their status (either a cup, charged (with an egg or larvae) or capped)

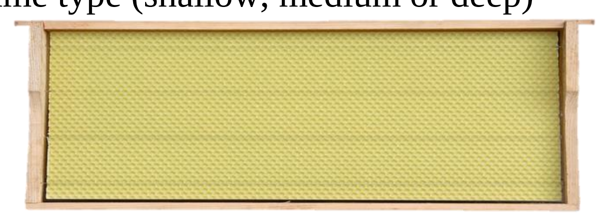
Supply list:

- Sticky boards
- Vegetable or canola oil (pam)
- Data sheet(s)

| colony ID | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | frame 1 | frame 2 | frame 3 | frame 4 | frame 5 | frame 6 | frame 7 | frame 8 | frame 9 | frame 10 |
| side A | Adult bee | | | | | | | | | | |
| side B | | | | | | | | | | | |
| side A | Sealed Brood | | | | | | | | | | |
| side B | | | | | | | | | | | |
| side A | pollen | | | | | | | | | | |
| side B | | | | | | | | | | | |
| side A | nectar | | | | | | | | | | |
| side B | | | | | | | | | | | |
| side A | honey | | | | | | | | | | |
| side B | | | | | | | | | | | |
| | eggs | | | | | | | | | | |
| | larvae | | | | | | | | | | |
| | Drone brood | | | | | | | | | | |
| | notes | | | | | | | | | | |

| temperament | |
|---|---|
| brood box type | brood pattern |
| medium | |
| Q cups | Q charge |
| | |
| Q capped | Q status |
| | |

STEP 1: Specify frame type (shallow, medium or deep)



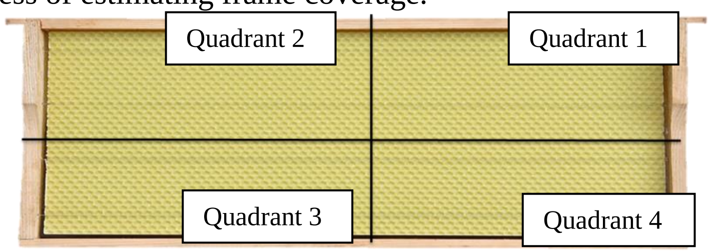
Medium Frame

## STEP 2: Split frame into quadrants

0 quadrants filled (0)
1 quadrant filled (1/4)
2 quadrants filled(1/2)
3 quadrants filled (3/4)
4 quadrants filled (1)

It is easiest to estimate frame coverage by splitting each side of the frame into quadrants. Estimate the number of quadrants that each variable will fill (bees, brood, pollen, honey and nectar) to nearest quarter (0, 1/4, 1/2, 3/4, 1).

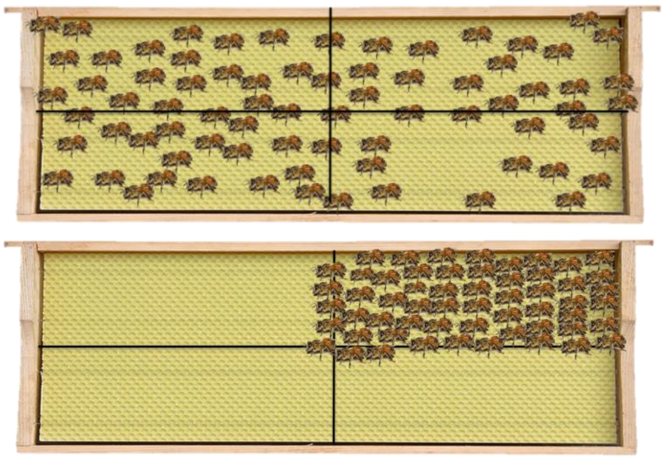
Be conservative on your estimates. Unless more than 3/4 of a quadrant is filled it is rounded DOWN to the nearest quarter. Splitting the frame into quadrants will simplify in the process of estimating frame coverage.




