ABSTRACT

Title of Dissertation: CHARACTERIZING EFFECTIVENESS OF AND OBSTACLES TO BEST BEEKEEPING MANAGEMENT PRACTICES

Kelly Kulhanek, Doctor of Entomology, 2020

Dissertation directed by: Associate Professor, Dr. Dennis vanEngelsdorp, Department of Entomology

Honey bees (*Apis mellifera, L.)* provide critical pollination services to many US crops, but decades of high colony loss rates have strained beekeepers' ability to provide sufficient colonies for crop production. In a national survey of colony losses for the 2015-2016 season, beekeepers reported losses averaging at 37.4%, and that the parasitic mite *Varroa destructor* was a leading cause of mortality. Survey results were used to create empirical best management practices (BMPs) to reduce colony loss rates. Best practices were the top four practices which correlated to significant reductions in winter colony loss. This set of BMPs was tested on 140 colonies in 7 locations across the US, compared to average beekeeping practices. At the end of 3 years, apiaries managed according to BMPs exhibited reduced *Varroa* loads, which resulted in reduced fall

viral loads and reduced winter mortality. However, colony loss rates still exceeded rates that beekeepers have deemed acceptable.

A prominent factor affecting colony health and mortality in the BMP study was *Varroa*. After identifying *Varroa* treatment as a preventative measure, the effects of *Varroa* management were evaluated in non-experimental apiaries. Citizen scientist beekeepers participating in the Sentinel Apiary Program provided *Varroa* samples and *Varroa* management information. Out of 192 *Varroa* treatments applied to 155 apiaries over 2 years, only 45 treatments resulted in reduced *Varroa* loads. Common hypotheses of factors affecting *Varroa* population growth failed to explain the rapid increases in *Varroa* loads experienced by beekeepers in critical fall months. Finally, a more novel explanation for rapid increases in *Varroa* load was explored: horizontal transmission of mites between apiaries. Colonies that were visited by non-natal bees experienced larger increases in *Varroa* loads than unvisited colonies, but not as a result of visitation to or from high mite colonies. High mite colonies in the landscape represent a threat to nearby colonies, and cooperative *Varroa* management is likely to mediate colony losses resulting from *Varroa*. This dissertation supports the critical need for proactive, cooperative *Varroa* management to improve colony health and reduce mortality.

CHARACTERIZING EFFECTIVENESS OF AND OBSTACLES TO BEST BEEKEEPING MANAGEMENT PRACTICES

by

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Preface

This dissertation is comprised partially of manuscripts in various stages of submission, and of chapter written specifically for the dissertation. Chapter 1 is published in the Journal of Apicultural Research as the most recent installation in annual reports of colony loss from the Bee Informed Partnership. Chapter 2 was prepared with collaboration from coauthors, and is ready for submission for publication in a journal. Chapters 3 and 4 are currently in formats tailored to the dissertation, and will be reformatted for publication in the future.

Dedication

I dedicate this dissertation to my parents, who have supported me in countless ways through 20 years of education. To my sister, my role model. And to my nephew Jack, the light of our lives.

Acknowledgements

Chapters 1 and 2 were funded by Coordinated Agricultural Project (CAP) grants distributed by the USDA-NIFA, and supported by the Bee Informed Partnership. Chapter 3 was also supported by the Bee Informed Partnership, and also by beekeepers participating in the Sentinel Apiary Program. Chapter 4 was funded by the Eastern Apicultural Society (EAS), a Northeast Sustainable Agriculture Research and Education (NE SARE) Graduate Student Grant, and a USDA-NIFA Agriculture and Food Research Initiative Critical Agricultural Research and Extension (AFRI-CARE) grant.

I would like to extend my thanks to the entire vanEngelsdorp bee lab. Special thanks to Heather Eversole and Rachel Kuipers for coordinating the processing of samples. Undergraduates Max O'Grady, Franklin Olmstead, and Mike Gladchuk provided substantial assistance in the field. Andrew Garavito taught me how to keep bees and took countless stings and miles in the truck to make this dissertation happen. Dr. Nathalie Steinhauer provided extensive theoretical, experimental, and statistical expertise, and laid the ground for much of this work. I would also like to thank the Bee Informed Partnership, and the many beekeepers who contributed directly and indirectly to these studies. Finally, I would like to thank Dan Reynolds, who was forced to help me in the field on many a weekend, and who's generous emotional support allowed me to succeed through these four years.

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General Introduction

Honey bees (*Apis mellifera, L.)* are the most prevalent pollinator in agricultural landscapes, providing essential pollination services to fruit, nut, and other specialty crops [1, 2]. The dependency of farmers on honey bees continues to increase, and the number of colonies available for pollination cannot keep up with demand [3, 4]. Shortages of colonies can be attributed in part to repeated high rates of colony loss. Every winter, US beekeepers lose an average of 35% of their colonies, and recouping those colonies is labor and resource intensive [5].

Major concern over colony loss rates originated in 2006, when beekeepers reported massive numbers of colonies collapsing with no dead bees left behind. This mysterious condition was termed Colony Collapse Disorder (CCD) [6]. While the set of symptoms comprising CCD is rarely seen today, the flurry of interest it started in honey bee health and loss rates identified many other colony health stressors. A major outcome of CCD was the initiation of the Bee Informed Partnership (BIP), and the annual Loss and Management Survey [7]. This annual survey of US beekeepers monitors seasonal colony loss rates, and beekeeper reported causes of colony mortality. In Chapter 1: A national survey of managed honey bee 2015–2016 annual colony losses in the USA, results of the tenth consecutive BIP Loss and Management Survey are reported. This report establishes current loss rates beekeepers are facing, as well as identifies relevant colony health stressors. These results serve as a starting point for the

development of germane management recommendations to combat relevant colony health stressors.

Honey bees are exposed to a multitude of interacting and synergizing stressors in the field [8]. Four main categories of stressors have been identified, including poor nutrition, pesticide exposure, pathogens, and parasites [8-12]. The parasitic mite *Varroa destructor* is considered a top cause of colony mortality by researchers and beekeepers, particularly due to the suite of viruses it vectors [13, 14].

Another major contributing factor to colony health that is often overlooked is the beekeeper. The beekeeper has a unique opportunity to mitigate the effects of colony heath stressors though the application of good beekeeping management practices. Beekeepers can apply *Varroa* management techniques to improve colony health and survival [15, 16]. The trouble, however, is that beekeeping can be a very opinion based practice. Many self-proclaimed online experts have opinions to offer, few of which have been tested or proven effective, leaving beekeepers at the mercy of trial and error. Beekeepers need science-based best management practices to avoid wasting time and resources on ineffective practices.

The BIP Loss and Management survey affords the opportunity to correlate colony loss rates to management practices. Using four years of survey data, empirical best practices correlated with reduced winter losses were developed [17]. In Chapter 2: Survey-derived best beekeeping management practices improve colony health and reduce mortality, these practices were tested on

colonies to evaluate their effectiveness compared to average beekeeping practice. One practice in particular was hypothesized to have the greatest impact on colony health: frequent *Varroa* management. Thus Chapter 3 and 4 focus on further characterizing *Varroa* management practices among US beekeepers, and identifying obstacles to successful *Varroa* management.

In Chapter 3: Factors contributing to excessive fall Varroa destructor populations: a citizen science approach, citizen science data collected from the BIP Sentinel Apiary Program is used to characterize typical *Varroa* population growth in US apiaries. Whether *Varroa* treatments provide the expected level of control is assessed. Factors impacting *Varroa* treatment outcome are explored, including the state of the apiary during treatment, and differing treatment practices. One of these potential factors is studied in depth in Chapter 4: A honey bee (Apis mellifera) colony's *Varroa* destructor population increases not because it robs, but because it is visited. The possibility of horizontal transmission of mites between apiaries in the fall leading to rapid increases in *Varroa* load is investigated. The extent to which bees move between apiaries, and the resulting change in *Varroa* load are assessed. Overall, this dissertation aims to characterize US beekeeping management practices, giving special attention to *Varroa* management. Obstacles to effective *Varroa* management are investigated, and best management recommendations are given.

Chapter 1: A national survey of managed honey bee 2015–2016 annual colony losses in the USA

Abstract:

Managed honey bee colony losses are of concern in the US and globally. This survey, which documents the rate of colony loss in the US during the 2015–2016 season, is the tenth report of winter losses, and the fifth of summer and annual losses. These results summarize the responses of 5725 valid survey respondents, who collectively managed 427,652 colonies on 1 October 2015, an estimated 16.1% of all managed colonies in the US. Responding beekeepers reported a total annual colony loss of 40.5% [95% CI 39.8–41.1%] between 1 April 2015 and 1 April 2016. Total winter colony loss was 26.9% [95% CI 26.4–27.4%] while total summer colony loss was 23.6% [95% CI 23.0–24.1%], making this the third consecutive year when summer losses have approximated to winter losses. Across all operation types, 32.3% of responding beekeepers reported no winter losses. Whilst the loss rate in the winter of 2015–2016 was amongst the lowest winter losses recorded over the ten years this survey has been conducted, 59.0% (n = 3378) of responding beekeepers had higher losses than they deemed acceptable.

Introduction:

Managed honey bees (*Apis mellifera*) add $15 billion worth of pollination services to US agriculture annually [18]. Insect pollinators provide over 153 billion euros (€153 billion) in crop production worldwide [3] including estimates of values ranging from $0.38 billion in the UK [19] to $6.4 billion in the EU [20]. Ongoing

high rates of colony mortality threaten the supply of sufficient colonies needed to pollinate fruit, nut and other specialty crops [1]. For instance, US honey bee populations declined by 61% between 1947 and 2008 [6, 21]. Despite high levels of severe colony losses over the last 10 years, the total number of colonies managed in the US has, however, increased from 2.39 million in 2006, when colony collapse disorder (CCD) was first reported [22], to 2.59 million in 2016 [23]. This increase can be explained by the ability of beekeepers to replace dead colonies through splitting existing colonies into two or more units [6]. Since splitting colonies involves labor and financial costs, particularly for large commercial operations who perform hundreds or thousands of splits in a year, the long-term sustainability of operations that suffer these high loss rates is threatened.

Colony mortality can result from a multitude of interacting factors including forage availability [24], pesticide exposure [25], issues associated with the ectoparasitic mite *Varroa destructor* [26], other pests, parasites and diseases [14], as well as various other socioeconomic factors [3]. With the initial concern raised by CCD, beekeepers and scientists began monitoring colony loss rates annually [7, 27-34], giving context to annual mortality rates, which then allows for identifying potential causes of and solutions to poor bee health.

The Bee Informed Partnership (BIP, beeinformed.org) has conducted winter colony loss surveys in the US since 2006–2007. The present survey, like previous BIP surveys, calculates colony loss rates indirectly, by quantifying the number of colonies alive on a specific date and obtained over specific time

periods [6, 35]. Total winter loss has ranged from a low of 22% (2011–2012, 2014–2015) to a high of 36% (2007–2008). Total summer loss has ranged from 24 to 25% (2012–2014). Finally, annual loss has ranged from 34% (2013–2014) to 45% (2012–2013) [7, 27-34]. Beekeeper-defined acceptable annual losses in previous US surveys have ranged from 13.2 to 19.1% [7, 27-34].

Surveys conducted by BIP do not solicit responses randomly, and thus are potentially biased, as the demographics of its respondents may not be reflective of the industry as a whole. To conduct a random survey, a national public registry of all beekeepers is needed from which to select respondents. The National Agricultural Statistics Service (NASS) maintains a list of all known farming operations in the US, including beekeepers. NASS does lend technical assistance and conducts surveys for private organizations and other government agencies. This can, however, be prohibitively expensive depending on the amount of work NASS is required to perform. To address concerns over potential biases of the BIP survey, the “National strategy to promote the health of honey bees and other pollinators” released by the White House [36] tasked NASS to produce annual, US and state level estimates on the number of honey bee colonies, colonies lost, and colony health. NASS had already been surveying beekeepers for its Honey report, using a stratified random sample of all known beekeeping operations with five or more colonies that also qualified as a farm. A panel was chosen from this sample and tracked on a quarterly basis throughout the year to produce the Honey Bee Colonies report. While BIP personnel were consulted during the development of the NASS survey, not all questions were

identical and so direct comparisons of results must be made with caution. Nevertheless, the questions and results pertaining to these two surveys are sufficiently similar to permit some comparisons. NASS recently published results [23] allowing a one-year comparison of results between these two different efforts.

As with previous BIP loss reports, here summer, winter, and annual colony losses that were self-reported by beekeepers across the US from 1 April 2015 to 1 April 2016 are documented. This is the fifth survey to include the summer and annual time periods and the tenth survey reporting winter losses. Beekeepers are classified by operation type based on the number of colonies they managed as “backyard” (≤50 colonies), “sideline” (51–500 colonies), or “commercial” (>500 colonies), and compared colony loss rates between these three groups. Furthermore, as done previously, colony loss rates are compared among beekeepers in different groupings, including those grouped by state, migratory practice, participation in California almond pollination, self-reported causes of loss, and self-declared acceptable annual loss rate. Annual data on the estimated percent of colonies lost in the US enabled the comparison the current survey results to those of prior years. Such comparisons help monitor the status of colony losses and honey bee health at the population level.

Methods:

Survey:

Beekeepers were invited to participate in the annual colony loss survey via email through distribution lists maintained by two national beekeeping

organizations (American Beekeeping Federation and American Honey Producer's Association), a beekeeping supply company (Brushy Mountain Bee Farm), two honey bee brokers, two beekeeping journals (American Bee Journal and Bee Culture), two subscription listservs (Catch the Buzz and ABFAlert), and the BIP mailing list (n = 15,328). The email directed participants to an online survey hosted via www.SelectSurvey.net. As a survey of convenience with a snowballing recruitment, emails asking beekeepers to participate in the survey also requested that respondents forward the survey invitation to fellow beekeepers who may also want to participate. Requests to distribute the survey were also sent to the Apiary Inspectors of America, state extension apiculturists, industry leaders including the American Beekeeping Federation (ABF) and the American Honey Producers Association (AHPA), and to a number of regional beekeeping clubs, including the Eastern Apicultural Society (eastern US), the Heartland Apicultural Society (central US), and the Western Apicultural Society (western US). To ensure adequate representation from commercial beekeepers, paper surveys were mailed to commercial beekeepers identified by state apiary inspectors (n = 1100). The survey was available online from 1 April 2016 to 30 April 2016. Paper surveys were mailed by the end of March and were accepted through to 29 July 2016.

The "loss survey" asked quantitative questions about the number of colonies in an operation and objective questions about perceived causes of loss and acceptable annual loss rates. This was followed by an optional "management survey." The present study addresses only responses to the loss

survey, which has included the same core questions for summer, winter, and annual losses since 2013–2014 [7, 27-34]. Loss seasonal periods are defined as 1 April 2015 to 1 October 2015 (summer), 1 October 2015 to 1 April 2016 (winter), and 1 April 2015 to 1 April 2016 (annual) [7, 27-34].

Duplicate responses and responses from non-US beekeeping operations were filtered out from the database. Responses with insufficient or illogical answers were also excluded. The “cause of loss” question included an open “Other: please specify” response. Specified “Other” causes of loss were either kept separate if they were truly unique, or were re-categorized into the appropriate cause of loss response. For example, a respondent who chose “Other” and specified “Flood” was re-categorized into the “Natural Disaster” cause of loss category.

Once the invalid responses were filtered out of the database, three subsets for analysis were created of valid summer, winter, and annual colony losses. Creation of these subsets was necessary because not all respondents answered all questions. Respondent’s results were only included in a given period if they had at least one colony at the start of a given period. Respondents were also categorized into three “operation type” groups, determined by the number of colonies they managed on 1 October 2015. “Backyard beekeepers” managed 50 or fewer colonies, “sideline beekeepers” between 51 and 500 colonies, and “commercial beekeepers” more than 500 colonies.

Statistics:

Total and average colony losses for summer, winter, and the annual period were calculated for all operations based on vanEngelsdorp et al. (2013) and R code first used in Steinhauer et al. (2014). The percentage of operational losses for each respondent was first calculated by dividing the number of colonies lost by the number of colonies at risk during each time period (summer, winter, annual). Total loss rate was then calculated by dividing the total number of colonies lost by the number of colonies at risk in that time period, and then multiplying the resulting number by 100. Total loss calculations count each individual colony without factoring in operation size, meaning that responses from beekeepers with larger operations exert a greater weight in total loss calculations than beekeepers with smaller operations. Total loss percentages are more representative of commercial beekeepers because they manage significantly more colonies (n = 378,693) than the smaller operations (sideline and backyard) combined (n = 48,959).

For comparison, average loss was also calculated, where the total loss of each operation is calculated and all operational total losses are summed and divided by the number of responding operations. Average loss facilitates better comparison between subsets of beekeepers. Average loss was calculated by adding each operational loss for a given period, then dividing that sum by the number of valid respondents in that time period.

Ninety-five percent confidence intervals (95% CI) for total losses were calculated using a generalized linear model with a quasi-binomial distribution (R

Development Core Team, 2016). Average loss 95% confidence intervals (CIs) were calculated using the Wald formula [35].

Differences in loss rates between operational sizes were identified with the Kruskal-Wallis rank sum test. Differences in loss rates were evaluated between operation size, migratory vs. stationary beekeepers, participation vs. non-participation in almond pollination, acceptable vs. higher than acceptable loss, and between various self-reported causes of death. When multiple comparisons were conducted, the Kruskal–Wallis test was followed by the Mann–Whitney U test (also known as Wilcoxon Rank Sum test) for a pairwise check of significance using a Bonferroni correction. Chi squared tests were used to check for differences between operation types, and for other groupings. All statistical tests were performed using the statistical program R (R version 3.3.1 (21 June 2016)) and all tests used a significance level of $\alpha = 0.05$.

We followed the USDA-NASS method to report state colony losses by counting colonies of multistate beekeepers in each state which the beekeeper reported having colonies (USDA-NASS, 2016). If a state had five or fewer respondents, the losses for that state were not reported to maintain the anonymity of the respondent(s).

Self-reported causes of loss:

To understand the potential impact on colony loss rates by different reported causes of loss, the percentage of total winter losses attributable to each reported cause of loss was calculated. The top three reported risk factors were considered, meaning those self-reported factors that directly cause colony loss.

These factors were – “*Varroa*” [37, 38], “Queen failure” [35, 39] and “Pesticides” [10]. How many colonies were lost to these risk factors was then estimated by counting how many colonies were lost by each beekeeper who reported each cause. For example, if a beekeeper who lost 50 colonies reported only “Queen Failure,” 50 colonies of the total winter colony losses were attributed (n = 145,106) to “Queen Failure.” If a beekeeper reported more than one of the top three risk factors (i.e., reported “Queen failure” and “*Varroa*”), his loss was divided equally among the categories “Queen failure + Other” and “*Varroa* + Other.” A beekeeper who lost 50 colonies would have 25 lost colonies attributed to each of the two categories. The “+ Other” categories also include beekeepers who selected a top three risk factor and one or more causes of loss other than the top three risk factors (i.e., a beekeeper who lost 50 colonies and reported “Queen failure,” and “Starvation” would give 50 lost colonies to “Queen Failure + Other”). The “All Other” category contains beekeepers who had a winter loss and reported one or more causes of loss other than the top three risk factors. This was done for each beekeeper who reported a cause of loss.

Comparison to USDA-NASS survey:

In 2015–2016, NASS collected and reported loss data for the first time. There are a few notable differences in the numbers reported and the methodology used to calculate losses between NASS and BIP loss reports. First, NASS divides the year into quarterly time periods as opposed to the half year breakdown (summer and winter). For each quarter, NASS reports the number of colonies at the start of the period, the number of colony additions, and the

number of colonies lost for each quarter. A state level “maximum” number of colonies is also calculated by adding all colonies that were in the state on the 1st of the quarter, plus all those which moved in during the quarter.

NASS calculates loss by directly asking each respondent how many colonies died over a given time period in each state an operation was in during the quarter. A state level loss ratio is calculated by dividing the number of colonies lost in a state during the quarter by the number of colonies with the potential to be lost in a state during that quarter (defined by NASS as the “Maximum colonies”.) At the US level, no Maximum colonies exists due to duplication, so the national loss ratio is total number of colonies lost divided by the total number of colonies on the first of the quarter. BIP calculates loss indirectly by calculating change in colony numbers over time to include fluctuations caused by splitting. BIP calculation methods could not be used to compare losses by quarter because they did not include colony counts for each quarterly start date. To compare NASS loss numbers with BIP’s (Table 1.1), the quarterly numbers published by NASS were combined to correspond to BIP’s division of the seasons into “summer” “winter” and “annual”).

The seasonal Total Loss (G) was calculated using NASS data and NASS methods using Equation (1):

$$G = \frac{F}{A}$$

where the number of colonies lost over the season (F) was the sum of the NASS reported number of colonies lost over the quarter, and the number of colonies at risk of dying (A) was the NASS reported number of colonies at the start of the

season. Seasonal Total Loss (G) was also calculated using BIP methods and NASS numbers using Equation (1). BIP methods calculates the total number of colonies at risk of dying (A) using Equation (2):

$$A = S + C - D$$

where S is the number of colonies at the start of a season, C is the number of colonies added (splits and additions), and D is the number of colonies sold during a period. However, NASS does not report the number of splits or purchases made. Nor does NASS report the loss rate of splits made during a quarter. Therefore, when calculating the total loss rate with BIP-like methods using NASS numbers, the number of colonies lost (F) was calculated using Equation (3):

$$F = A - (S_2)$$

where A is the number of colonies at the start of the period, and S2 is the number of colonies at the start of the next period. In the case where NASS has not yet reported the S2 (e.g., after 4th quarter), S2 was estimated by summing the number of colonies remaining after the period (e.g., colonies at start of period – lost colonies during the period + added colonies during the period) and the total number of additions made during the period. In other words, it was assumed that none of the additions died during the fourth quarter (e.g., January– March).

Table 3.1 Summary of NASS-published data including number of colonies at the start of each season (Colonies Start), colonies added (Added), number of colonies at risk (Total colonies at Risk = Colonies Start + Added), colonies lost (Lost), and Total Loss (%). Total loss is calculated using both NASS and BIP-like methodologies for comparison of results.