## Step 3: Estimate frame coverage of each variable

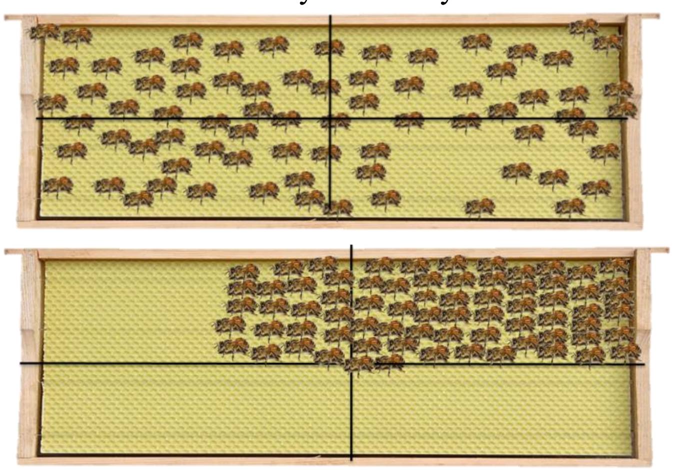
To accurately estimate percent frame coverage of each variable it is best to rearrange and group each variable on the frame. It is similar to piecing together a puzzle to determine how many quadrants each variable fills. In each example below there is a "what you actually see" and "how you would report" the variables on a frame.
STEP 3a: Estimate how many quadrants the bees will fill. It is important to condense the bees so there is minimal space between each bee.

What you actually see How you would report



Bee coverage fills only 1 quadrant (1/4).

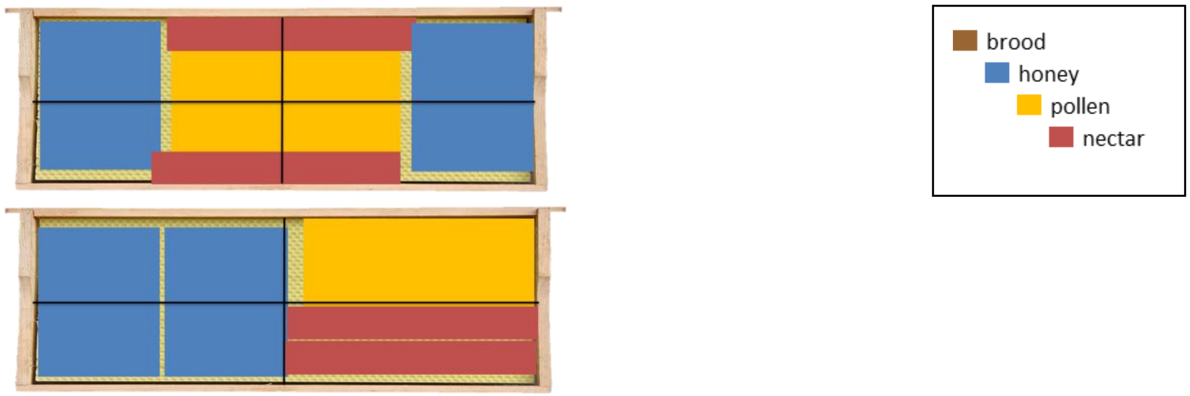
What you actually see How you would report



Bee coverage, although fills 1.5 quadrants will be rounded down to filling only 1 quadrant (1/4).

STEP 3b: Estimate the number of quadrants that brood, pollen, nectar and honey fill. This is different than estimating bee coverage because it is based upon cell coverage. Each variable should be grouped and condensed to where there are no empty cells. If there is pollen, honey, nectar or brood on the frame and it does not fill 1 quadrant than place a check mark in the box (not applicable to bees). Brood estimations does NOT include drone brood, only worker brood.

.



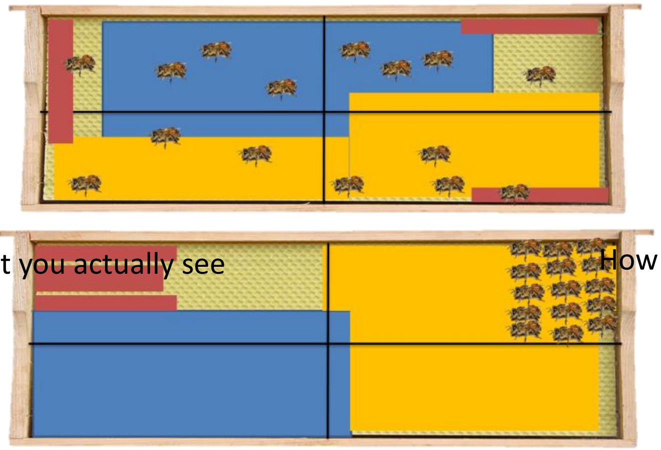

What you actually see　　　　　　　　How you would report

| | Adult bee | Sealed brood | pollen | nectar | honey |
|---|---|---|---|---|---|
| side A | 0 | 0 | 1/4 | 1/4 | 1/2 |

Not every frame is as straightforward and easy to estimate as the above examples. Many times the frame is a mosaic of variables arranged throughout the frame and this is when estimating becomes more difficult, as in the following examples.