NASS Numbers, NASS Periods, NASS Reported Losses						
Season	Colonies at Start	(Added)	.	.	Lost	Total Loss (%) (=Lost/Colonies Start)
Apr-Jun	2,849,500	(661,86)			352,860	12.38
Jul-Sep	3,152,880	(172,99)			457,100	14.50
Oct-Dec	2,874,760	(117,15)			412,380	14.34
Jan-Mar	2,594,590	(376,16)			428,800	16.53
NASS Numbers, BIP Seasons, NASS-modified method for loss calculation						
Season	Colonies at Start	(Added)	.	(Total Colonies at Risk)	Lost	Total Loss NASS method (%) (=Lost/Colonies Start)
Summer	2,849,500	(661,86) (172,99)		(3,511,360)	809,960	28.42
Winter	2,874,760	(117,15) (376,16)		(2,991,910)	841,180	29.26
Annual	2,849,500	(661,86) (172,99) (117,15) (376,160		(3,801,500)	1,651,140	57.94
NASS Numbers, BIP seasons, BIP – like method for loss calculation						
Season	Colonies at Start	Added	Total Colonies at end of season	Total Colonies at Risk	Lost	Total Loss BIP method (%) (=Lost/Total Colonies at Risk)
Summer	2,849,500	834,850	2,874,760#	3,684,350	809,590	**21.97**
Winter	2,874,760	493,310	2,541,950#	3,368,070	826,120	**24.53**
Annual	2,849,500	1,328,160	2,541,950	4,177,660	1,635,710	**39.15**

#Estimate

For annual loss estimates, using NASS numbers and BIP-like methods, the additions from the first three quarters were added to the starting colonies. In each case, as per NASS standards, splits made during the most recent quarter (most recent splits) are not considered in the pool of colonies at risk (Table 1.1).

Results:

Average and total losses:

There were 7535 beekeepers who responded to this survey. A total of 399 duplicates and 341 non-US respondents were identified and invalidated, leaving 6795 valid responses to comprise the analytical data-set. After invalidating illogical and insufficient responses, the data-set included 5725 valid winter responses, 4875 summer responses and 4624 annual responses. These respondents managed a total of 427,652 colonies on 1 October 2015. Based on USDA-NASS (2016) estimates, this survey represents 16.1% of all managed honey producing colonies in the US in the summer of 2016. Of the 5725 valid winter loss respondents, 5499 were back-yard beekeepers, 137 were sideline beekeepers, and 89 were commercial beekeepers. On 1 October 2015, the respondent backyard, sideline, and commercial beekeepers managed 33,254, 15,705, and 378,693 colonies, respectively.

Total colony loss in 2015–2016 was 23.6% [95% CI 23.0–24.1%] in summer, 26.9% [95%CI 26.4–27.4%] in winter, and 40.9% [95% CI 39.9–41.1%] annually. Average loss per beekeeper was 16.5% [95% CI 15.8–17.2%] in summer, 37.7% [95% CI 36.8–38.7%] in winter, and 44.2% [95% CI 43.2–45.2%] annually (Table 1.2). Across all operation types, a total of 32.3% of responding

beekeepers reported no winter loss, 99.5% of which were backyard beekeepers who managed an average of 3 ± 0.1 colonies.

Table 1.4. A summary of the three colony loss periods (summer, winter, and annual) of the self-reported colony loss data from 1 April 2015 to 1 April 2016, with the total number of respondents, the total number of colonies on each date, the total number of colonies increases (+) and decreases (−), and the total loss and average loss for each period (%) [95% CI].

		Total colonies alive on:						
Season	N	April 1, 2015	Net interim increase	October 1, 2016	Net interim increases	April 1, 2016	Total Loss (%)	Average Loss (%)
Summer	4,875	399,055	138,787	411,167	-	-	23.6 [23.0-24.1%]	16.5 [15.8-17.2%]
Winter	5,725	-	-	427,652	112,222	394,768	26.9 [26.4-27.4%]	37.7 [36.8-38.7%]
Annual	4,624	373,710	137,603	511,313	98,544	362,954	40.5 [39.9-41.1%]	44.2 [43.2-45.2%]

Notes: Sample size (n) is the number of beekeepers providing valid responses. Net interim changes include the numbers of increases (+) by splits or purchases and decreases (–) through selling or giving away during a time period.

State losses:

The number of respondents varied between states across all seasons. Puerto Rico had only one valid respondent for the winter loss season, while Pennsylvania had 777. State total losses also varied, from 5.3 to 55.2% in summer, 2.4 to 60.1% in winter, and 24.5 to 71.3% annually (Figure 1.1, Supplemental Figures 1.1a, 1.1b). State average losses ranged from 8.2 to 29.5%, 11.2 to 55.9%, and 18.8 to 60.9% in summer, winter, and annually, respectively (Figure 1.2, Supplemental Figures 1.2a, 1.2b).

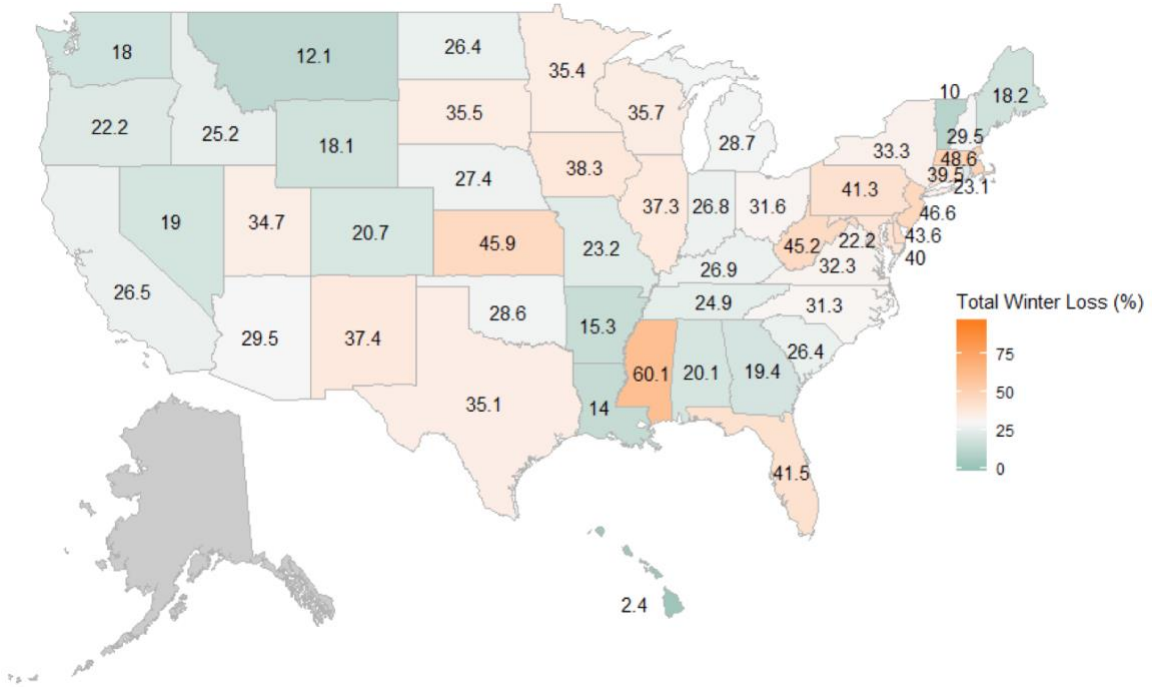


Figure 1.1. Total colony winter losses (%) reported for each state in the USA.

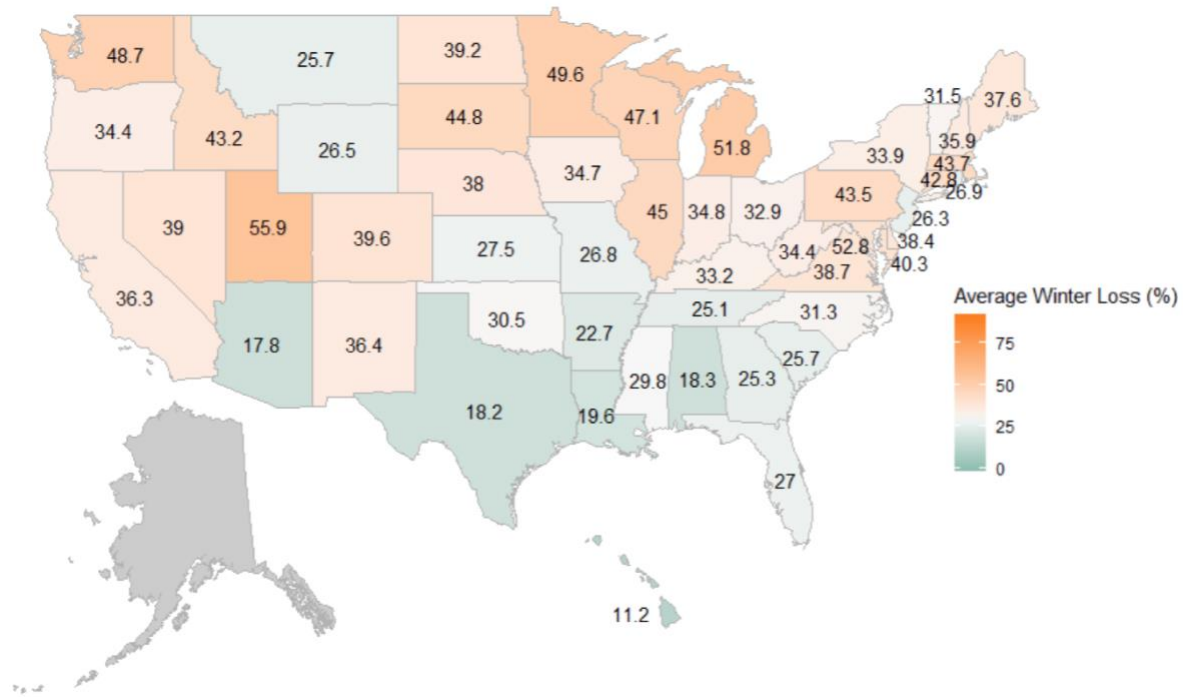


Figure 1.2. Average colony winter losses (%) reported for each state in the USA.

Losses by operation type:

Each operation type had different numbers of respondents. Because the majority of beekeeping operations in the US are small, backyard beekeepers predominate the survey respondents, representing 96.1% (n = 5499) of winter respondents, 95.7% (n = 4670) of summer and 95.7% (n = 4426) of annual respondents. There were 116 valid sideline beekeepers in summer, 173 in winter, and 114 in the annual portion. There were 89 valid commercial beekeepers in summer 84 in the winter and annual season.

In summer, sideline beekeepers lost on average the fewest number of colonies (15.1% [95% CI 11.7–18.5%]), followed by backyard beekeepers (16.5% [95% CI 15.6–17.2%] $p < 0.005$). Commercial beekeepers reported the highest rate of loss (21.1% [95% CI 17.3– 24.9%]) compared to the other two operation types [vs. backyard: $p < 0.0001$, vs. sideline: $p < 0.005$]. Summer loss was the only period for which all operation types differed significantly [$\chi 2 = 45.39$, $p < 0.0001$]. Average losses were the same for all beekeeping groups over the winter [$\chi 2 = 1.91$, $p = 0.3849$] and annually [$\chi 2 = 3.05$, $p = 0.2174$]. Average losses were 38.2% [95%CI 37.2–39.1%] in winter and 44.5% [95% CI 43.4–45.5%] annually for backyard beekeepers, 28.7 [95% CI 24.6–32.8%] in winter and 37.6% [95% CI 32.9–42.4%] annually for sideliners, and 26.3% [95% CI 22.2–30.3%] in winter and 38.8% [95% CI 34.3–43.2%] annually for commercial beekeepers (Table 1.3, Figure 1.3).

Migratory operations were composed primarily of commercial beekeepers (83.7%, n = 72). Commercial operations also composed most of the population of

respondents who reported using their colonies for almond pollination (81.4%, n = 70). Beekeepers who reported moving across state lines were categorized as migratory, and experienced average winter loss (28.4% [95% CI 24.7–32.5%]) that trended lower than stationary beekeepers (38.0% [95% CI 37.0–39.0%]) [$\chi 2 = 3.242$, $p = 0.072$]. Beekeepers pollinating almonds lost the same number of colonies (28.1% [95% CI 23.7–32.6%]) on average as those who reported as not pollinating almonds (27.5% [95% CI 23.5–31.5%]) [$\chi 2 = 0.021$, $p = 0.8853$].

Table 1.5. 2015–2016 US colony loss by operation type (total and average loss (%) [95% CI]), showing the number of respondents (n), the total number of colonies at the start of the respective period (# Colonies (start)) for each of the operation type categories: backyard beekeepers (1–50 colonies), sideline beekeepers (51–500 colonies) and commercial beekeepers (>500 colonies).

Season	Operation type	n	# Colonies (start)	% Colonies (start)	Total Loss (%)	Average Loss (%)
	Backyard	4,670	21,679	5.4	17.7 [17.1-18.4%]	16.4 [15.7-12.2%]
Summer	Sideline	116	11,275	2.8	25.5 [20.8-30.7%]	15.1 [11.7-18.5%]
	Commercial	89	366,101	91.7	23.9 [20.3-27.7%]	21.1 [17.3-24.9%]
	Backyard	5,499	33,254	7.8	34.3 [33.5-35.2%]	38.2 [37.2-39.1%]
Winter	Sideline	137	15,705	3.7	28.4 [25.0-32.5%]	28.7 [24.6-32.8%]
	Commercial	89	378,693	88.6	26.3 [22.8-30.0%]	26.3 [22.2-30.3%]
	Backyard	4,426	20,530	5.5	43.5 [42.6-44.4%]	44.5 [43.4-45.5%]
Annual	Sideline	114	9,771	2.6	41.6 [36.5-46.8%]	37.6 [32.9-42.4%]
	Commercial	84	343,409	91.9	40.3 [36.0-44.6%]	38.8 [34.3-43.2%]

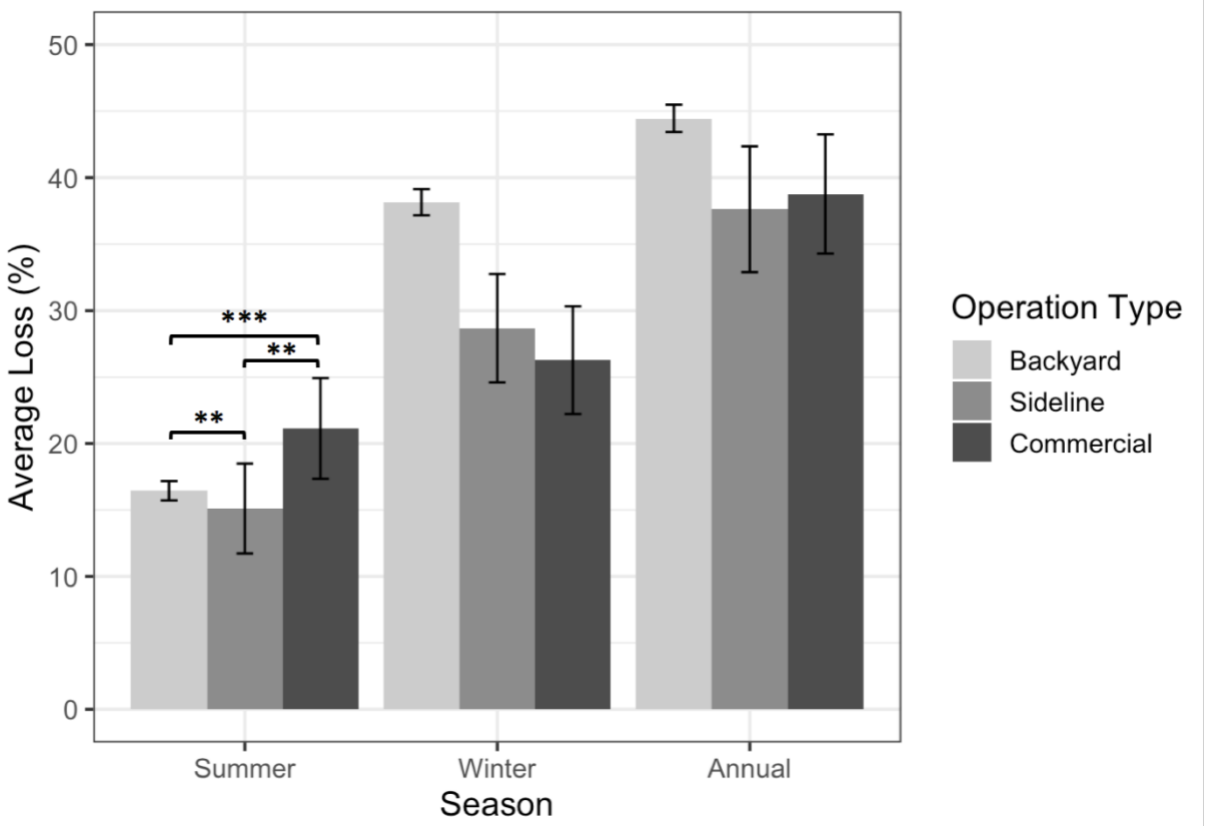


*Figure 1.3 Average (%) summer (1 April 2015 to 1 October 2015), winter (1 October 2015 to 1 April 2016), and annual (1 April 2015 to 1 April 2016) colony losses (with 95% CI) of three beekeeping operation types (backyard, sideline, and commercial). Notes: Bars represent 95% CI. **p < 0.01, ***p < 0.001*

Acceptable loss:

On average, survey respondents indicated that a loss rate of 19.0% [95% CI 18.5–19.4%] (n = 5,726) was acceptable. Commercial beekeepers reported that a 16.5% [95% CI 14.0–19.1] loss rate was acceptable, where sideline and backyard beekeepers reported that 17.4% [95% CI 15.1–19.8%] and 19.0% [95% CI 18.6–19.5%] loss rates were acceptable respectively. Using the average reported acceptable loss of 19.0%, 59.0% (n = 3378) of beekeepers observed higher losses than they deemed acceptable. These beekeepers had an average

loss of 62.2% [95% CI 61.3–63.2%], which was much higher than beekeepers who lost fewer colonies than the average acceptable loss rate (2.5% [95% CI 2.3–2.7%]) [$\chi 2 = 4324.2$, $p < 0.0001$].

Fifty-four percent of responding beekeepers had higher colony loss rates than their own standard of acceptable loss rates. These beekeepers experienced a 62.0% average loss [95% CI 61.0–63.0%] compared to a 7.1% average loss [95% CI 6.5–7.8%] for those who experienced loss they considered acceptable [$\chi 2 = 3{,}583$, $p < 0.0001$].

Self-reported causes of loss:

Of the 5725 valid winter loss respondents, 3369 (3459 backyard, 131 sideline, 79 commercial) lost at least one colony and reported at least one cause of loss. “Weak in the fall” (n = 1210), “*Varroa*” (n = 1181), “Don’t know” (n = 952), and “Queen failure” (n = 933) were the most commonly selected causes of loss across all operation types (Table 1.4). Self-reported causes of death differed between operation types. Backyard and sideline beekeepers were more likely to report “Weak in the fall” (reported by 36 and 44% of backyard and sideline respondents, respectively) and “*Varroa*” (33, 62% respectively), while commercial usually reported “Queen failure” (70%) and “*Varroa*” (84%). Backyard beekeepers, often the least experienced group (www.beeinformed.org, 2015), were also very likely to report “Don’t know” (30%) (Figure 1.4).

Table 1.6. Causes of death and association with each commercial type and average loss.

Cause of death	n (total)	n (backyard) (%)	n (sideline) (%)	n (commercial) (%)	Average Loss % [95% CI]
Queen Failure	933	823 (88.2%)	60 (6.4%)	50 (5.4%)	47.3 [45.3-49.2]
Starvation	766	709 (92.6%)	44 (5.7%)	13 (1.7%)	53.4 [51.2-55.5]
Varroa	1181	1042 (88.3%)	82 (6.9%)	57 (4.8%)	55.9 [54.2-57.7]
Nosema	142	116 (81.7%)	14 (9.9%)	12 (8.4%)	52.4 [47.5-57.2]
Small Hive Beetle	162	150 (92.6%)	6 (3.7%)	6 (3.7%)	58.8 [54.1-63.5]
Poor Winter	603	583 (96.7%)	15 (2.5%)	5 (0.8)	65.7 [63.3-68.1]
Pesticides	274	232 (84.7%)	17 (6.2%)	25 (9.1%)	66.1 [62.5-69.6]
Weak in Fall	1210	1133 (93.6%)	55 (4.6%)	22 (1.8%)	52.1 [50.4-53.8]
CCD	401	355 (88.5%)	21 (5.3%)	25 (6.2%)	64.0 [61.0-66.9]
Disaster	103	88 (85.4%)	10 (9.7%)	5 (4.9%)	56.0 [50.1-61.9]
Don't Know	952	920 (96.6%)	16 (1.7%)	16 (1.7%)	65.2 [63.3-67.1]
Other Pests	104	102 (98.1%)	2 (1.9%)	0	62.1 [56.0-68.1]
Mismanagement	21	20 (95.2%)	1 (4.8%)	0	54.5 [41.7-67.3]
Other Disease/Virus	31	24 (77.4%)	4 (12.9%)	3 (9.7%)	51.8 [39.8-63.8]
Other	183	171 (93.5%)	9 (4.9%)	3 (1.6%)	54.5 [50.0-59.1]

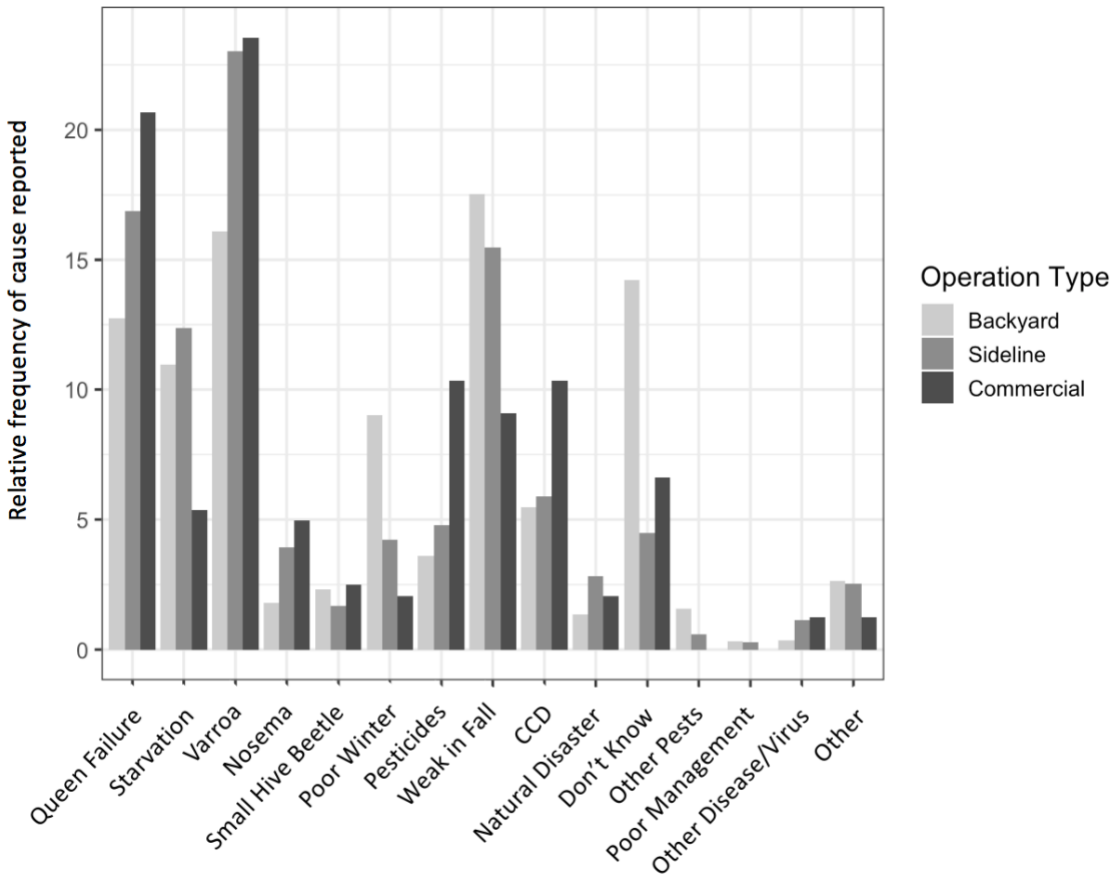


Figure 1.4. Relative frequency of respondents reporting each cause of loss by operation type.

Average losses differed between those who reported different self-diagnosed causes of loss. Beekeepers who reported "Don't know" as a cause of loss, lost more colonies on average (65.2% [95% CI 63.3–67.1%]) than those who did not (52.8% [95% CI 63.6– 67.1%]) [$\chi 2 = 113.2$, $p < 0.05$]. Average loss for "Weak in fall" reporters was 52.1% [95% CI 50.4–53.8%], which is lower than those who did not report "Weak in fall" (58.0% [95% CI 50.4–53.9%]) [$\chi 2 = 28.885$, $p < 0.05$]. Those who reported "Queen failure" as a cause lost 47.3% [95% CI 45.3–49.3] of colonies on average, which was lower than those who did not report "Queen failure" (59.0% [95% CI 57.8–60.2%]) [$\chi 2 = 102.88$, $p < 0.05$]. Average loss by those who reported "*Varroa*" as a cause was 55.9% [95% CI

54.2–57.7%], and was about the same as those who did not list "*Varroa*" as a main contributor to their losses (56.1% [95% CI 54.8–57.3%]) [$\chi 2 = 0.006$, $p > 0.05$].

Beekeepers who reported one or more of the three most commonly reported risk factors associated with colony mortality ("Queen failure," "*Varroa*," and "Pesticides") experienced a combined loss of 132,463 colonies (Figure 1.5). These calculations suggest that beekeepers who reported Queen Failure, *Varroa*, and/or Pesticides lost 91.3% of total number of colonies lost over the winter (n = 145,106).

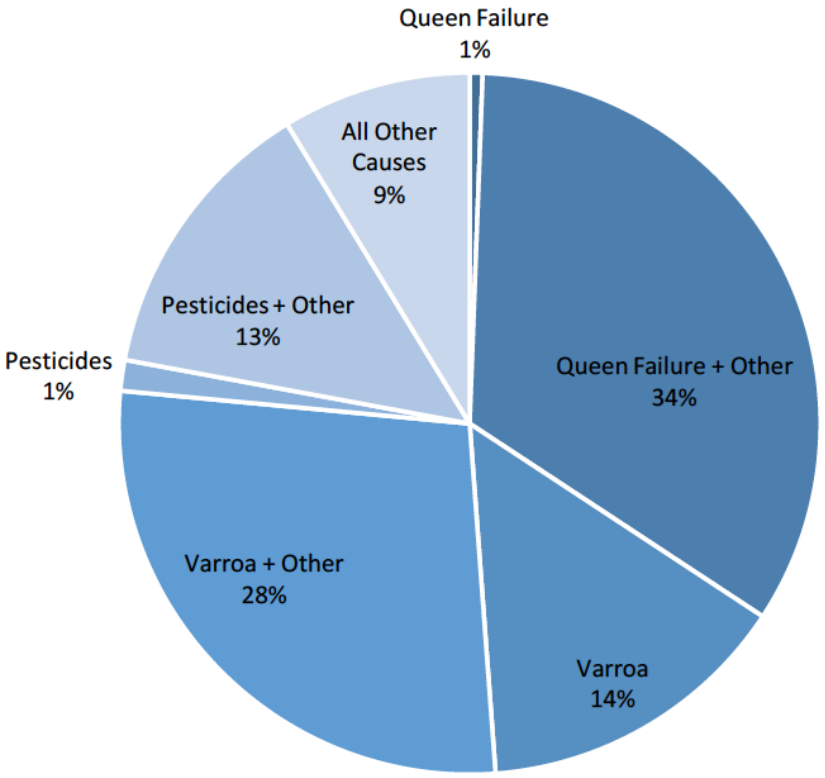


Figure 1.5. Estimated (see methods) number of colonies lost (n = 132,463) due to each commonly self-reported colony health risk factors.

One survey question asked specifically if the beekeeper lost colonies with the symptom "no dead bees in the hive or apiary," a known symptom of CCD. Of the 3675 valid responses to this question, 1619 (1474 backyard, 77 sideline, 68

commercial) beekeepers reported that this symptom was a prominent cause of colony death in their operation. The average loss for those that reported the symptom was 58.8% [95% CI 57.3–60.3%], which is significantly higher than those who did not report it who, on average, lost 53.8% of colonies [95% CI 52.5–55.2%]) [$\chi 2 = 23.35$, $p < 0.0001$].

Comparison to USDA-NASS Survey:

To explore potential differences between the BIP and NASS survey results, four sets of calculations were performed using either numbers collected by BIP or numbers collected by NASS (as described in Methods; Table 1.1c) (Table 1.5). Total loss numbers (%) were calculated using the BIP numbers using BIP loss calculation methodology, NASS numbers using NASS methods and BIP seasons, NASS numbers and BIP loss calculation methods, and BIP numbers using NASS-modified calculation methodology.

Table 1.7. Summary of Total colony losses (%) as calculated by BIP and NASS for each time period.

Total Loss Estimates (%)		BIP numbers BIP method	NASS numbers NASS method	NASS numbers BIP method	BIP Numbers NASS Method
Summer	Apr 2015-Sep 2015	23.55	23.07	21.97	22.65
Winter	Oct 2015-Apr 2016	26.88	28.12	24.53	23.93
Annual	Apr 2015-Apr 2016	40.49	43.43	39.15	-

As BIP data are collected for every 6-month period, these results do not compare BIP results with NASS published results directly. Furthermore, because NASS divides losses into quarters, it is not possible to calculate the total annual loss using BIP numbers and NASS calculation methodology.

Discussion:

This is the tenth consecutive survey to report winter colony losses, and the fifth to report summer and annual losses. Total winter loss of 26.9% this year is slightly higher than the 25% total winter loss reported last year [28] and the 10-year total winter loss average of 24.6%. This year's average winter loss of 44.2% is consistent with the two highest years of average winter loss in 2012–2013 and 2013–2014 [27, 30]. Average winter loss, and summer losses that rivaled that of winter losses, emphasize the need for surveys that encompass the entire year to understand bee health.

Beekeepers reporting no winter colony losses were primarily backyard beekeepers (n = 1838, 99.5%). These backyard beekeepers had an average operation size of 3.7(± 0.1) colonies. Furthermore, 17.0% of backyard beekeepers reported 100% loss, while only one sideline and zero commercial beekeepers reported 100% loss. Smaller operations are more likely to retain or lose all of their colonies because they have a smaller margin for error. There were commercial (n = 5) and sideline (n = 5) beekeepers who reported no loss. This may be a result of the subjective nature of this survey, as beekeepers may approximate or misremember data. Some beekeepers reporting no winter loss did experience a summer loss, indicating they may split heavily in fall and assume they compensated for any potential winter loss.

It is useful to compare colony losses in the US to those experienced by beekeepers in other countries. These comparisons put the severity of US colony mortality rates into context on a global scale and help to identify broader trends. US beekeepers experienced higher winter loss than 27 of the 29 countries

included in the 2015–2016 COLOSS survey, surpassed only by Ireland (29.5%) and Northern Ireland (28.2%) [39]. However, these comparisons are very tenuous, because the European survey allows beekeepers to self-define “winter,” meaning that some beekeepers in southern countries could report losses over a two-week period and northern beekeepers report losses over a period of two or more months. Winter losses estimated in China (10.1%, 2010–2013 [40]) and Uruguay (20.2%, 2013–2014 [41]) over past years were also consistently lower than in the US. Estimates conducted in South Africa (29.6% 2009– 2010, 46.2% 2010–2011 [42]) however, were more similar to losses experienced in the US. Direct comparisons between datasets should be made with caution as methodologies, sample sizes, and operation types differ between these surveys and the countries represented.

Differences in state losses are explained, at least in part, by differences in climate. Stationary beekeepers who keep colonies in northern states are expected to have higher loss rates as overwintering colonies are more vulnerable to starvation [9] and parasite pressures [43] due to harsher overwintering conditions such as lack of forage availability, reduced colony size, and cold temperatures. Other, more variable climatic conditions probably played a role in elevated losses. For instance, recent drought experienced in some western states [44, 45] probably affected winter colony mortality. A warm, dry climate has a pronounced effect on vegetation, which in turn affects honey bee foraging and colony health [46].

Typically, commercial beekeepers have lower loss rates than do backyard beekeepers. This year, while numerically true for all seasons, only total and average summer losses were significantly different for the beekeeper groups, with commercial beekeepers losing more colonies in the summer than backyard beekeepers.

Commercial beekeepers manage more colonies and are the most likely to migrate colonies and participate in California almond pollination. These activities expose colonies to stresses such as transport, pesticide exposure, and nutritional monocultures [47-49]. In the ten years of this survey including this year, operations categorized as migratory or participating in almond pollination had the same or lower losses compared with those who did not migrate or pollinate almonds [7, 27-34]. Migratory beekeepers and those pollinating almonds are typically commercial, and these beekeepers generally tend to experience lower loss.

Almost 60% of beekeepers reported losing more than the average loss deemed acceptable (19.0%) by beekeepers in 2016. In the past, average acceptable loss has ranged from 13.2% to a high of 19.0% annually [7, 27-34]. Prior to 2013–2014 when average acceptable loss was also 19.0%, acceptable loss rates were never higher than 14.6%. The upward trend of reported acceptable loss suggests that beekeepers are expecting higher losses than in the past.

Frequent media reports of high colony losses could have an effect on beekeeper outlook, influencing their perception of their loss rates. Adaptation of

the beekeeping industry to continuous years of higher than acceptable loss rates may have also increased beekeeper expectation of loss. As annual colony loss rates remain high in the US, beekeeper attitude and acceptability of colony loss may also remain high.

The self-reported cause of loss survey question provides a unique opportunity to track trends in what beekeepers think the underlying colony health issues are in their operations. What beekeepers report tends to differ between operation types. Commercial beekeepers tend to report direct and known risk factors that correlate to colony losses such as "*Varroa*" [37, 38] and "Queen failure" [35, 39]. Backyard beekeepers, on average, are more likely to assign factors that are more easily mitigated by good management, such as "Weak in the fall" or "Starvation," both of which can be mediated by timely and proper feeding strategies. For those who reported losing colonies with the CCD symptom of no dead bees, it is important to note that this is only one symptom of CCD, and does not mean that CCD was the actual cause of death.

This year, for the first time, backyard beekeepers reported "*Varroa*" as one of the leading causes of colony loss. This may indicate that outreach efforts aimed at promoting *Varroa* control are penetrating the backyard beekeeper community. Ideally, increased awareness of *Varroa* issues will increase the adoption of year-round *Varroa* monitoring and management plans.

The "National strategy to promote the health of honey bees and other pollinators" released by the White House [36] called for national honey bee winter loss of under 15% within ten years. Using the assumptions outlined above: Self-

reported Cause of Loss, the removal of the three most commonly identified direct risk factors (“Queen failure,” “*Varroa*,” and “Pesticides”) reduces this year’s total winter loss of 26.9–2.4%. This emphasizes the impact of these risk factors on national winter colony losses, as well as the need for further research into quantifying the impact of various risk factors associated with colony mortality and development of strategies to mitigate these risks.

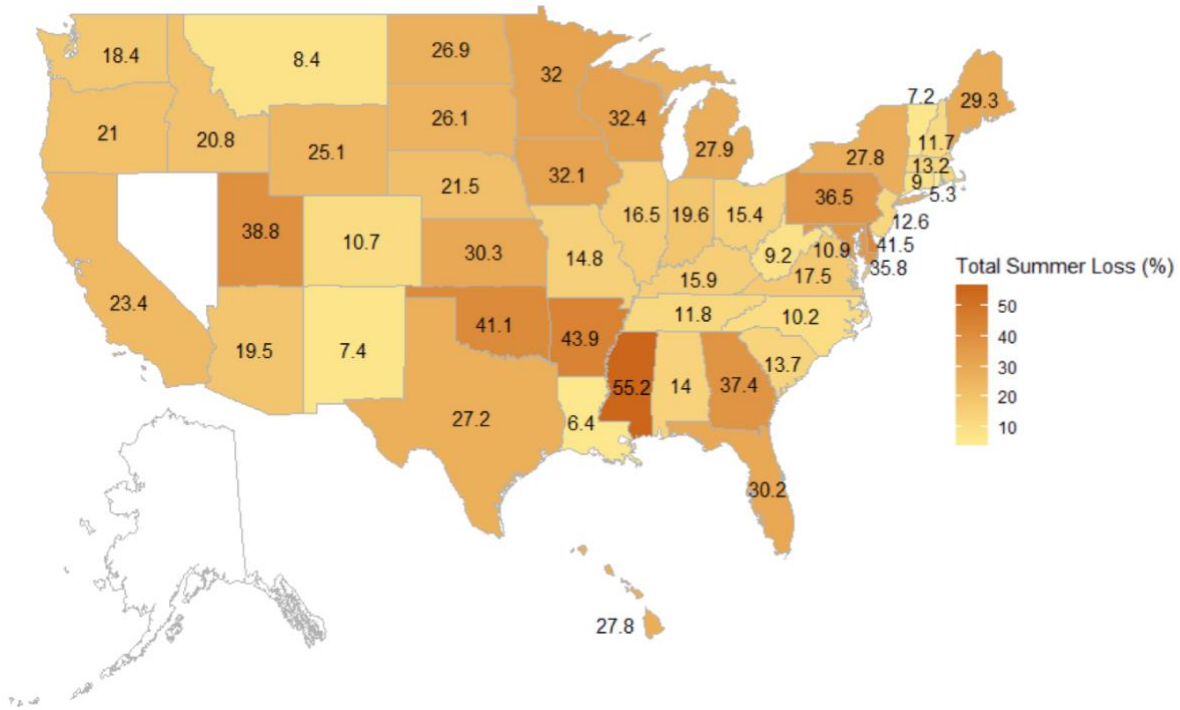
While both the BIP and NASS survey share the goal of estimating loss rates in managed honey bee colonies, both surveys differ somewhat in their approach and reporting. NASS’s survey differed from ours in questions asked, delivery of surveys, data presentation, and methodology of loss calculations. NASS divides, collects and reports loss numbers and rates in quarterly time periods only (1 April–30 June, 1 July–30 September, 1 October–31 December, 1 January–31 March) as opposed to BIP’s reporting of summer, winter, and annual loss numbers and rates. NASS also calculates loss by directly asking the beekeeper how many colonies died in each quarter, while BIP indirectly calculates the number of colonies lost by calculating the difference in expected and actual colonies reported alive at the end of a specific period. This means that NASS’s approach would not include the death of colonies that resulted from splits made within a survey period, while BIP methods would account for such colonies. Despite this difference, NASS loss numbers, once transformed to BIP seasons (e.g., summer and winter), are strikingly similar (Table 1.5). In fact, summer losses reported by NASS fall within the 95% CI of BIP summer losses, while winter losses reported by NASS are just above the upper bound of the BIP

total winter loss 95% CI (Table 1.2). The advantage of having two different survey methods conducted on this large scale to generate the same estimates lies in the ability to compare results. Regardless of stark differences in methods, both survey results were comparable. The two surveys serve to validate the assumption that different methods can be used to generate valid, representative estimates of colony loss.

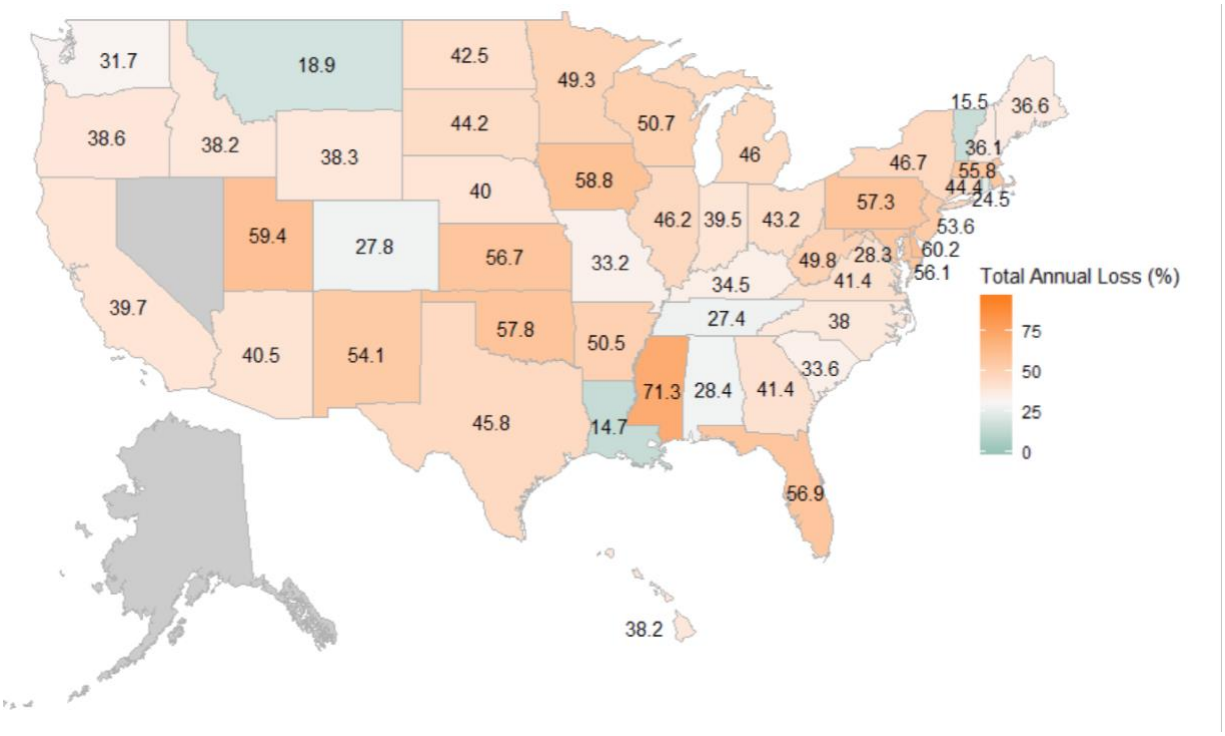
This survey further contributes to the long-term monitoring of trends in honey bee colony losses in the US. It demonstrates the importance of tracking both winter and summer losses, as summer losses have rivaled winter losses for the last three years. Although losses recorded in this survey are only slightly higher than previous loss averages, these losses still remain higher than those which beekeepers consider acceptable, even as this level of acceptable losses self-reported by beekeepers continues to climb.

Apparent growing awareness of the role of *Varroa* in colony losses, especially among backyard beekeepers, is encouraging, as these losses are probably responsible for the plurality of colony loss in the US. Continued colony loss surveys and monitoring are essential for documenting both negative and positive changes in the US beekeeping industry.