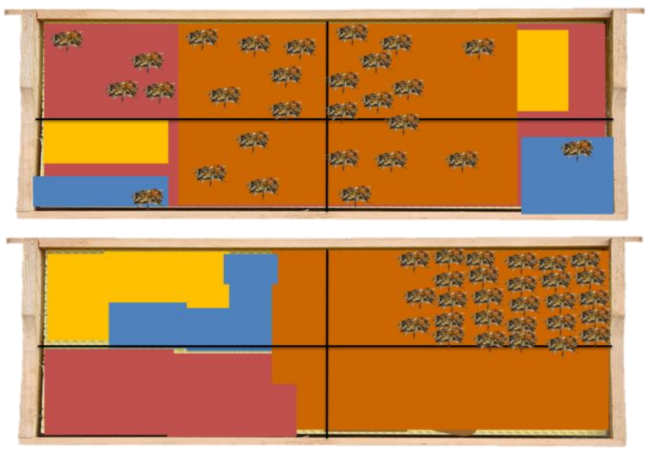
What you actually see　　　　　　　　How you would report

| | Adult bee | Sealed brood | pollen | nectar | honey |
|---|---|---|---|---|---|
| side A | 1/4 | 1/2 | 1/4 | ✓ | 0 |



What you actually see How you would report

| | Adult bee | Sealed brood | pollen | nectar | honey |
|---|---|---|---|---|---|
| side A | 0 | 0 | 1/2 | ✓ | 1/2 |



What you actually see How you would report

| | Adult bee | Sealed brood | pollen | nectar | honey |
|---|---|---|---|---|---|
| side A | 1/4 | 1/2 | ✓ | 1/4 | ✓ |

Below is an example of a data entry sheet that shows the general format and what data to collect during each assessment period. The frames that had drone brood, eggs or larvae received a check mark.

| colony ID 4 | | frame 1 | frame 2 | frame 3 | frame 4 | frame 5 | frame 6 | frame 7 | frame 8 | frame 9 | frame 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| side A | Adult bee | 1/2 | 1/4 | 1/4 | 1/4 | 1/4 | 1/2 | 1 | 1/4 | 1/2 | 1/2 |
| side B | | 1/4 | 3/4 | 1/4 | 1/2 | 1/2 | 3/4 | 1/2 | 3/4 | 1 | 1 |
| side A | Sealed Brood | 1/4 | 1/4 | 1/4 | 1/4 | 0 | 3/4 | 0 | 1 | 0 | 0 |
| side B | | 0 | 0 | 0 | 1/4 | 0 | 1/2 | 0 | 3/4 | 0 | 0 |
| | pollen | 1/4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1/4 |
| | nectar | 1/2 | 0 | 0 | 1/2 | 1/4 | 0 | 3/4 | 0 | 0 | 1/4 |
| | honey | 3/4 | 1/4 | 1 | 3/4 | 3/4 | 3/4 | 1/2 | 0 | 1/4 | 1.5 |
| | eggs ✓ | | | | ✓ | ✓ | ✓ | | | | |
| | larvae ✓ | | | ✓ | | ✓ | | ✓ | | ✓ | |
| | Drone brood | | ✓ | | | | | ✓ | | | |
| | notes | | | | | | | | | | |

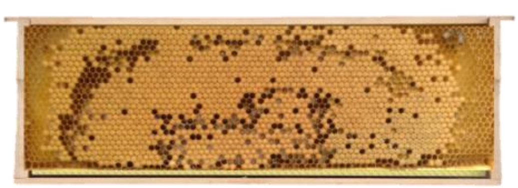
| temperament | 2 |
|---|---|
| brood box type | brood pattern |
| medium | 3 |
| Q cups | Q charge |
| 2 | 0 |
| Q capped | Q status |
| 0 | queen laying |

## STEP 4: Brood patterns

Brood pattern is not measured for each frame. It is an overall estimate of the colony.