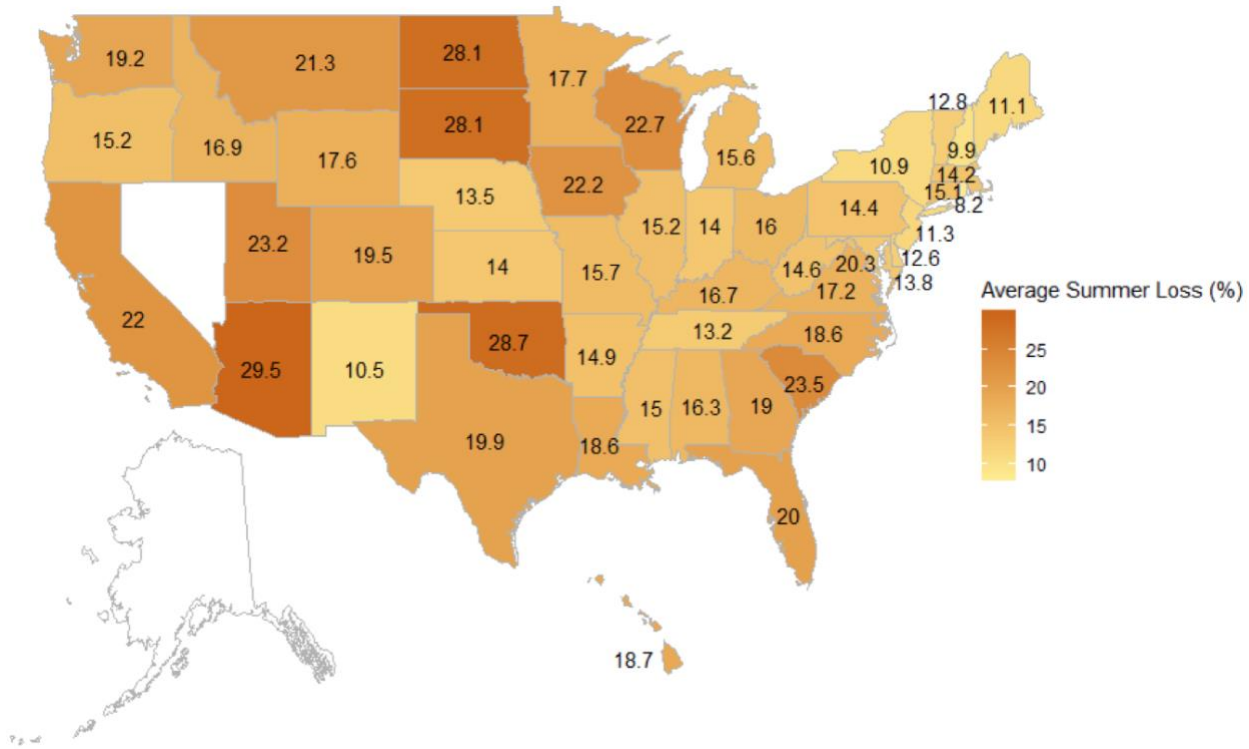
Supplemental Figures:



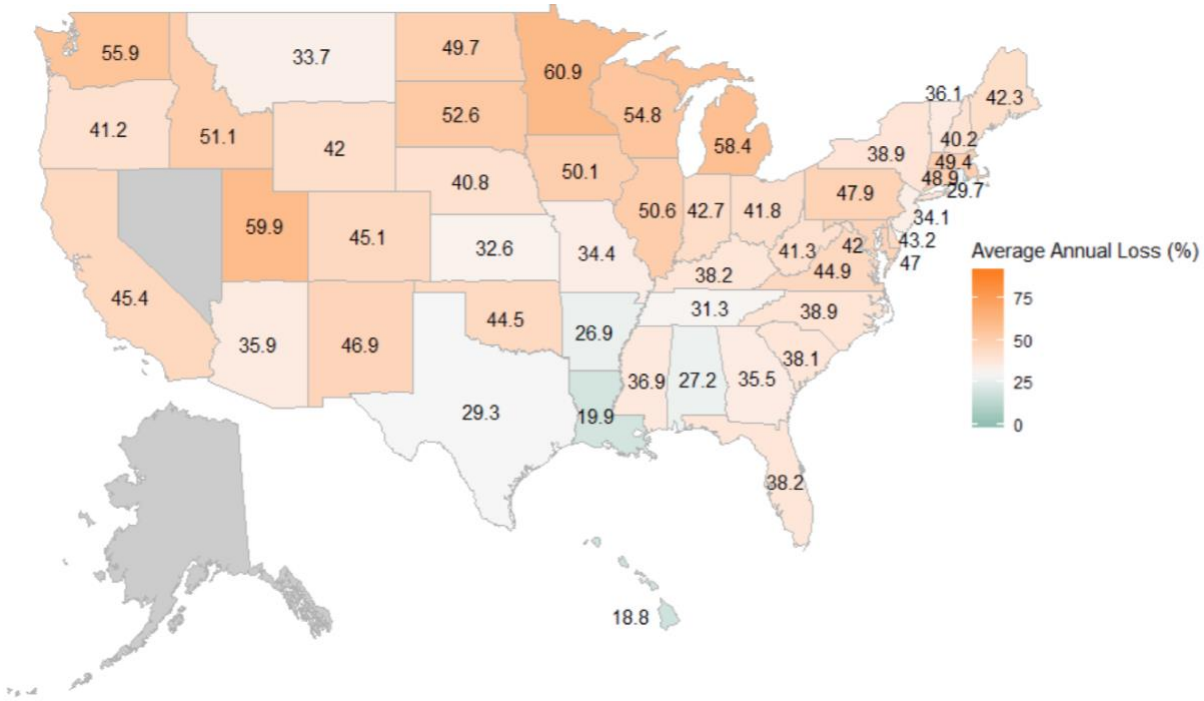
Supplemental Figure 1.1a. Map of total summer losses (%) reported for each state.



Supplemental Figure 1.1b. Map of total annual losses (%) reported for each state.



Supplemental Figure 1.2a. Map of average summer losses (%) reported for each state.



Supplemental Figure 1.2b. Map of average annual losses (%) reported for each state.

Supplemental Table 1.1. Estimates of total and average summer, winter and annual colony loss by US states, showing the number of operations (or number of valid respondents), number of colonies at the start of the period of interest, total colony loss (%), and average colony loss (%), by state of operation, for each season (summer, winter and annual). Each loss estimate (%) is presented along with its 95% CI. Data for states with fewer than five respondents are withheld. Total Loss was calculated by dividing the sum of colonies lost by the sum of colonies at risk of all participants combined. Colonies Lost: the sum of colonies at risk minus the sum of the number of colonies managed on April 2016. Colonies at risk: the sum of the total number of colonies managed on October 2015 and colonies bought or made between October 2016 and April 2016 subtracting the total number of colonies sold between October 2015 and April 2016. Average Loss was calculated as the mean of all individual winter loss (a mean of proportions.

	Summer Loss				Winter Loss				Annual Loss			
	n(# of operations)	Total # of colonies lost	Total Loss mean [95% CI]	Average Loss [95% CI]	n(# of operations)	Total # of colonies lost	Total Loss mean [95% CI]	Average Loss [95% CI]	n(# of operations)	Total # of colonies lost	Total Loss [95% CI]	Average Loss [95% CI]
US	5243	493205	23.6 [23.0-24.1%]	16.5 [15.8-17.2%]	6118	573127	26.9 [26.4-27.4%]	37.7 [36.8-38.7%]	4976	986553	40.5 [39.9-41.1%]	44.2 [43.2-45.2%]
STATE:												
Alabama	46	73	14.04 [8.65-20.92]	16.31 [9.01-23.6]	47	101	20.12 [13.4-28.19]	18.27 [10.66-25.88]	43	153	28.44 [20.97-36.8]	27.2 [18.97-35.43]
Alaska	2	-	-	-	5	18	72 [42.95-91.94]	79.09 [53.96-104.23]	2	-	-	-
Arizona	7	8	19.51 [2.79-52.19]	29.52 [-0.63-59.68]	8	13	29.55 [10.56-55.35]	17.78 [-3.95-39.51]	6	15	40.54 [16.52-68.27]	35.93 [6.85-65]
Arkansas	40	880	43.93 [39.09-48.86]	14.89 [7.31-22.47]	43	201	15.34 [11.83-19.36]	22.72 [14.31-31.14]	36	1020	50.5 [45.77-55.21]	26.91 [17.54-36.27]
California	202	97023	23.37 [20.92-25.94]	21.97 [18.51-25.43]	204	113586	26.47 [24.13-28.9]	36.27 [31.82-40.72]	182	192402	39.66 [36.75-42.62]	45.43 [41.02-49.85]

Colorado	138	363	10.69 [8.97-12.58]	19.48 [14.77-24.2]	185	679	20.73 [18.26-23.35]	39.61 [34.18-45.04]	130	944	27.8 [24.99-30.73]	45.06 [38.92-51.2]
Connecticut	69	77	9.01 [5.9-12.92]	15.06 [8.93-21.19]	75	320	39.46 [34.19-44.89]	42.77 [34.57-50.97]	66	384	44.39 [38.67-50.22]	48.92 [40.5-57.34]
District of Columbia	7	14	10.94 [5.24-19.22]	20.28 [-6.45-47]	8	26	22.22 [11.71-35.89]	52.78 [23.49-82.07]	6	36	28.35 [19.42-38.59]	41.96 [13.99-69.93]
Delaware	23	7559	41.54 [38.72-44.4]	12.6 [3.32-21.87]	24	7916	43.64 [41.43-45.87]	38.45 [24.38-52.51]	21	15458	60.21 [58.88-61.53]	43.18 [28.69-57.67]
Florida	66	14984	30.16 [26.9-33.56]	20.04 [14-26.07]	61	15796	41.48 [38.57-44.42]	26.99 [19.57-34.42]	58	29387	56.92 [54.38-59.44]	38.19 [30.36-46.02]
Georgia	73	855	37.35 [32.38-42.51]	19.03 [12.99-25.06]	71	434	19.38 [15.79-23.35]	25.31 [19.57-31.05]	65	1255	41.36 [37.5-45.3]	35.51 [29-42.03]
Hawaii	57	1412	27.81 [21.76-34.45]	18.72 [11.28-26.17]	51	160	2.4 [1.21-4.18]	11.17 [5.49-16.85]	45	1395	38.16 [34.24-42.18]	18.83 [11.76-25.9]
Idaho	46	25936	20.78 [16.24-25.86]	16.9 [11.25-22.55]	53	29557	25.18 [21.67-28.92]	43.17 [33.98-52.37]	45	54208	38.2 [33.14-43.43]	51.07 [42.19-59.94]
Illinois	129	214	16.49 [13.21-20.16]	15.19 [11.39-18.99]	155	465	37.32 [32.38-42.44]	44.99 [39.14-50.83]	127	609	46.21 [40.69-51.78]	50.61 [44.66-56.56]
Indiana	157	332	19.58 [16.3-23.15]	14 [10.28-17.71]	198	477	26.8 [22.79-31.08]	34.84 [29.68-40]	152	674	39.48 [34.7-44.4]	42.74 [36.92-48.56]
Iowa	49	405	32.12 [23.65-41.45]	22.21 [15.09-29.33]	55	351	38.28 [32.99-43.76]	34.68 [26.08-43.28]	48	739	58.84 [52.02-65.44]	50.06 [41.59-58.54]
Kansas	34	1753	30.32 [26.37-34.48]	13.97 [7.06-20.88]	36	3219	45.94 [43-48.89]	27.48 [17.96-37.01]	32	4953	56.69 [53.03-60.3]	32.61 [22.18-43.05]
Kentucky	75	132	15.9 [11.38-21.25]	16.68 [11.19-22.16]	81	211	26.95 [21.77-32.59]	33.17 [25.86-40.49]	71	286	34.5 [28.93-40.36]	38.23 [31.2-45.26]

			6.42	18.64			13.96	19.57			14.68	19.92
			[2.99-	[6.79-			[7.43-	[8.23-			[9.15-	[8.34-
Louisiana	24	43	11.6]	30.49]	24	99	22.86]	30.91]	21	102	21.67]	31.49]
			29.31	11.1			18.16	37.55			36.64	42.26
			[26.83-	[7.26-			[14.42-	[30.24-			[31.97-	[34.34-
Maine	82	12376	31.88]	14.93]	112	7713	22.35]	44.87]	81	20044	41.48]	50.19]
			35.8	13.79			39.99	40.29			56.13	47.03
			[33.49-	[10.01-			[37.68-	[34.51-			[53.66-	[41.07-
Maryland	131	8233	38.16]	17.58]	154	9026	42.34]	46.07]	128	17184	58.57]	52.98]
			13.18	14.21			48.62	43.69			55.75	49.4
			[10.76-	[10.08-			[44.05-	[37.47-			[50.89-	[42.75-
Massachusetts	114	169	15.88]	18.33]	153	633	53.2]	49.9]	113	727	60.54]	56.05]
			27.92	15.62			28.65	51.83			46.04	58.42
			[25.36-	[12.52-			[25.8-	[47.23-			[43.38-	[53.47-
Michigan	199	1647	30.59]	18.73]	256	1573	31.62]	56.44]	195	3026	48.72]	63.38]
			31.99	17.65			35.38	49.57			49.26	60.89
			[28.6-	[12.95-			[33.24-	[43.43-			[46.31-	[54.09-
Minnesota	116	13637	35.51]	22.36]	170	15843	37.55]	55.71]	109	26587	52.23]	67.69]
			55.16	14.99			60.1	29.81			71.32	36.93
			[50.65-	[2.78-			[49.61-	[16.72-			[62.18-	[20.77-
Mississippi	12	6286	59.63]	27.19]	15	9693	69.99]	42.89]	12	15975	79.42]	53.09]
			14.83	15.73			23.24	26.78			33.23	34.44
			[11.67-	[11.42-			[19.74-	[21.54-			[28.88-	[28.98-
Missouri	126	147	18.42]	20.04]	138	244	27]	32.03]	120	330	37.78]	39.9]
			8.38	21.34			12.06	25.7			18.86	33.66
			[4.46-	[7.29-			[8.04-	[11.5-			[12.28-	[17.31-
Montana	19	3842	13.89]	35.4]	18	5225	17.04]	39.91]	17	8855	26.89]	50.02]
			21.48	13.48			27.43	38			40	40.82
			[13.54-	[4.15-			[22.79-	[22.44-			[31.77-	[24.27-
Nebraska	16	7310	31.19]	22.8]	18	9543	32.41]	53.55]	16	16810	48.61]	57.36]
			26.68	25.99			19.04	39.03			40.08	42.27
			[25.04-	[6.2-			[14.95-	[17.78-			[37.89-	[16.82-
Nevada	5	808	28.38]	45.77]	9	431	23.62]	60.29]	5	1214	42.3]	67.72]
			11.68	9.86			29.5	35.92			36.07	40.19
New			[7.92-	[4.69-			[22.6-	[26.76-			[28.77-	[30.48-
Hampshire	49	34	16.32]	15.04]	64	95	37.1]	45.08]	48	110	43.82]	49.9]
			12.62	11.34			46.65	26.31			53.57	34.13
			[11.32-	[7.88-			[43.36-	[20.86-			[50.4-	[28.56-
New Jersey	114	1142	14.01]	14.8]	129	3841	49.95]	31.77]	112	4939	56.72]	39.71]

New Mexico	17	11	7.38 [1.65-19.09]	10.49 [-0.38-21.36]	19	74	37.37 [24.25-51.9]	36.45 [23.04-49.86]	17	80	54.05 [40.65-67.07]	46.91 [32.05-61.78]
New York	148	8916	27.85 [25.17-30.63]	10.95 [7.91-13.99]	189	10791	33.27 [30.52-36.11]	33.89 [28.8-38.99]	145	18683	46.73 [43.59-49.88]	38.86 [33.3-44.41]
North Carolina	267	1087	10.24 [9.04-11.53]	18.59 [15.32-21.86]	271	3036	31.33 [29.71-32.98]	31.3 [27.3-35.3]	246	4038	37.97 [36.29-39.67]	38.91 [34.94-42.89]
North Dakota	28	62781	26.89 [21.31-33.02]	28.12 [21.58-34.66]	31	61501	26.35 [20.51-32.81]	39.16 [28.84-49.47]	26	120660	42.46 [35.4-49.73]	49.65 [40.51-58.8]
Ohio	241	409	15.35 [13.03-17.89]	16.04 [13.09-18.99]	296	977	31.64 [28.57-34.82]	32.85 [28.73-36.98]	236	1207	43.15 [39.58-46.77]	41.84 [37.54-46.13]
Oklahoma	44	3006	41.14 [38.25-44.06]	28.67 [19.86-37.47]	46	1255	28.57 [22.75-34.91]	30.46 [19.78-41.14]	38	4222	57.78 [54.09-61.41]	44.49 [35.4-53.58]
Oregon	137	10055	20.96 [18.36-23.73]	15.22 [11.51-18.94]	159	7875	22.15 [19.74-24.69]	34.42 [28.91-39.93]	132	16465	38.6 [34.83-42.46]	41.21 [35.5-46.91]
Pennsylvania	619	8190	36.53 [35.35-37.72]	14.41 [12.49-16.33]	777	9415	41.26 [40.17-42.36]	43.55 [40.89-46.2]	599	17176	57.32 [56.21-58.44]	47.86 [45.02-50.69]
Puerto Rico	1	-	-	-	1	-	-	-	1	-	-	-
Rhode Island	19	5	5.32 [0.86-15.82]	8.25 [-0.78-17.27]	24	25	23.15 [13.67-34.9]	26.87 [12.64-41.09]	19	23	24.47 [12.78-39.46]	29.72 [12.62-46.83]
South Carolina	107	276	13.74 [10.45-17.54]	23.52 [17.91-29.14]	107	621	26.38 [23.77-29.11]	25.66 [19.99-31.33]	97	844	33.63 [30.67-36.67]	38.11 [32.31-43.91]
South Dakota	14	18106	26.13 [18.6-34.75]	28.06 [13.18-42.93]	16	34841	35.55 [28.75-42.76]	44.83 [27.54-62.12]	13	40944	44.21 [35.87-52.78]	52.63 [35.31-69.94]
Tennessee	104	145	11.82 [8.75-15.43]	13.23 [8.88-17.58]	113	384	24.85 [20.72-29.33]	25.06 [19.67-30.44]	98	342	27.43 [22.56-32.68]	31.32 [25.33-37.32]
Texas	103	40391	27.24	19.92	99	61197	35.06	18.15	95	86061	45.8	29.32

			[24.1-30.53]	[15.55-24.29]			[31.88-38.34]	[13.3-23]			[42.29-49.35]	[23.96-34.68]
			38.8	23.2			34.67	55.89			59.37	59.92
Utah	41	6144	[34.25-43.48]	[15.23-31.17]	46	3448	[31.26-38.2]	[45.76-66.02]	38	9424	[54.6-64.03]	[49.78-70.06]
			7.23	12.76			9.97	31.45			15.5	36.13
Vermont	51	173	[4.86-10.2]	[7.02-18.5]	58	224	[6.27-14.72]	[22.67-40.23]	48	368	[11.01-20.84]	[27.69-44.57]
			17.55	17.23			32.28	38.73			41.39	44.92
Virginia	595	710	[15.84-19.35]	[15.05-19.42]	692	1446	[30.12-34.5]	[35.92-41.54]	553	1723	[39-43.8]	[41.96-47.87]
			18.43	19.17			17.98	48.7			31.66	55.86
Washington	121	6486	[16.7-20.25]	[14.45-23.89]	136	5788	[16.21-19.86]	[42.72-54.67]	118	12214	[29.17-34.22]	[49.94-61.78]
			9.15	14.61			45.21	34.43			49.76	41.25
West Virginia	57	126	[6.59-12.22]	[9.43-19.79]	63	604	[38.32-52.23]	[26.26-42.61]	55	713	[43.12-56.39]	[33.93-48.58]
			32.38	22.7			35.68	47.09			50.75	54.83
Wisconsin	127	9358	[30.01-34.81]	[18.3-27.1]	165	10844	[33.83-37.56]	[41.5-52.69]	121	20006	[48.22-53.27]	[48.95-60.71]
			25.12	17.59			18.11	26.47			38.28	41.97
Wyoming	26	6503	[17.67-33.7]	[8.66-26.51]	28	3605	[12.37-24.99]	[15.74-37.21]	26	10107	[28.38-48.87]	[30.96-52.98]
			23.72	17.73			26.4	26.61			39.75	35.76
MultiStateOperation	149	102633	[21.01-26.59]	[14.75-20.72]	162	117687	[23.76-29.15]	[22.96-30.26]	143	201432	[36.48-43.09]	[31.81-39.71]

Chapter 2: Survey-derived best beekeeping management practices improve colony health and reduce mortality

Abstract:

Honey bee colony losses in the US have exceeded acceptable levels for at least a decade, leaving beekeepers in need of management practices to improve colony health and survival. Here, an empirical Best Management Practice (BMP) regime was tested, comprised of the top four management practices associated with reduced colony mortality in backyard beekeeping operations according to Bee Informed Partnership Loss and Management survey results. Seven study locations were established across the US, and each location consisted of ten colonies treated according to empirical BMPs and ten according to average beekeeping practice. After 3 years, colonies treated according to empirical BMPs experienced reduced *Varroa* infestation, viral infection, and mortality compared to colonies managed with average practices. In addition, BMP colonies produced more honey and splits. The colonies under average practices were treated for *Varroa* only once per year, and thus spent more months above treatment threshold of 3.0 mites/100 bees. Increased time spent above treatment threshold was significantly correlated to both increased viral infection and colony mortality. This study demonstrates the cumulative effects of management and colony health stressors over months and years, especially the dire importance of regular *Varroa* monitoring and management.

Introduction:

Honey bees are the most economically important pollinators in the world, providing billions of dollars in pollination services [1, 3, 18]. However, beekeepers consistently lose more colonies each year than they deem acceptable [5, 27, 29, 30, 34, 50], and the need for pollination units has grown more rapidly than the supply of honey bee colonies [51]. Thus, beekeepers struggle to keep their operations viable and provide sufficient colonies for crop production.

Research has identified many factors contributing to taxing rates of colony mortality [8]. The parasitic mite, *Varroa destructor* causes direct damage via feeding wounds [52-54] and vectors a suite of viruses [13, 55]. Prolonged exposure to pesticides reduces a colony's ability to combat other stressors [10, 56]. Poor nutrition further impacts colony health, particularly as landscapes are converted to monocultures that provide no or poor resources [57]. While these factors may not kill colonies in isolation, in concert these stressors can interact to manifest colony death [8, 58]. Over the past decade, substantial research has focused on identifying these stressors and assessing their impacts. More recently, scientists have begun to investigate interactions between and among stressors to better understand colony experiences in real world settings [59-61].

After identifying risk factors, the logical next step in an epidemiological challenge is to develop preventative strategies. Beekeepers have an opportunity to mitigate the effects of colony health stressors through the application of good beekeeping management practices. For example, beekeepers provide colonies

with supplemental food when natural pollen and nectar sources are scarce [5]. Additionally, interrupting *Varroa* population growth with various control measures is often required to reduce colony mortality [15]. For colonies and apiaries, it can be challenging to determine the effectiveness of management practices because of multiple interacting health stressors [8]. Science-based management recommendations can help beekeepers avoid using trial and error to reduce colony mortality.

Multiple groups have conducted surveys on colony losses and beekeeping management around the world (Germany: [37]; Canada: [62, 63] Europe: [39, 64]). The Bee Informed Partnership (or BIP; beeinformed.org) has conducted an annual Loss and Management Survey of US beekeepers since 2010. The survey consists of over 80 questions about the number of colonies lost and management practices employed by an operation over the previous year. Survey methods and results are published annually [5]. In total, the survey has collected over 50,000 responses, and has built the largest database of colony loss and management information in the world. These data can be analyzed to assess the effectiveness of management practices as they relate to reduced colony mortality.

One practice consistently associated with reduced mortality is *Varroa* control. Beekeepers who control *Varroa* consistently lose fewer colonies annually [15, 65]. Despite clear evidence of their benefits, only 48% of backyard beekeepers (beekeepers with 1-50 colonies) have reported using *Varroa* treatments over the duration of the BIP survey. While more backyard beekeepers report treating for *Varroa* every year (up to 78% of backyard beekeepers in

2018), there are many “treatment-free” beekeepers who do not employ effective mite-control strategies [66, 67]. Notwithstanding, backyard beekeepers who employ control measures typically only do so once per year [15], which is likely insufficient to reduce *Varroa* populations below economic thresholds. Backyard beekeepers experience the highest levels of colony loss each year [5], and improved *Varroa* control likely can reduce this mortality rate.

A full analysis of observational survey data was conducted to identify management practices that, if adopted, were predicted to have the largest reduction in colony loss rate. The top five of these empirical best management practices (BMPs; [17]), were developed for four different beekeeper demographics (southern backyard, northern backyard, stationary professional, and migratory professional). Four of the top five empirical BMPs were the same for northern and southern backyard beekeepers. However, before recommending these four practices to beekeepers, they needed to be field-tested to assess their effects on colony health and mortality. To this end, a 3-year study was conducted to assess the effectiveness of these four BMPs. It was hypothesized that apiaries treated according to the four empirical BMPs would reach larger colony sizes, and exhibit better brood patterns and fewer queen events. BMP apiaries were also hypothesized to experience lower *Varroa*, *Nosema*, and pathogen loads, reduced mortality, and produce more honey and splits.

Methods:

Apiaries:

This experiment was conducted at 7 locations in five states across the USA. Each state represented a different climatic region as designated by NOAA [68], and was chosen to test the effectiveness of empirical BMPs in different climates (Figure 2.1). Each location divided 20 colonies into two groups of ten colonies each. One group was treated according to average beekeeping practices, and the other was treated according to empirical BMPs as outlined in Steinhauer et al., 2017. The two groups were separated by 10-50 meters to minimize drift of bees between management groups at each location. Microclimates of the colony groups (*e.g.*, hours of shade, direction of colony entrance) were kept as similar as possible. Apiaries were established in spring 2016 and maintained until spring 2019. Each colony was established from packages on new plastic foundation to minimize initial differences in colony strength. After two years of the study, the BMPs were deemed unsuccessful in Minnesota, and those colonies were not included in 2018.

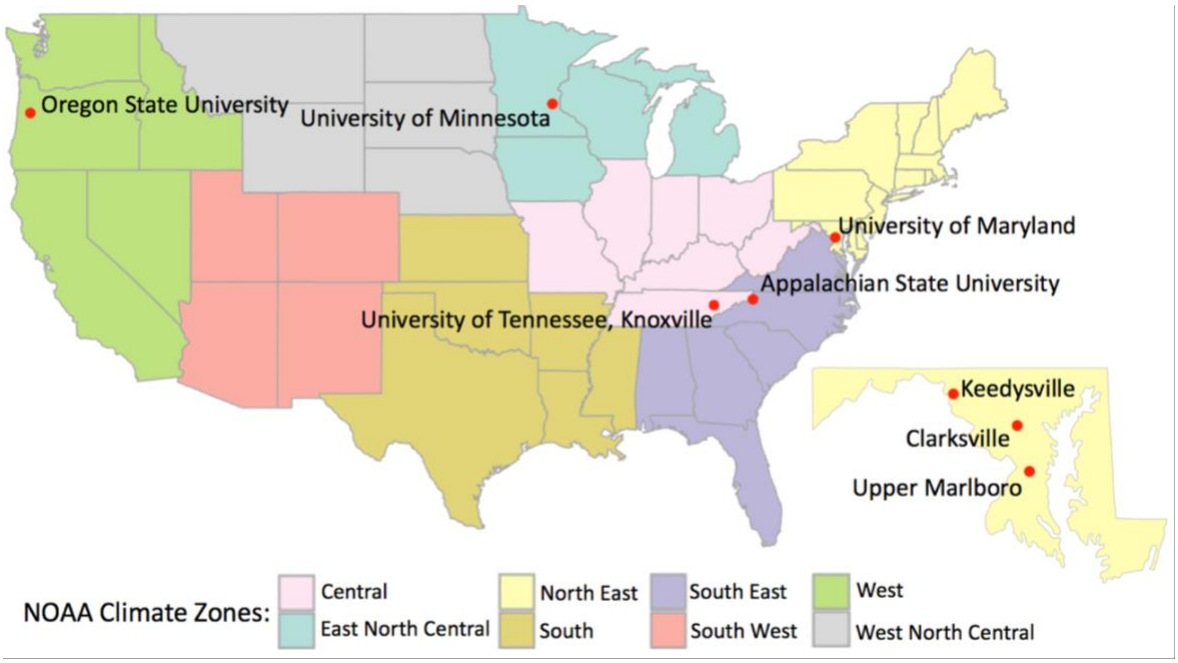


Figure 2.1. Map of apiary locations and corresponding NOAA climate zones.

Management Practices:

This experiment compared two different management regimes (average vs. BMP, Table 2.1) with four categories of management practices: action on deadouts, *Varroa* treatment, starting new colonies, and comb-culling technique. The BMP regime was derived from a combination of expert recommendations and survey results in Steinhauer et al., 2017. Beekeeper's survey responses were scored on how well they aligned with expert recommendations. Beekeeper's with higher scores (more aligned with expert recommendations) experienced significantly reduced winter colony loss, indicating that the expert's opinions were correct. Bootstrapped sensitivity analyses were performed to identify which management practices had the greatest effect on colony loss. The

BMP regime in this study corresponds to the expert recommendation for the top four practices that most affected colony loss. The “average practice” regime was derived from BIP Loss and Management Survey data as the most common practice employed by backyard beekeepers in the same four categories.

At each location, ten colonies were treated with the average practice of the top four management categories, and the other ten treated with the BMP. All other apiary management (feeding, requeening, honey harvest) was done on an as-needed basis according to standard beekeeping practices, which was kept consistent between the two groups. The only differences in management between groups were in the four categories of practices being tested, performed as follows.

Action on deadouts refers to how beekeepers respond to dead colonies discovered during the active season. The average practice is to remove that equipment from the apiary and store it for later use, typically the following spring when a new colony is established. The empirical BMP is to reuse that equipment immediately, either by making a new colony (split) using the equipment or by adding the boxes to another colony that needs more space. In reality, this BMP is difficult to enact because of the seasonality of discovering dead colonies (typically late fall), which does not correspond with the seasonality of needing equipment for new or expanding colonies (early summer). Deadout equipment was reused immediately when possible, and if not possible, combs were frozen, stored, and frozen again before reuse the following spring.

Varroa treatment refers to the frequency with which *Varroa* populations are controlled. The average practice is to treat the colony once per year in the Fall (typically in August or September). The BMP is to monitor *Varroa* on a monthly basis and treat whenever a single colony in the apiary exceeds 3.0 mites/100 bees. This practice was followed strictly throughout the study for the BMP colonies at each location. The choice of specific mite control product applied was left to the discretion of researchers in each state, as Varroacides have specific temperature and brood requirements and honey contamination risks. Once a colony exceeded the threshold of 3.0 mites/100 bees, treatments were applied to all colonies within that apiary.

Starting new colonies refers to the manner by which a new colony is formed at the beginning of the beekeeping season. The average hobbyist beekeeper starts new colonies by purchasing packages. The empirical BMP is to start new colonies by making splits from successfully overwintered colonies. If insufficient colonies are available to split, then purchasing nucleus colonies is the next best option. In the spring of 2016, all colonies were started from packages on new plastic foundation to equalize the starting point of both management groups. After initial installation, if a colony died over the year, it was not replaced until the following spring. In 2017 and 2018, new colonies installed in the spring came from packages in average apiaries and splits in best apiaries. Apiaries were always replenished to a size of 10 colonies each. If an insufficient number of BMP colonies survived the winter to make splits to reach 10 colonies, local nucleus colonies were purchased.

Finally, comb culling refers to how old brood comb is managed before it is reused in a new colony. Beekeepers often have a stock of old brood combs, typically from colonies that died previously or shrunk in population, so a secondary brood box could be removed. These combs are later reused by the beekeeper, either by adding to a growing colony that needs an additional brood box or installing a new colony into it the following spring. Beekeepers sometimes treat this old comb to kill persistent *Nosema* spores, small hive beetle, or wax moth adults or larvae by using chemicals (*e.g.* paradichlorobenzene crystals (moth crystals) or acetic acid), irradiation, or freezing. The average hobbyist beekeeper will not treat this brood comb before reusing it in a new colony. However, the empirical BMP is to freeze this comb at -20 °C for a minimum of 24 hours prior to adding it to a new colony. In this study, all brood combs added to best apiaries were frozen prior to use, while combs used in average apiaries were stored at room temperature. If a dead colony was discovered in a best apiary and the equipment could not be immediately reused, the combs were frozen immediately and then again before being added to a new colony.

Table 2.1. Average practices vs. BMPs to be tested in the field.

	Average Practice	*BMP*
Action on deadouts	Store equipment for later use	Reuse equipment immediately by adding to living colonies or using for a split
Varroa treatment	Treat once in fall	Monitor monthly and treat when above 3.0 mites/100 bees

Starting new colonies	Packages	Make splits when possible and buy nucs if splits impossible
Comb culling technique	Don't treat old brood comb before reuse	Freeze old brood comb before reuse

Sampling:

All colonies in this study were monitored from spring 2016 through spring 2019. Each year, colonies were inspected and sampled once per month for six months from spring to fall. The actual months when colonies were sampled varied somewhat based on weather and climate in each region. For example, in 2016 Minnesota colonies were sampled from April to September, and North Carolina colonies were sampled from June to November. In all analyses, only data from May to October were used to keep comparisons equal between groups.

Each inspection included a colony strength assessment and record of the typical metrics of frames of bees, queen status, and brood pattern [69]. Frames of bees were evaluated according to standard methods. One deep frame completely covered in adult bees on both sides was counted as one frame of bees. Mediums frames, if used, were counted as 2/3 of a full deep frame. Brood pattern was evaluated on a scale of 1-5, a 5 being a frame of contiguously capped brood. Brood pattern is a standard colony health metric used by beekeepers, where better brood patterns are considered indicative of queen and brood health. Queen status was judged as one of six options: queen seen, queen-right (queen not seen but fresh eggs observed), virgin queen, drone layer, queen not seen (no queen or fresh eggs seen but seems otherwise queen right),

or queen-less (clearly no queen present). If a colony experienced a queen issue, attempts were made to rectify it (*e.g.* adding a new queen or frame of eggs) but occasionally queen issues resulted in colony mortality.

A sample of adult bees was also taken from each colony at each sampling event. Approximately 300 bees were taken from a frame with partially capped brood and placed into a saltwater bottle. Super-saturated saltwater (2.5 lbs salt per 1-gallon water) was used in lieu of alcohol to decrease the cost of shipping, and all samples were processed before any decay occurred. Each participating researcher mailed their samples to the bee diagnostics lab at University of Maryland, where samples were processed for *Varroa* (mites/100 bees) by shaking and *Nosema* (millions of spores/bee) by microscope [70, 71].

A separate sample was taken from each colony for testing of viruses three times per year (spring, mid-summer, and fall). The precise timing of these samples varied based on regional climate, and only two samples were taken in the first year (mid-season and fall) as colonies were not established well enough to support an extra sample in spring. Viral samples consisted of placing approximately 100 bees from a frame with partially capped brood into a 50 mL Eppendorf tube. The tubes were immediately placed on dry ice and kept at -80 °C until they could be shipped on dry ice to the North Carolina State University Queen & Disease Clinic for processing. Samples were tested for copy numbers of the following viruses: Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus A (DWVA), Deformed Wing Virus B (DWVB), Israeli Acute Paralysis Virus (IAPV),

Lake Sinai Virus (LSV), *Trypanosoma* spp., and *Nosema* spp. Reverse transcription quantitative PCR (RT-qPCR) was performed for detection of all pathogens following previously described methods [72, 73].

Honey production and the number of colonies available to split were recorded as metrics of colony productivity. Honey production was measured in total kg and kg/colony for all honey harvested. Some splits were made directly, but the potential for splits was much higher than the actual number made because of logistical constraints of the experimental design. In order to better quantify split potential, a metric for splittable colonies was developed. A splittable colony is any colony that survived winter and had >10 frames of bees in May of the following year.

Colony mortality was assessed for three time periods per year: summer (April 1st – October 31st), winter (November 1st – March 31st), and annual (April 1st – March 31st). Dead colonies included colonies with zero or very few adult bees remaining, or colonies that were perpetually queenless.

Analyses:

All statistical tests were performed in R (version 3.3.3). All graphs present average apiary data in orange and BMP apiary data in blue. All summary statistics are reported as means ± SE unless otherwise noted. Time-series data (*i.e.*, those collected at multiple sampling months for *Varroa*, *Nosema*, viruses, frames of bees, and brood pattern) were analyzed with mixed effects models to account for the pseudo-replication in the data. Sampling month, year, and location were included as random effects in all models. Binomial response

variables (*e.g.*, queen events, colony mortality, splits) were fitted to general binomial mixed effect models with sampling month, year, and location as random effects. When comparing variables at a single time point (*e.g.,* at the start of the experiment) regular linear models were used. ANOVAs were used to compare goodness of fit in a stepwise selection procedure to remove non-significant terms. A relative risk analysis was performed to assess the change in risk of annual colony mortality under a BMP regime using the following equation, and 95% confidence intervals were calculated based on approximation (R function "riskratio", package "fmsb"):

$$RR = \left(\frac{BMP_{dead}}{BMP_{dead} + BMP_{alive}}\right) / \left(\frac{Average_{dead}}{Average_{dead} + Average_{alive}}\right)$$

Virus data were analyzed by prevalence (% infected) and intensity (copy numbers). Prevalence was analyzed with binomial mixed effects models with season, year, and location as random effects. ANOVAs were used to eliminate non-significant fixed effects in a stepwise fashion. Viral copy data is not suited to typical linear modeling because it is highly skewed (contains a high proportion of zeros) and has large variance. Viral copy data was log-transformed to better follow a normal distribution, but the high proportion of zeros in the data still prevented typical linear modelling. Rows containing zeros were then removed for each virus, and log copy numbers were analyzed for significant differences with mixed effects models. Year and location were included as random effects. ANOVAs were used to compare linear models to null models to generate p-values for the effect of management group. Where significant differences in viral prevalence or copy number were detected, associations with other variables

including mortality, months exceeding 3.0 mites/100 bees, average yearly *Varroa* load were checked with separate mixed effects models.

Results:

Colony strength (frames of bees, brood pattern, and queen status):

Over the 3 years, 2,244 colony strength inspections were performed. Colony health metrics were similar between management groups. The 3-year mean frames of bees in best apiaries was 11.48 ± 0.19 and in average apiaries 11.23 ± 0.20. Both groups peaked in colony size in July and were smallest in October. Although frames of bees varied among years ($F_2 = 19.97$, $p < 0.01$), months ($F_5 = 2.97$, $p = 0.02$) and locations ($F_6 = 39.6$, $p < 0.001$), there was no difference between management groups (Figure 2.2, $F_1 = 0.64$, $p = 0.41$).

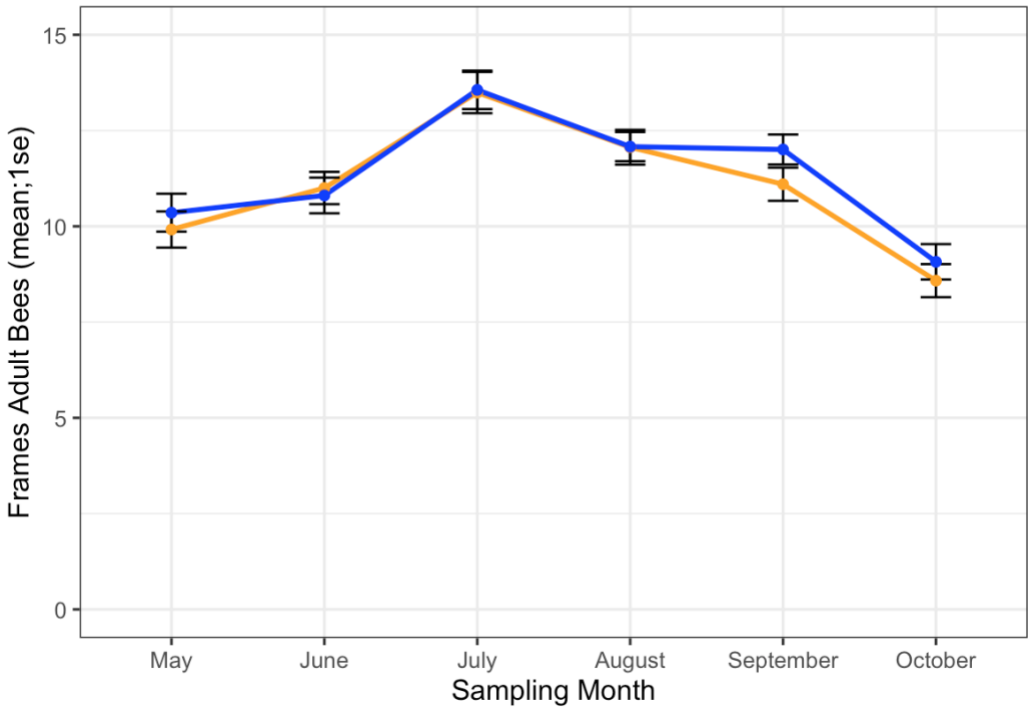


Figure 2.2. Mean frames of bee s+/- standard error for BMP (blue) and average (orange) apiaries over each sampling month. This graph represents all 3 years of data together

Brood pattern was also similar between management groups. The 3-year mean brood pattern rating in BMP colonies was 3.29 ± 0.03, and average colonies 3.26 ± 0.04. In both groups, brood pattern was lowest in fall when brood production slowed down and less capped brood was present. Brood pattern varied among years ($F_2 = 0.27$, $p = 0.02$), months ($F_5 = 10.15$, $p < 0.001$), and locations ($F_6 = 11.18$, $p < 0.001$), but not between management groups (Figure 2.3, $F_1 = 0.29$, $p = 0.51$).

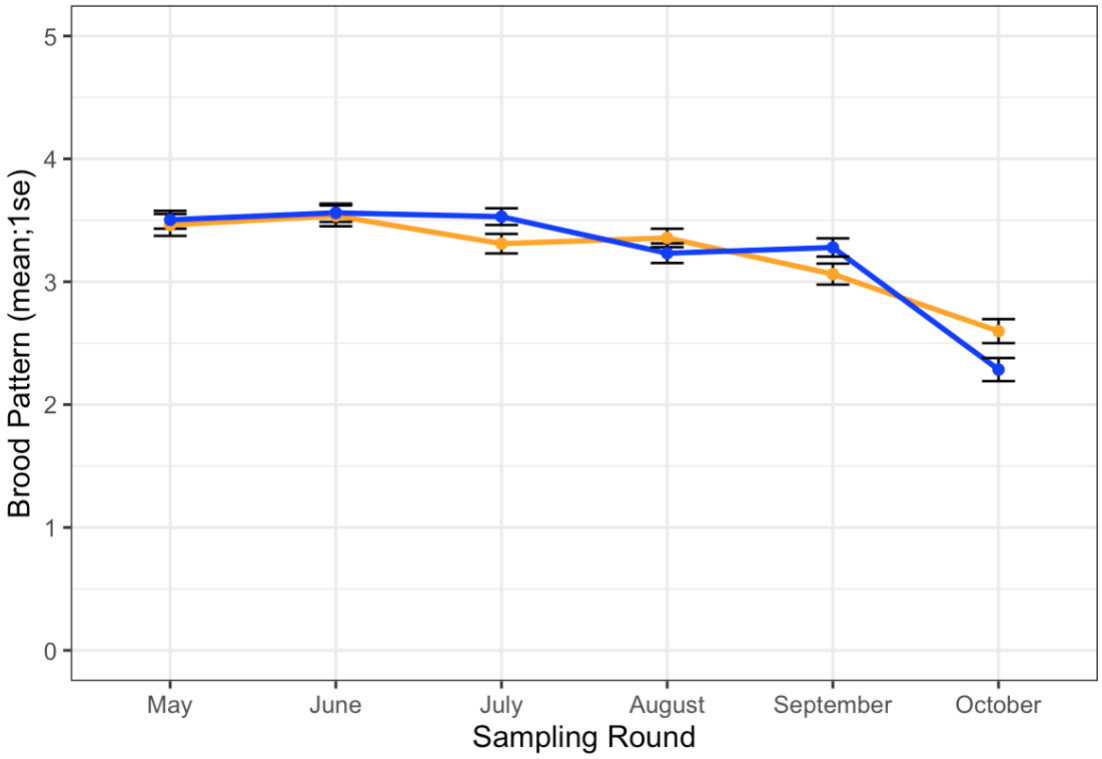


Figure 2.3. Mean brood pattern +/- standard error for BMP (blue) and average (orange) apiaries over each sampling month. This graph represents all 3 years of data together.