Brood guidelines

- Brood pattern quality does not depend on the amount of the frame that is covered in brood, but on the total amount and consistency of the pattern of brood, larvae or eggs throughout the entire colony
- The number of empty cells (not containing eggs, larvae nor brood) and the shape of the brood area define a pattern
- The inner frames will have more brood typically than the outer frames that are reserved for pollen, nectar and honey
- Brood pattern is based upon a scale of 1-3
    - A score of (3) will have minimal empty cells; a score of (1) the brood, eggs or larvae will be scattered and inconsistent



Brood pattern: 3

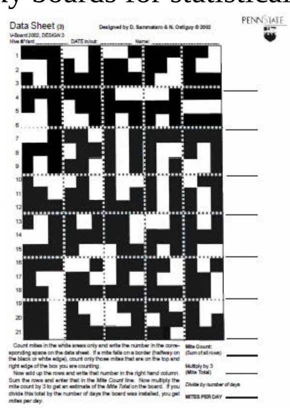
Brood pattern: 3
The interior cells are filled with eggs or larvae

Brood pattern: 1

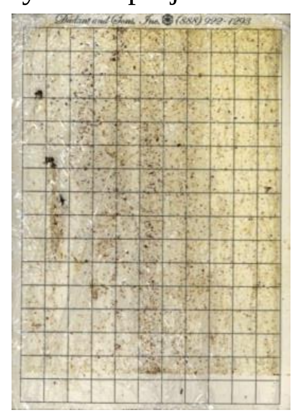
## STEP 5: *varroa* measurements

The sticky board is the only mandatory monitoring method for measuring *varroa* mites. There are two styles of sticky boards and they have different thresholds. The checkered sticky board has a mite threshold of 60 mites; the full white sticky board has a threshold of 120 mites. IPM thresholds are specific to the region and so it may be difficult to compare thresholds to colonies in the south, Midwest or west coast. It is recommended to use the full white sticky boards for statistical purposes and accuracy of the project.

Thresholds for sticky board types



Threshold of 60 varroa



Threshold of 120 varroa

*Varroa* counts should be done at 30 day intervals. They are performed typically one day after the hive assessment. One day at minimum must be allocated between assessment and sticky board insertion because it will give the bees time to calm down.

## Sticky board monitoring

1. The sticky board is covered with a sticky substance (vegetable oil or petroleum jelly)

2. Insert the board sticky-side up below screen bottom boards or in the hive entrance with a screen cover on top to protect from bees for solid bottom boards
3. As mites are dislodged during grooming or removal from the capped cells they will fall through the screen cover and adhere to the board
4. The board is removed after 72 hours (3 days)
5. The mites are counted

Screen bottom board

Solid bottom

## Colony division (Split), applicable to only treatment hives

Below I have provided a recommended guideline for splitting, although you may have your own method that you feel more comfortable with following. However, it is important to follow the natural pattern of colony swarming. There are two requirements: (1) the parent queen must be given to the daughter colony (the split) and you must allow the parental colony to rear a new queen; (2) the splits must mimic the natural timing and behavior of a swarming colony. It is also important to remember that you do not need to split every colony in your apiary, for example if the colony is not showing signs of swarming then it is better not to split them.

### When to split:

- When you notice signs that a colony is making preparation to swarm
- Over five charged queen cells
- At least five or more frames of brood in the colony and 3-5 frames of honey (more or less running out of room), although it can vary

### Colony division

- At least 1/3 of the brood frames with clinging bees should be given to the daughter colony and 2/3 left in the parental colony, replacing the frames in the parental colony with drawn comb
- The queen is given to the daughter colony and the parental colony will be left to rear a new queen

- Split the honey and nectar frames between the colonies and feed the daughter colony
- It is recommended to not split after the end of June because the colony may not be able to properly build up and store enough honey for winter use

If you decide to host a control apiary you must prevent your colonies from swarming. Swarming is related to space availability; here are several techniques that can keep the incidence of swarming low: 1). removing queen cells 2). providing ample room for queen egg laying and expansion of the brood nest 3). providing ample room for pollen, nectar and honey storage.