Queen status data were subdivided into two categories: colonies that experienced a “queen event” or no “queen event”. A colony was considered to have experienced a queen event if during colony inspection it was found to be

queenless, a drone layer, a virgin queen, or no queen or eggs were seen [74]. Colonies without queen events either had eggs present or the queen was seen. Over all 3 years, a total of 79 (39.7%) BMP colonies and a total of 83 (41.7%)

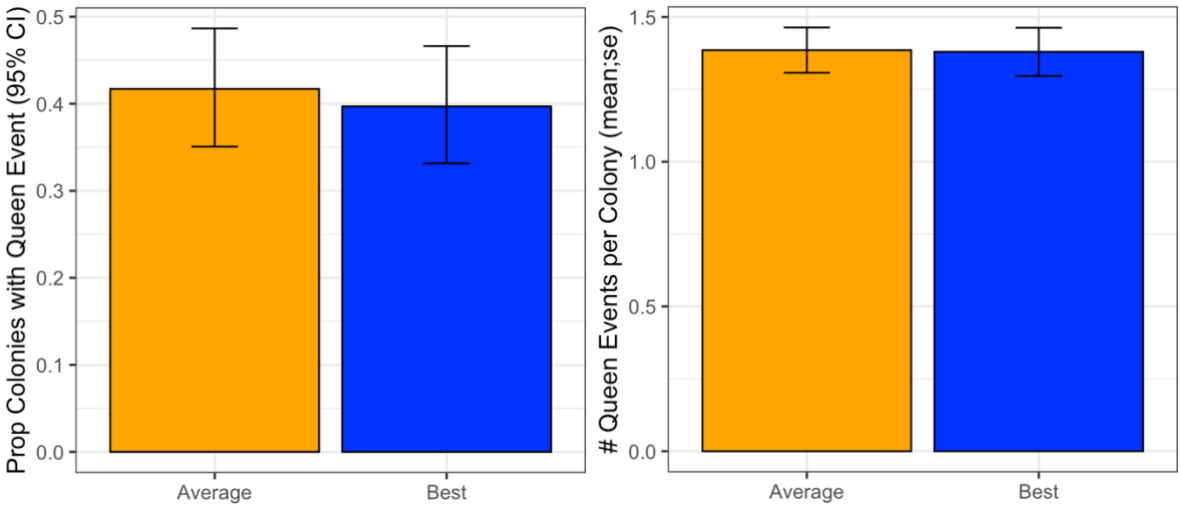


Figure 2.4. Proportion of colonies that had a queen event, and the average number of queen events colonies had once they became queenless +/- 95% CI in BMP (blue) and average (orange) apiaries.

average colonies had queen events. The number of queen events differed among years ($F_2 = 3.48$, $p = 0.05$), months ($F_5 = 2.70$, $p = 0.03$), and locations ($F_6 = 3.69$, $p < 0.01$), but not between management groups (Figure 2.4, $F_1 = 0.45$, $p = 0.43$). Some colonies were subject to repeated queen events, where a colony would become queenless and remain queenless for several subsequent colony inspections. There was no difference in the number of repeated queen events between management groups ($F_1 = 0.13$, $p = 0.71$).

Measures of Morbidity (Varroa, Nosema, and pathogens):

Varroa:

Across all 3 years, 2,244 *Varroa* samples were collected. The 3-year average *Varroa* load in BMP apiaries was 2.67 ± 0.14 and 3.62 ± 0.18 in average

apiaries. *Varroa* loads did not differ among years ($F_2 = 0.01$, $p = 0.98$). *Varroa* loads did differ among sampling months, and were lowest in May and highest in October ($F_5 = 9.25$, $p < 0.001$). *Varroa* loads differed between management groups ($F_1 = 10.85$, $p < 0.001$), and there was an interaction between sampling month and management group ($F_5 = 4.08$, $p < 0.01$). BMP apiaries exhibited lower *Varroa* loads than average apiaries across all sampling months ($F_{1,5} = 23.43$, $p < 0.001$), except in October when there was no difference in *Varroa* load detected between management groups ($F_1 = 0.90$, $p = 0.21$), indicating a convergence of *Varroa* infestation between groups after average colonies were treated for *Varroa* in the fall. *Varroa* also differed among locations ($F_6 = 8.6$, $p < 0.001$), but there was no interaction between location and management group ($F_6 = 0.20$, $p = 0.40$), with BMP apiaries exhibiting lower *Varroa* loads at each location.

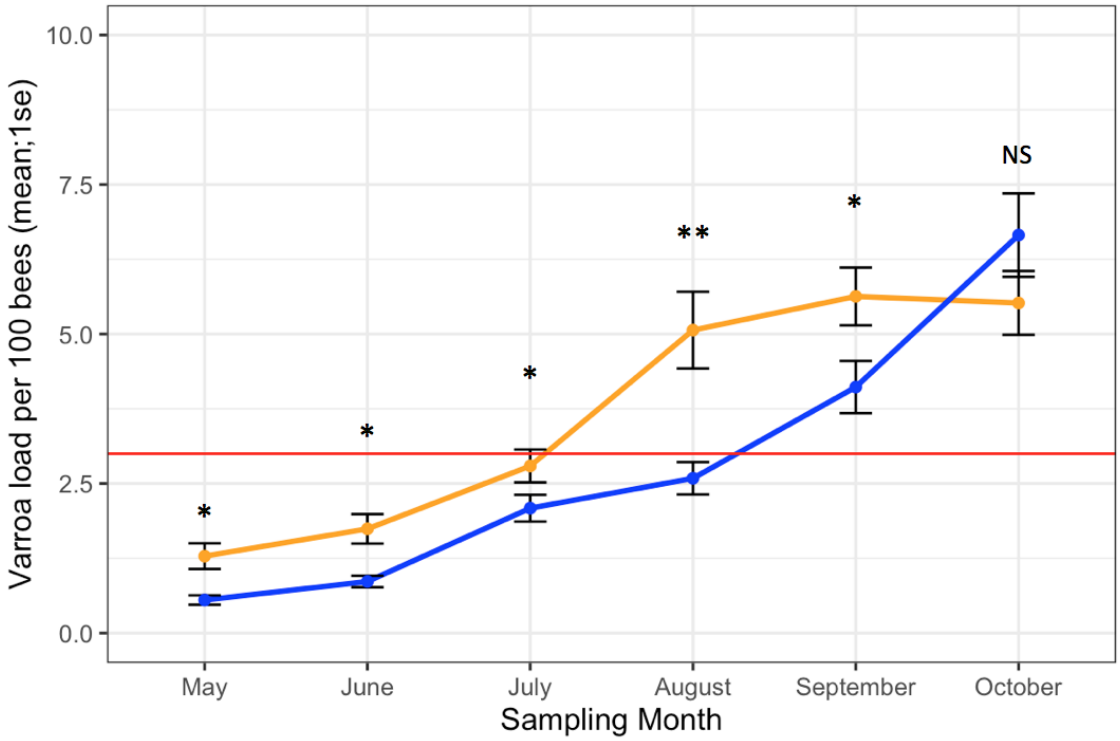


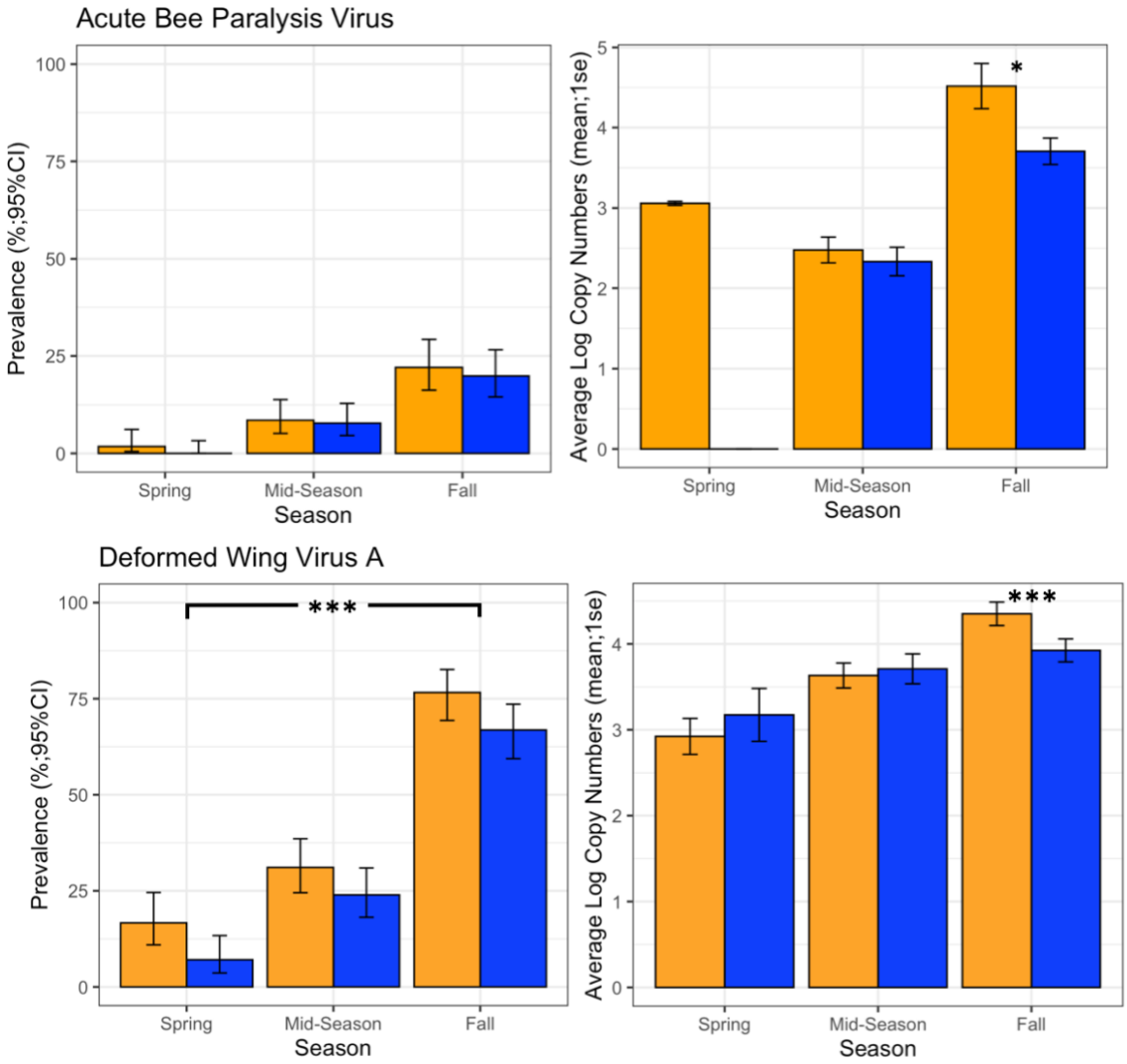
*Figure 2.5. Mean Varroa loads +/- standard error for BMP (blue) and average (orange) apiaries over each sampling month. This graph represents all 3 years of data together. The red line represents the treatment threshold of 3.0 mites/100 bees. *p < 0.05, **p < 0.01.*

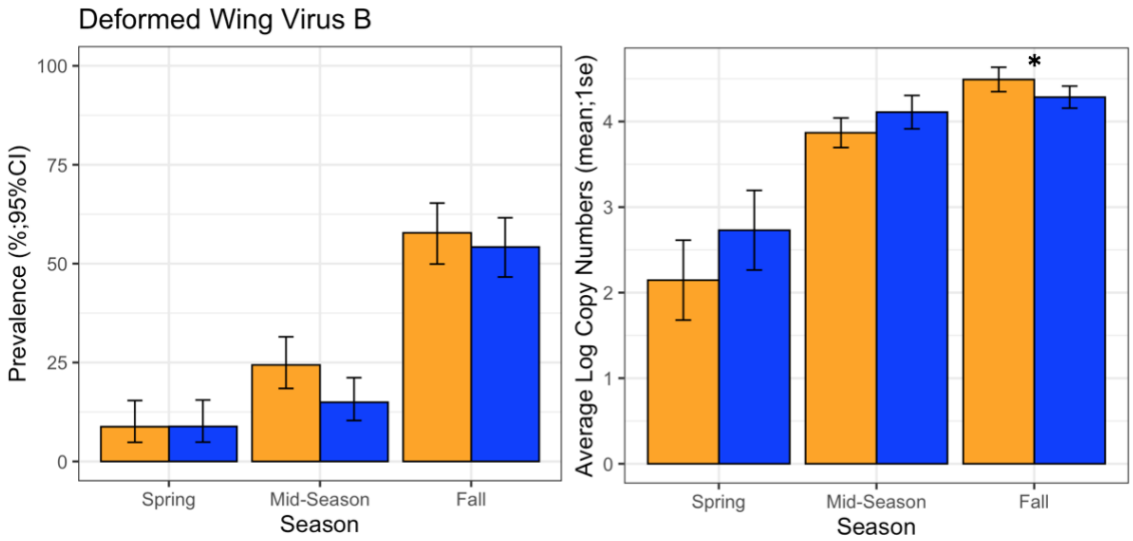
There was no difference in *Varroa* load between management groups at the start of the experiment ($F_1 = 2.46$, $p = 0.12$). In the second and third years, average apiaries started the season with higher *Varroa* loads than BMP apiaries in May (1.24 ± 0.02 mites/100 bees compared to 0.56 ± 0.07, respectively, $F_1 = 0.93$, $p = 0.001$). This inflated *Varroa* population persisted through each season, resulting in average apiaries exceeding 3.0 mites/100 bees one sampling month prior to best apiaries each year. Additionally, average apiaries spent more months above threshold (1.81 ± 0.09) compared to 1.34 ± 0.08 months in best apiaries (Figure 2.5, $F_1 = 21.62$, $p < 0.001$).

Pathogens:

A total of 878 samples were taken for pathogen analyses. Prevalence was similar between management groups, with only Deformed Wing Virus A (DWVA) being significantly lower in BMP apiaries over all seasons across all years (Figure 2.6, $F_1 = 3.38$, $p < 0.001$). Fall intensity was lower in BMP apiaries for Acute Bee Paralysis Virus (ABPV) ($F_1 = 6.87$, $p = 0.01$), DWVA ($F_1 = 12.89$, $p < 0.001$), and Deformed Wing Virus B (DWVB) ($F_1 = 4.30$, $p < 0.05$) (Figure 2.6). These metrics did not differ between best and average apiaries at the start of the experiment (Prevalence DWVA $F_1 = 1.06$, $p = 0.31$; Copy Numbers DWVA $F_1 = 2.18$, $p = 0.09$; DWVB $F_1 = 2.46$, $p = 0.12$; ABPV $F_1 = 0.03$, $p = 0.85$), confirming that these differences developed after management practices were employed. For the four viral metrics that significantly differed between BMP and average apiaries (prevalence of DWVA and the fall intensity of ABPV, DWVA, DWVB), separate mixed effects models were performed with year and location as random effects to determine if other variables were associated with increased viral pressure. A colony's average yearly mite load was associated to fall copy numbers of ABPV, DWVA, DWVB, as well as the prevalence of DWVA ($F_1 = 21.50$, $p < 0.001$; $F_1 = 18.94$, $p < 0.001$; $F_1 = 23.70$, $p < 0.001$; $F_1 = 25.24$, $p < 0.001$, respectively). Additionally, the number of months a colony spent above 3.0 mites/100 bees was also associated with these same viral metrics ($F_1 = 4.33$, $p = 0.04$; $F_1 = 10.68$, $p = 0.001$; $F_1 = 6.13$, $p = 0.01$; $F_1 = 25.26$, $p < 0.001$, respectively).

*Figure 2.6. Prevalence +/- 95% CI and Average Log Copy Numbers +/- standard error for the 3 viruses which differed between BMP (blue) and average (orange) apiaries. These graphs represent all 3 years of data together. *p < 0.05, ***p < 0.001*





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Nosema:

A total of 2,244 samples were taken for *Nosema*. The 3-year average *Nosema* load in BMP apiaries across all sampling months was 0.31 ± 0.04 million spores/bee and in average apiaries across all sampling months was 0.32 ± 0.04 million spores/bee. *Nosema* pressure in this experiment was generally low compared to other surveys [75], and averages never exceeded the recommended treatment threshold of 1.0 million spores/bee. Average *Nosema* load in both treatments followed typical *Nosema* seasonal patterns, with loads highest in spring, lowest in summer, and rising again in fall [75]. Mixed effects models showed differences among locations ($F_6 = 7.27$, $p < 0.001$) and years ($F_2 = 0.92$, $p = 0.05$), but not among sampling month ($F_5 = 1.02$, $p = 0.17$) or management groups (Figure 2.7, $F_1 = 0.03$, $p = 0.86$).

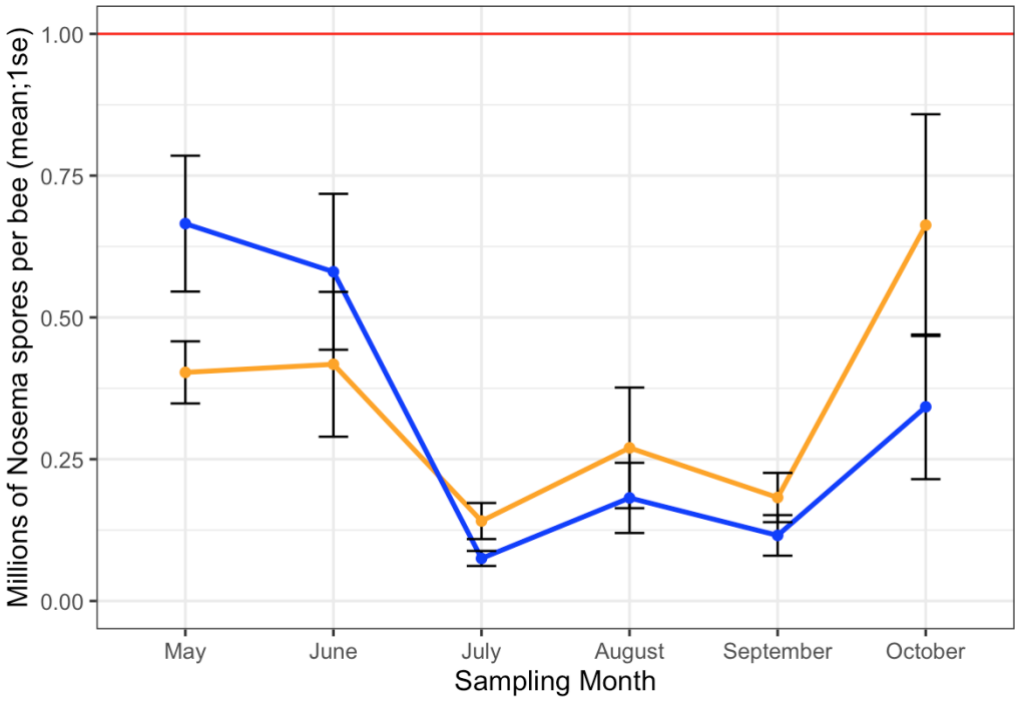


Figure 2.7. Mean Nosema loads +/- standard error for BMP (blue) and average (orange) apiaries over each sampling month. This graph represents all 3 years of data together. The red line represents the recommended treatment threshold of 1.0 million spores/ bee.

Colony Outcomes (mortality, honey production, and split production)

Mortality:

Total summer mortality for all years in BMP apiaries was 15.2% (95% CI 10.8-20.8%) and 20.6% (95% CI 15.6-26.6%) in average apiaries. Summer mortality was highest in both groups in 2016. Binomial mixed effects models found differences among years ($F_2 = 4.77$, $p = 0.02$) and locations ($F_6 = 4.42$, $p < 0.01$), but no effect of management group on summer loss ($F_1 = 1.35$, $p = 0.13$).

Total winter mortality for all years in BMP apiaries was 30.8% (95% CI 24.8-37.6%) and 45.2% (95% CI 38.5-52.2%) in average apiaries. Winter loss in average apiaries increased each year while in BMP apiaries winter loss

decreased each year. Binomial mixed effects models found differences among locations ($F_6 = 2.55$, $p = 0.03$) and management groups across all years ($F_1 = 3.70$, $p < 0.01$). There was no interaction between location and management group ($F_6 = 1.27$, $p = 0.09$), indicating that the effects of management were similar in all locations. A separate analysis of individual years found BMP apiaries lost significantly fewer colonies in 2018 ($F_1 = 7.04$, $p = 0.001$).

Total annual mortality for all years in BMP apiaries was 46.0% (95% CI 39.2-53.0%) and 65.8% (95% CI 59.9-72.1%) in average apiaries. Binomial mixed effects models found no differences among locations ($F_6 = 1.03$, $p = 0.39$), but did find an effect of management across all years ($F_1 = 15.78$, $p < 0.001$). A separate analysis of individual years found BMP apiaries lost significantly fewer colonies in 2018 (Figure 2.8, $F_1 = 10.94$, $p < 0.01$). A relative risk analysis of mortality showed that using this set of best management practices reduced the risk of colony mortality by 30% (RR = 0.70, 95% CI 0.58 - 0.84, $p < 0.001$).

Separate binomial mixed effects models with year as a random effect were used to check for regional differences in the effect of management on mortality. Considering separate regions is different than considering separate locations because Maryland represents one region but three locations. Regional analyses were only performed for winter and annual loss, as management had no effect on summer loss across all regions ($F_1 = 1.10$, $p = 0.21$). Region did not change the effect of management on winter ($F_4 = 1.86$, $p = 0.18$), or annual loss ($F_4 = 1.36$, $p = 0.24$). However, in Minnesota and Oregon, the number of colonies lost in BMP and average apiaries across all years was similar (Supplemental

Figure 2.2), suggesting these management practices may not be as effective in northern climates. Because these practices seemed ineffective in Minnesota after the first 2 years, they did not continue this experiment in 2018.

Associations between colony mortality and risk factors that differed between management groups were also assessed. A colony's average yearly mite load was associated with colony mortality ($F_1 = 15.32$, $p < 0.001$). Additionally, the number of months a colony was above 3.0 mites/100 bees was associated with mortality ($F_1 = 16.61$, $p < 0.001$). Finally, prevalence of DWVA was associated with mortality ($F_1 = 05.13$, $p = 0.02$).

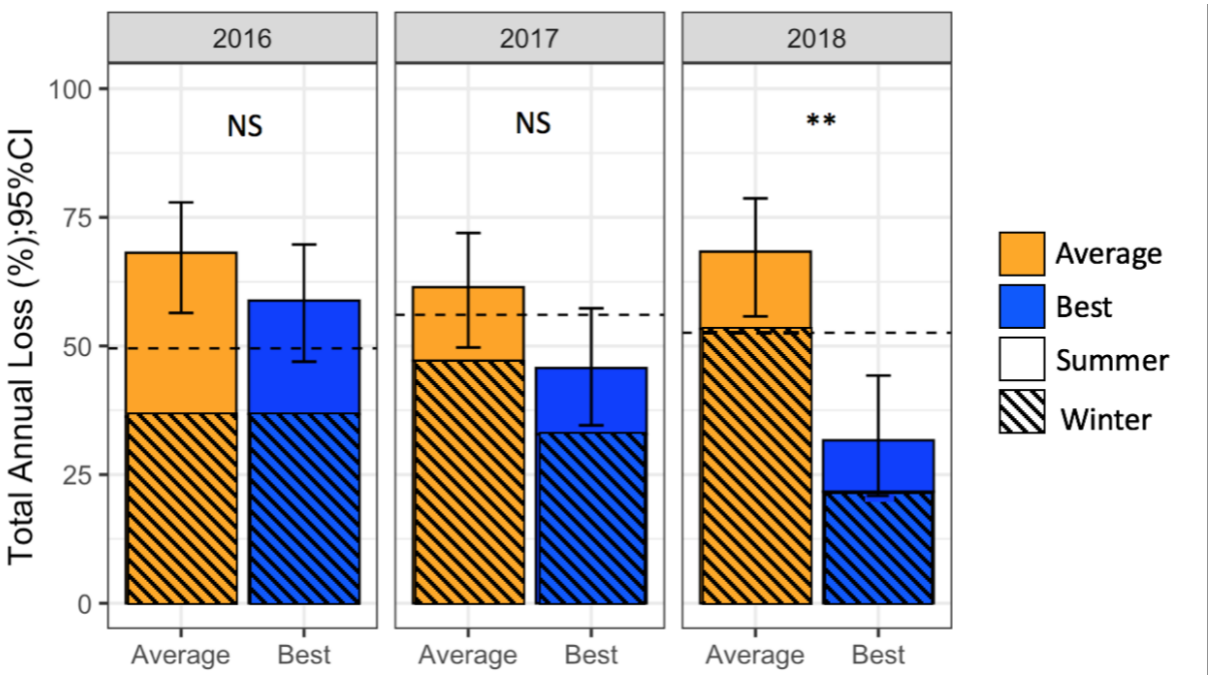


*Figure 2.8. Total annual loss +/- 95% CI in each BMP (blue) and average (orange) apiaries over the 3-year experiment. Summer loss is represented by solid colors, and winter loss by striped colors. Dashed horizontal lines represent the national total winter loss for backyard beekeepers each year. **p < 0.01.*

Honey Production:

In total, 3,699 kg of honey was harvested. Average apiaries produced a total of 1,541 kg, and BMP apiaries produced a total of 2,158 kg. No honey was harvested in 2016 as colonies had to invest significant energy in wax production in their first year (all colonies were started on bare foundation). The average honey produced per colony was 21.8 ± 4.6 kg and 27.2 ± 7.4 kg in average and BMP colonies, respectively. Linear mixed effects models showed no differences between management group in the total honey produced, (Figure 2.9, $F_1 = 1.96$, $p = 0.23$) mean honey produced per colony (Figure 2.10, $F_1 = 0.02$, $p = 0.85$) or the proportion of colonies harvested from (Figure 2.11, $F_1 = 1.00$, $p = 0.22$). However, BMP apiaries did produce 617 kg more honey than average apiaries. There was a small number of BMP colonies that produced far above average honey in 2018, making the total kg produced much higher, but not significantly affecting the average produced per colony.

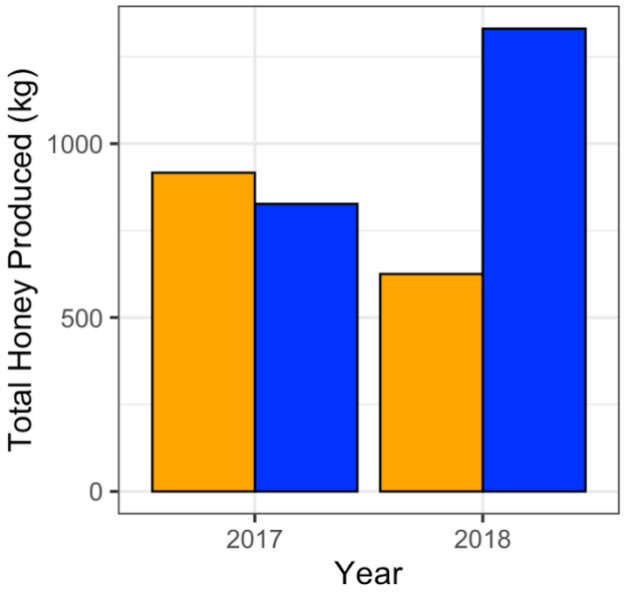


Figure 2.9. Total honey produced (kg) in BMP (blue) and average(orange) apiaries in 2017 and 2018.

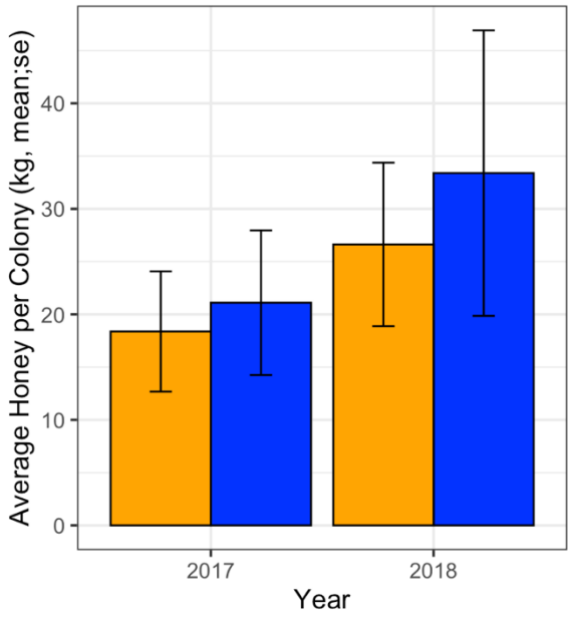


Figure 2.10. Average honey produced per colony +/- standard error in BMP (blue) and average (orange) apiaries in 2017 and 2018.

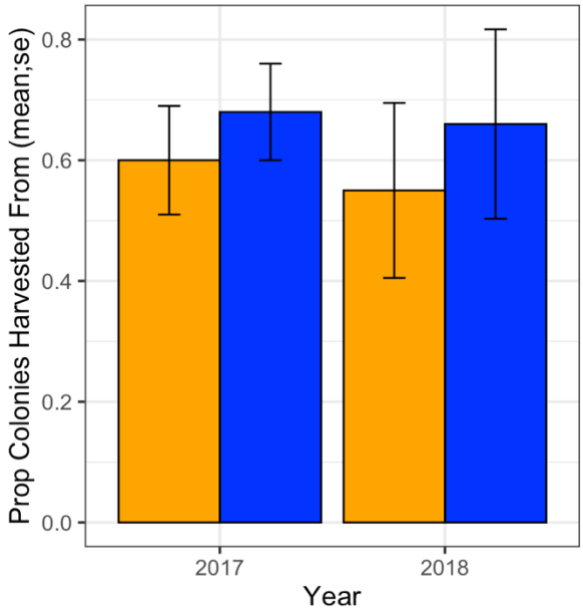


Figure 2.11. Proportion of colonies honey was harvested from +/- 95% CI in BMP (blue) and average (orange) apiaries in 2017 and 2018.

Split Production:

Across all 3 years, BMP apiaries produced 79 splittable colonies and average apiaries produced 46. A generalized binomial model found best apiaries produced more splittable colonies ($F_1 = 8.14$, $p < 0.01$). There was an effect of year ($F_2 = 6.61$, $p = 0.03$) and separate analyses conducted on each year showed that this trend increased over time. Best apiaries produce numerically more splits each year, finally producing significantly more in 2018 ($F_1 = 4.43$, $p = 0.048$).

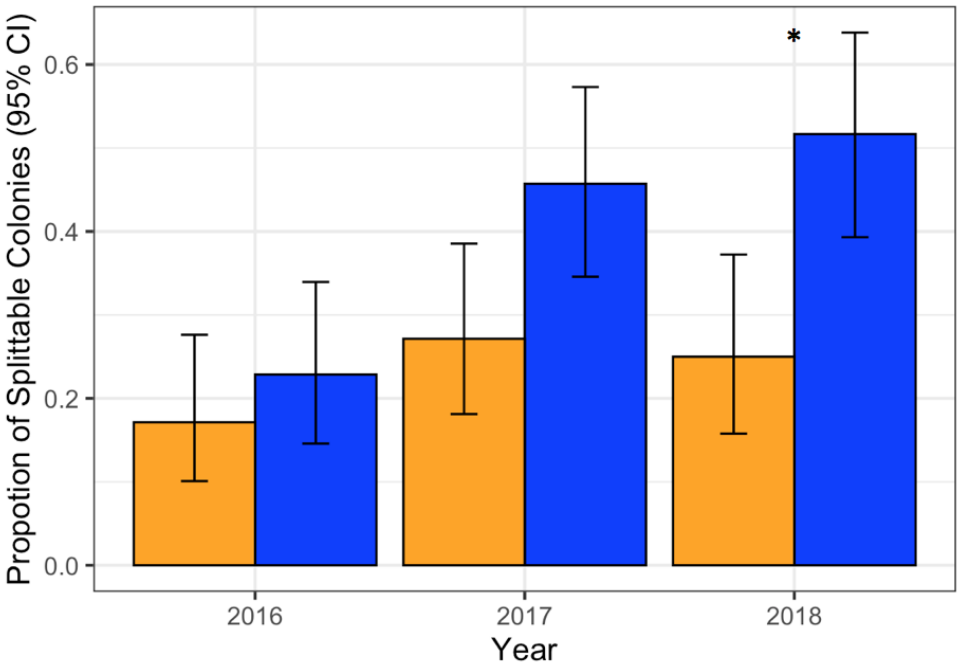


*Figure 2.12. Proportion +/- 95% CI of colonies that survived winter and were splittable the following spring in BMP (blue) and average (orange) apiaries. *p < 0.05.*

Discussion:

It was hypothesized that BMP apiaries would outperform average apiaries in colony strength metrics, productivity, and survival. There were no differences between BMP and average apiaries in colony size, brood pattern, queen status, or *Nosema* load. However, BMP apiaries did experience reduced *Varroa* loads, reaching the threshold of 3.0 mites/100 bees one month later than average apiaries and spending fewer months above threshold overall. BMP apiaries also exhibited reduced infection levels of ABPV, DWVA, and DWVB in the fall. BMP apiaries also produced more honey, and by the third study year, produced more splits and experienced lower mortality than average apiaries.

It was proposed that BMP colonies would reach larger population sizes and exhibit better queen health and productivity. BMP colonies were started from nucleus colonies or splits, which in theory should reach larger population sizes my mid-season because of greater establishment at installation. Thus, the similarity in colony size between management groups was unexpected but supports the idea that colony size is not representative of colony health or productivity, and that other colony health metrics such as *Varroa* load and/or viral load may be better predictors of colony survival [57, 76]. The frequency of queen events between management groups was almost identical, indicating that these management practices did not affect queen mortality. Brood pattern, thought to be an indicator of queen productivity, was also similar between management groups. The biggest predictor of brood pattern was season, as brood production dropped off sharply in all apiaries in October as colonies prepared for winter. It is surprising that average colonies did not exhibit diminished brood patterns as a result of their elevated *Varroa* and viral loads, as these stressors often result in brood not surviving to emergence [77, 78]. However, recent work indicates brood pattern may be a result of some unknown feature of a colony's environment as opposed to queen quality or *Varroa* or viral loads [72].

Regardless of the similarities in colony strength metrics, *Varroa* loads were significantly lower in BMP apiaries throughout the season. However, in October, mean *Varroa* population appeared to become similar between groups. One potential cause of this occurrence is horizontal transmission of mites among colonies. Horizontal transmission could have occurred if healthy colonies from

BMP apiaries were robbing out weaker colonies in nearby apiaries [79]. It is known that drifting of mites and bees across colonies increases in the fall, concurrent with an increase in *Varroa* population [80]. This phenomenon can also help explain why on occasion after treatments, BMP apiaries reached *Varroa* loads above the treatment threshold of 3.0 mites/100 bees the following month. Treatments may have been effective immediately after application, but the intense mite pressures within the adjacent landscape caused rapid re-infestations before the next sampling event. These re-infestations may have inflated *Varroa* measurements, so the fact that significant differences were observed in spite of this shows the strength of the effect of management. Further, this finding emphasizes the importance of monitoring for mites as often as possible, especially after treatments to ensure their effectiveness.

Despite comparable mean fall *Varroa* loads, BMP apiaries exhibited reduced winter mortality compared to average apiaries, and significantly so in the third year of the study. This is likely because if BMP apiaries were exceeding 3.0 mites/100 bees in October, they would receive a critical pre-winter treatment in November or December. These pre-winter treatments likely reduced mite loads below damaging thresholds. However, weather conditions did not permit sampling for *Varroa* late in the season to confirm this supposition. Still, the average beekeeping practice of applying a single *Varroa* treatment in late summer is insufficient to adequately control mite populations in overwintering colonies.

Another consequence of insufficient *Varroa* control was demonstrated in the viral results. Prevalence of most pathogens was similar between management groups. Only DWVA was less prevalent in BMP apiaries. However, the intensity of the *Varroa*-vectored viruses (ABPV, DWVA, and DVWB) in the fall was higher in average apiaries. This indicates that average colonies were more likely to succumb to these infections than BMP apiaries. This supports the supposition that *Varroa* and other stressors can weaken colony level immune defenses [81]. It is also possible that the elevated mite populations in average colonies were more effective at transmitting viruses at higher rates. Models of *Varroa*-virus interactions support the hypothesis that increased mite numbers would lead to increased viral intensity in a colony [55, 82].

Furthermore, after the first year, average apiaries began each spring with a higher *Varroa* load than BMP apiaries, suggesting that high fall infestations from the prior year persist in a colony over winter. These *Varroa* populations remained inflated throughout the season, resulting in average apiaries exceeding 3.0 mites/100 bees one month earlier than best apiaries. The number of months spent above threshold and average *Varroa* load were positively associated to viral infection and mortality. Time spent above threshold is therefore a good predictor of mortality, presumably because it is also related to viral infection. The longer a colony is above threshold, the higher the risk of experiencing *Varroa*-vectored viruses and at higher levels. This relationship can likely explain much of the mortality exhibited in average apiaries. The strong effect of time spent above threshold suggests that there is a cumulative effect of management and its

impact on colony health. While a beekeeper can conceivably control their mite load in the fall after significant mite population build up, the damage incurred from viruses is much harder to rectify. Management needs to be proactive, and is just as critical early in the season as it is when preparing for winter.

The cumulative effect of management can also be seen over multiple years. The amount of honey and the number of splits produced in BMP apiaries increased each year. Winter mortality in average apiaries increased each year, while in BMP apiaries it decreased, becoming significantly lower by the third study year. One explanation for these cumulative effects may be that new BMP colonies were started from nucs or splits in 2017 and 2018. It is well documented that nucs and splits are less likely to die than packages [65]. It is also possible that the brood break resulting from splitting overwintered BMP colonies provided extra *Varroa* control by reducing initial mite populations in parent colonies, resulting in reduced *Varroa* population growth over entire seasons [15, 83]. Another important cumulative factor is likely the elevated residual mite populations left in average colonies in the spring of 2017 and 2018. Although mite populations in overwintered average colonies were low enough to avoid immediate colony mortality, the overwintered mite populations negatively impacted colony health for months afterward. The resulting elevated viral loads still increased colony mortality, just over a longer time period. These results indicate that the effects of management and of colony health stressors occur over longer time periods than previously documented.

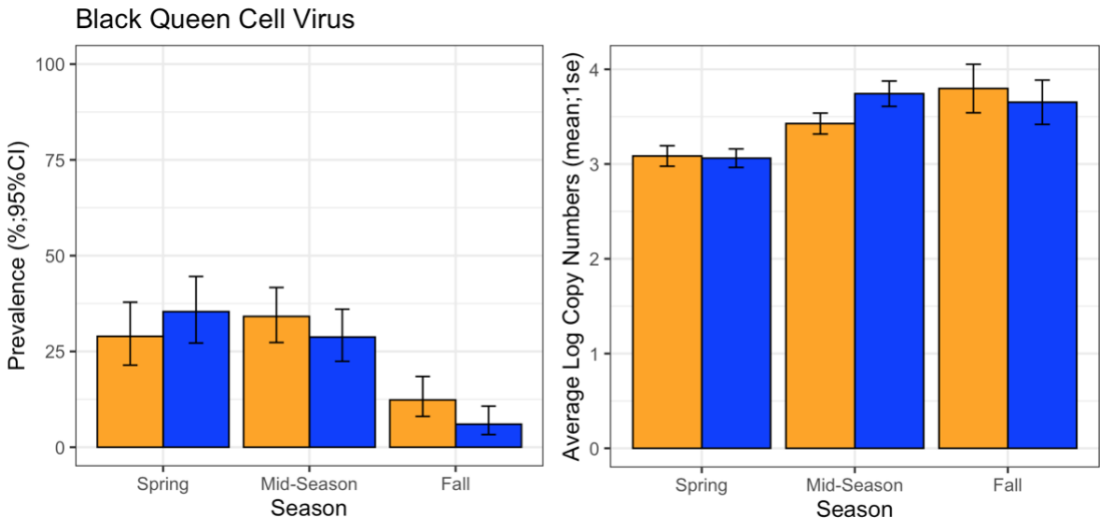
As a result of reduced *Varroa* and viral pressure, the BMP apiaries outperformed average apiaries in honey and split production and winter survival in the third year of the study. While the difference in honey production per colony was not statistically significant, the absolute difference in kgs produced still represents a real benefit to the beekeeper. At an average farmer's market price of $10 per lb, this represents an extra $13,590, or $97 per colony for a beekeeper using BMPs. BMP apiaries also produced 33 more splittable colonies than average apiaries. When factoring in the average cost a backyard beekeeper would pay to replace a dead colony, or the price at which a beekeeper could sell a nucleus, these splits are worth $175 each for a total of $5,775. Furthermore, BMP practices lowered the relative risk of mortality by 30%. This represents a substantial reduction in the labor and cost of replacing dead colonies each year, assuming a beekeeper would have to replace 1/3 fewer colonies.

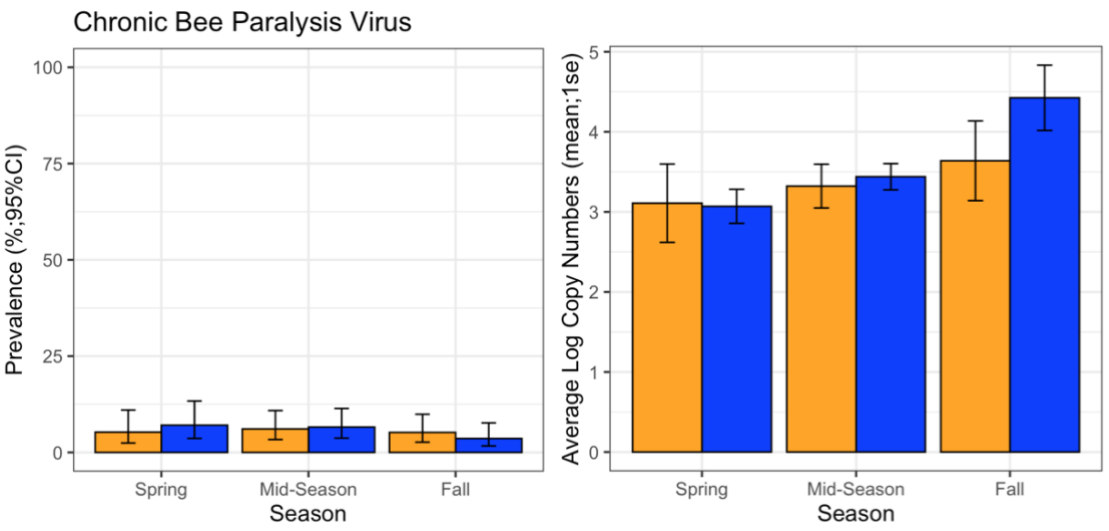
It is important to note that although BMPs improved colony productivity and reduced mortality, after 3 years, total loss in BMP apiaries still exceeded 30%. This is still well above the level of colony loss that beekeepers report as acceptable (~20% in 2019 [65]). This study demonstrates that while management can help inhibit some colony health stressors, it cannot prevent all colony mortality. There are environmental factors that management cannot control, such as heavily *Varroa* infested colonies nearby, landscape nutritional quality and pesticide exposure [10, 57, 73, 84, 85]. Even with an aggressive *Varroa* control strategy, BMP apiaries faced significant *Varroa* pressure and frequently exceeded treatment threshold, likely as a consequence of heavily infested

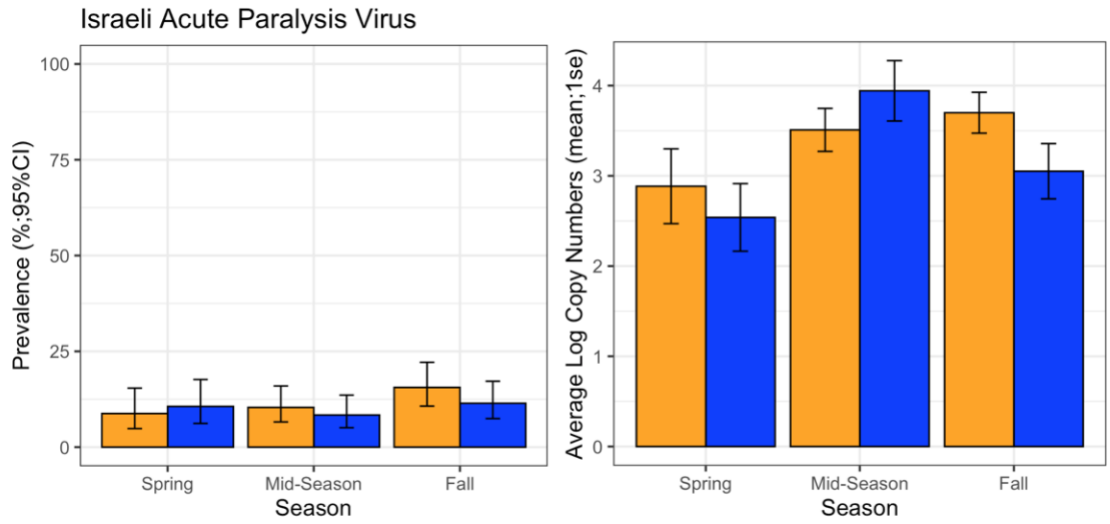
colonies nearby. Supplemental feeding was often required, however, supplements are not as nutritious as resources from flowers [86]. Pesticide exposure could have interacted with other colony health stressors to inhibit the effects of management [87-89]. While management alone cannot prevent all colony losses, the BMPs tested in this study are meant to act as additional tools for beekeepers to bolster their colony health. This study focused on aspects of colony health that beekeepers can control, in an attempt to arm them with practical methods that can be readily integrated into their current practices to further improve colony health and reduce colony mortality across the US.

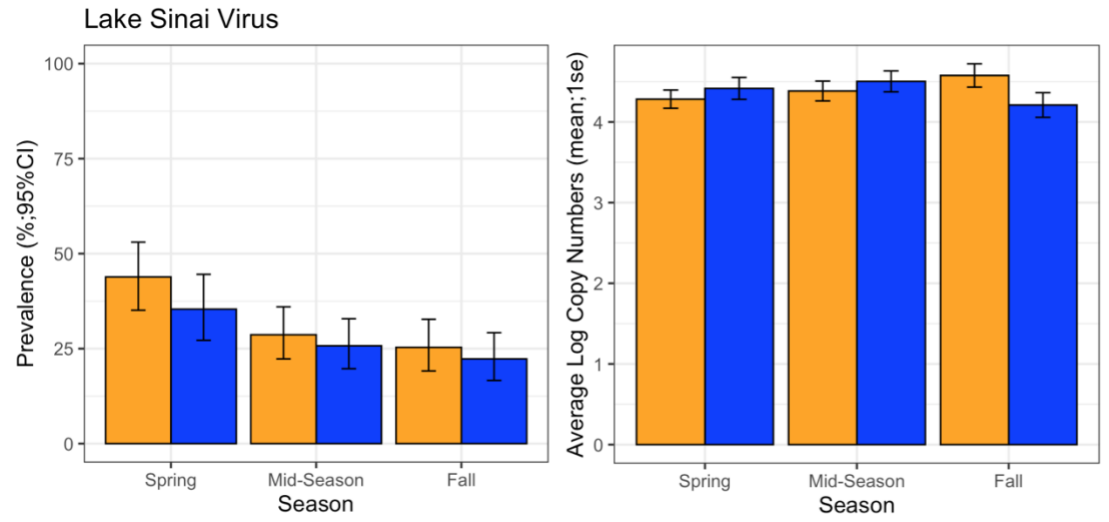
Supplemental Figures:

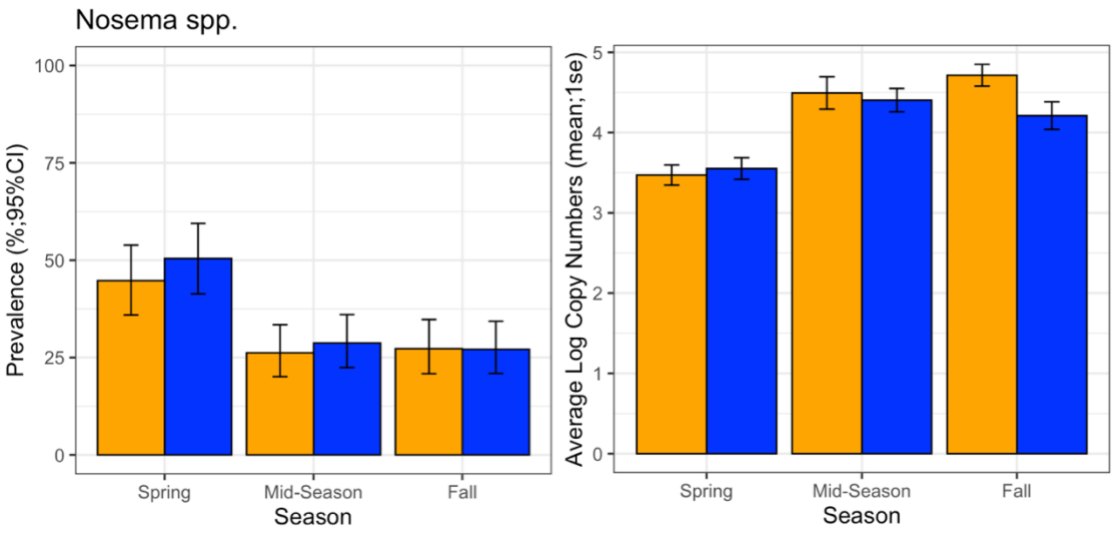
Supplemental Figure 2.1. Prevalence +/- 95% CI and average log copy numbers +/- standard error over the season (all years combined) for viruses, Trypanosome spp. and Nosema spp. that did not significantly differ between BMP (blue) and average (orange) apiaries.

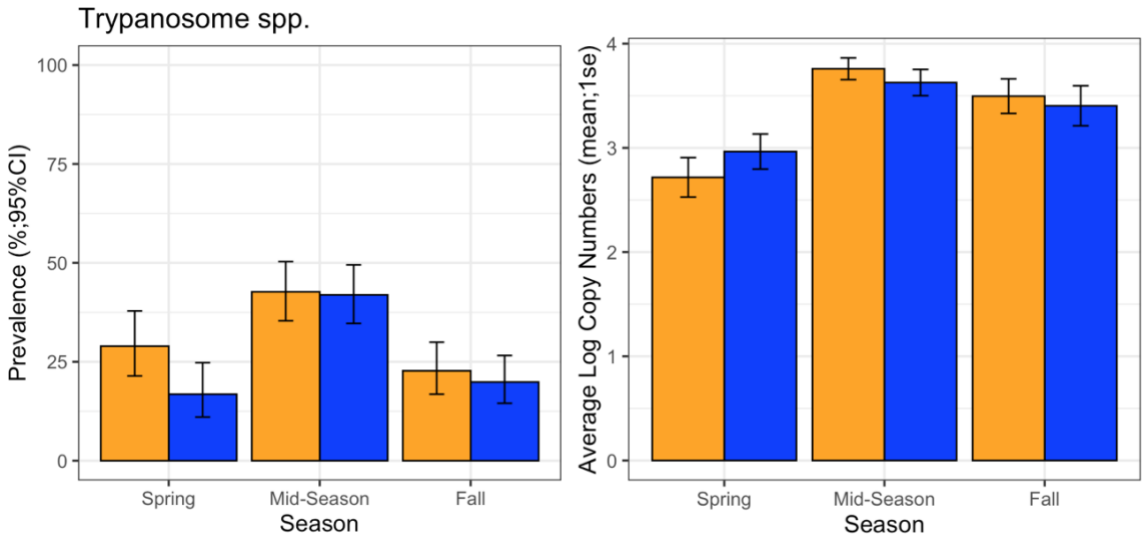



Chronic Bee Paralysis Virus
Prevalence (%;95%CI)
100
75
50
25
0
Spring
Mid-Season
Fall
Season
Average Log Copy Numbers (mean;1se)
5
4
3
2
1
0
Spring
Mid-Season
Fall
Season

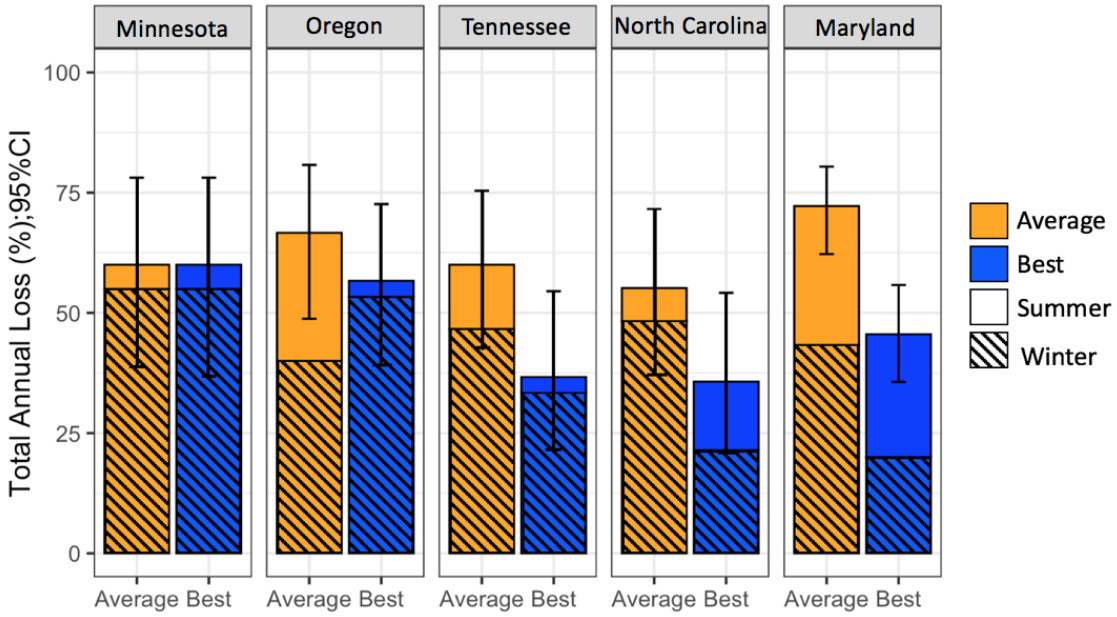

Israeli Acute Paralysis Virus
Prevalence (%;95%CI)
100
75
50
25
0
Spring
Mid-Season
Fall
Season
Average Log Copy Numbers (mean;1se)
4
3
2
1
0
Spring
Mid-Season
Fall
Season


Lake Sinai Virus
Prevalence (%;95%CI)
100
75
50
25
0
Spring
Mid-Season
Fall
Season
Average Log Copy Numbers (mean;1se)
4
3
2
1
0
Spring
Mid-Season
Fall
Season


Nosema spp.
Prevalence (%;95%CI)
100
75
50
25
0
Spring
Mid-Season
Fall
Season
Average Log Copy Numbers (mean;1se)
5
4
3
2
1
0
Spring
Mid-Season
Fall
Season


Trypanosome spp.
Prevalence (%;95%CI)
100
75
50
25
0
Spring
Mid-Season
Fall
Season
Average Log Copy Numbers (mean;1se)
4
3
2
1
0
Spring
Mid-Season
Fall
Season

Supplemental Figure 2.2. Total annual loss +/- 95% CI in average (orange) and BMP (blue) apiaries (all years combined) by region. Summer loss is represented by solid colors, and winter loss by striped colors



Chapter 3: Factors contributing to excessive fall *Varroa destructor* populations: a citizen science approach

Abstract:

The Bee Informed Partnership's Sentinel Apiary Program is a citizen science colony health monitoring program for US beekeepers. Between 2017-2018, Sentinel Apiary participants submitted 6,001 samples from 155 apiaries in 30 US states. Here, Sentinel Apiary *Varroa* samples and *Varroa* management information were used to assess *Varroa* treatment effectiveness throughout the active beekeeping season. Compared to untreated apiaries, apiaries treated for *Varroa* exhibited lower *Varroa* loads and slower *Varroa* population growth than untreated apiaries only in the fall, and still exceeded expected values. *Varroa* loads increased in 77.3% of recently treated apiaries. These increases in *Varroa* loads were likely not a result of treatment failure, but of other factors making treatments seem ineffective. Reduction in capped brood may explain some, but not all of these increases. The percent of colonies treated in an apiary and treatment method used did not affect the resulting change in mite load. These results suggest that rapid increases in mite population resulting in unexpected treatment outcomes were caused by an external source of mites, such as immigration of mites from highly infested colonies in nearby apiaries.

Introduction:

Since the onset of Colony Collapse Disorder (CCD) in 2006, multiple factors contributing to poor honey bee colony health have been identified [8]. While CCD is rarely observed today, the urgency it instilled in beekeepers and

researchers led to extensive work on a more prevalent colony health stressor: the parasitic mite *Varroa destructor* (Anderson and Trueman, [12]). *Varroa* is considered a top colony health stressor by researchers, and beekeepers consistently report it as a leading cause of colony mortality [5, 15, 90, 91]. *Varroa* feed on immature and adult bees, weakening bee immune systems, spreading a suite of viruses, and making colonies more susceptible to other health stressors [13, 53, 55, 92, 93]. Effective *Varroa* control is essential in the fall to prepare colonies for the overwintering period. Overwintering bees have to survive up to 12 weeks, and *Varroa* feeding during that period can shorten bee lifespan and increase the likelihood of overwinter colony mortality [14, 94, 95]. Several miticides and other tactics are available to combat *Varroa* population growth, including effective organic and synthetic chemical options [15]. Non-chemical options can also be effective, but require precise timing and proper execution [96]. Thresholds for *Varroa* treatment have been developed; 3.0 mites/100 bees is considered the standard treatment threshold in temperate climates [97, 98].

The life cycle of *Varroa* relies on the presence of capped brood. An adult female mite (the “foundress”), will enter a brood cell within 24 hours before it is capped. Once capped, the foundress feeds on the developing bee pupae and lays her first egg, always a male. She then lays up to 3-4 female eggs, of which only 1-2 typically mate with the male and survive to adulthood [12, 99]. When the bee emerges from the cell as an adult, so do the adult female mites, and the cycle begins again. Several mathematical models of this process have been developed to predict the population growth rate of *Varroa*. Most of these studies

agree that *Varroa* population growth over a single season follows an exponential curve, and that populations are expected to double approximately every 30 days [100-103]. However, in recent years, these models significantly underestimate the rate of *Varroa* population increases recorded by monitoring efforts in the fall [104].

The Bee Informed Partnership (BIP) has monitored *Varroa* infestation loads and *Varroa* control strategies with various survey efforts. The annual Loss and Management survey is a voluntary survey of US beekeepers on management practices and associated levels of colony mortality [5]. From 2018-2019, 78.5% of beekeepers reported treating for *Varroa*, and beekeepers who treated for *Varroa* lost 12.8 percentage points fewer colonies over the winter than beekeepers who did not treat for *Varroa* (38.3% compared to 51.1%; [65]). However, beekeepers who treated for *Varroa* still lost 15.8 percentage points more colonies over winter than they deem acceptable (22.5%; [65]). Furthermore, the USDA-APHIS National Honey Bee Disease Survey reports that *Varroa* levels, on average, exceed the recommended treatment threshold in August (3.2 mites/100 bees), September (4.5 mites/100 bees), and October (6.7 mites/100 bees), despite most beekeepers attempting to control mites [75]. These monitoring efforts demonstrate that *Varroa* treatments are not providing the expected and necessary level of *Varroa* control. Understanding factors contributing to rapidly increasing *Varroa* loads and unexpected treatment outcomes is a critical knowledge gap in attempts to reduce *Varroa* mediated honey bee colony losses.

Some of the most notable factors affecting *Varroa* loads include the amount of capped brood present in a colony and the application of *Varroa* control products. The rate of *Varroa* reproduction declines as the amount of capped brood in the colony declines, as *Varroa* rely on the presence of capped brood to complete their reproductive cycle [105, 106]. Application of miticides can help reduce *Varroa* populations, assuming effective methods are applied properly [100, 105]. The typical recommended best practice for applying miticides is to treat all colonies in an apiary at the same time, assuming this reduces the possibility of mites spreading between colonies [65]. A more recent hypothesis to explain increasing fall mite loads is the immigration of mites into colonies from other colonies and apiaries [79, 80, 104]. The immigration of mites into colonies is well demonstrated, and is known to increase in the fall and when colonies are crowded in apiaries and in the landscape [84, 107]

To investigate these factors, results from the BIP citizen science Sentinel Apiary Program can be leveraged. The Sentinel Apiary Program began as an extension effort to educate beekeepers on how *Varroa* populations fluctuate over the season, and how best to control them. Participating beekeepers sample 4 or 8 colonies once per month for six months, typically from May to October. Sentinel participants perform colony health inspections, record colony strength metrics, and provide a sample of adult bees from each colony. Samples are processed at the diagnostics lab at the University of Maryland, and results are provided to the beekeeper within two weeks to help inform their management decisions. Sentinel Apiaries help beekeepers improve *Varroa* monitoring and management skills, act

as regional benchmarks for colony health, and provide valuable longitudinal colony health data. It is the only BIP program where physical samples, colony strength metrics, and management information are collected from the same colonies over an entire beekeeping season. This allows the evaluation of *Varroa* management strategies on colony health changes over long time periods.

Here, Sentinel Apiary data was used to characterize seasonal *Varroa* population growth changes, assess treatment effectiveness, and investigate factors affecting treatment outcome. It was hypothesized that *Varroa* loads on average would exceed the recommended treatment threshold of 3.0 mites/100 bees in the fall, and that *Varroa* population growth would exceed the model-predicted rate of 100% in the fall. It was also hypothesized that recently treated apiaries would exhibit reduced *Varroa* loads and *Varroa* population growth compared to pre-treatment levels and recently untreated apiaries. When initial analyses found that *Varroa* loads in recently treated apiaries typically increase and are often not lower than in untreated apiaries, possible factors affecting treatment outcome were investigated. It was hypothesized that lower reduction in capped brood during a treatment, higher proportion of treated colonies in the apiary, and more effective treatment methods would result in reduced increases in *Varroa* load. Finally, the possibility of mite immigration from highly infested apiaries nearby affecting treatment outcome was considered.

Methods:

The Sentinel Apiary Program:

Piloted in 2015, the Sentinel Apiary Program has involved 295 beekeepers in 30 US states to date. This study includes data from years 2017-2018. Participants were recruited with advertisements placed in beekeeping magazines (Bee Culture, American Bee Journal), blogs on the BIP Website, and emails to local beekeeping clubs and state apiarists. Beekeepers enrolled in the Sentinel Apiary Program via beeinformed.org/sentinel. They could participate at a 4 or 8 colony level. The program's enrollment fee ($275 USD for 4 colonies, $499 USD for 8 colonies) included a sampling kit containing protocols and materials needed to perform six monthly colony health inspections and take samples of adult bees for *Varroa* and Nosema processing from each of their Sentinel colonies (Appendix). Beekeepers were encouraged to sample between May and October, so their results would be comparable with other beekeepers. After they performed and recorded their monthly health inspections and collected samples, they sent these to the BIP diagnostics lab at University of Maryland for processing.

Samples were processed for *Varroa* and Nosema load quantification using standard methods [70, 71]. Results were summarized in a report, which also included the information the beekeeper recorded on their data sheet (Appendix) Reports were returned to the beekeeper within two weeks of receiving the samples so they could use results to inform their management decisions. Beekeepers recorded queen status, frames of bees, and brood pattern according to standard methods and according to program protocol (Appendix [69]). They also included apiary level management information such as *Varroa* treatments,

feeding, or harvesting honey. Beekeepers reported the proportion of colonies an action was performed on, but the specific colony numbers an action was performed on was not reported.

Treatment groups:

Initial analyses were conducted on two groups: recently treated and recently untreated apiaries. Recently treated apiaries included any apiary where a recognized *Varroa* control technique was completed between the prior month's sample and the most recent sample. *Varroa* control techniques included application of chemicals (amitraz, coumaphos, fluvalinate, formic acid, hop oil, oxalic acid, and thyme oil) and non-chemical methods (drone brood removal, brood break via splitting or queen removal). Screened bottom boards were not included as treatments in these analyses, as they are used for the entire duration of the sampling season and before and after mite infestation levels could not be evaluated.

Recently treated apiaries were compared to recently untreated apiaries by mean monthly *Varroa* load and mean monthly percent change in *Varroa* load. Note: percent change was calculated as mean percent change of all apiaries, not percent change in the all apiary mean. The percent change between the mean *Varroa* load in May and the mean *Varroa* load in June is not the same as the mean percent change in *Varroa* load between May and June. This becomes evident when comparing Figures 2 and 3, and Figures 5 and 6. Apiary mean *Varroa* load and brood pattern percent change per month was calculated according to the following formula:

$$\%\ Change = \left(\frac{Value_{End} - Value_{Start}}{Value_{Start}}\right) * 100$$

To avoid zero denominators in these calculations, starting means of zero were replaced with small biologically appropriate arbitrary values. Standard methods assume that every colony in the US has more than zero mites present. Thus for apiary mean *Varroa* load, zero starting values were replaced with 0.5 mites/100 bees. As beekeepers commonly rate colonies with almost no brood with a brood pattern of 0 or 1, zero starting values for brood pattern were also replaced with 0.5.

Factors affecting treatment outcome:

Three potential factors affecting treatment outcome were assessed: change in brood area, proportion of treated colonies in an apiary, and treatment method. Change in brood area was calculated as the monthly percent change in apiary mean brood pattern during treatment application. Brood pattern is a metric commonly used by beekeepers to evaluate the reproductive capacity of their colonies. Rated on a scale of 0-5, brood pattern is a measure of the amount of brood that is capped on each frame. A rating of 5 would signify all brood frames are almost completely covered in capped brood. A rating of 0 indicates almost no capped brood. The proportion of treated colonies in an apiary was self-reported by beekeepers with each treatment they applied. If 2 colonies were treated in an apiary of 8 colonies total, the proportion of treated colonies equaled 0.25. The treatment method used was also self-reported by the beekeeper on monthly data sheets.

Statistics:

All statistical analyses were performed in R (version 3.2.1). All summary statistics are reported as mean ± standard error unless otherwise noted. Linear mixed effects models with apiary as a random effect were used to check for differences between variables measured repeatedly (*i.e. Varroa* load, % change in *Varroa* load per month). Binomial mixed effects models were used to compare between binomial response variables (*i.e.* proportion of treatment outcomes each month). Regular linear models were used to compare between variables at a single time point. Models were eliminated in a stepwise fashion with ANOVAs until the best fit model was identified. Linear regressions were performed to assess differences in changes in *Varroa* load between variables (*i.e.* % change in brood pattern, percent of treated colonies in an apiary, and treatment method). Where significant differences were detected between all groups, post-hoc pairwise Tukey tests with a Bonferroni correction were performed to assess differences between pairs. Only significant results from Tukey tests ($\alpha = 0.05$) are reported.

Results:

In 2017 and 2018, the Sentinel Apiary Program consisted of 94 beekeepers sampling 155 apiaries in 30 states (Figure 3.1). A total of 6,001 samples were submitted from 1,198 colonies in this time period.

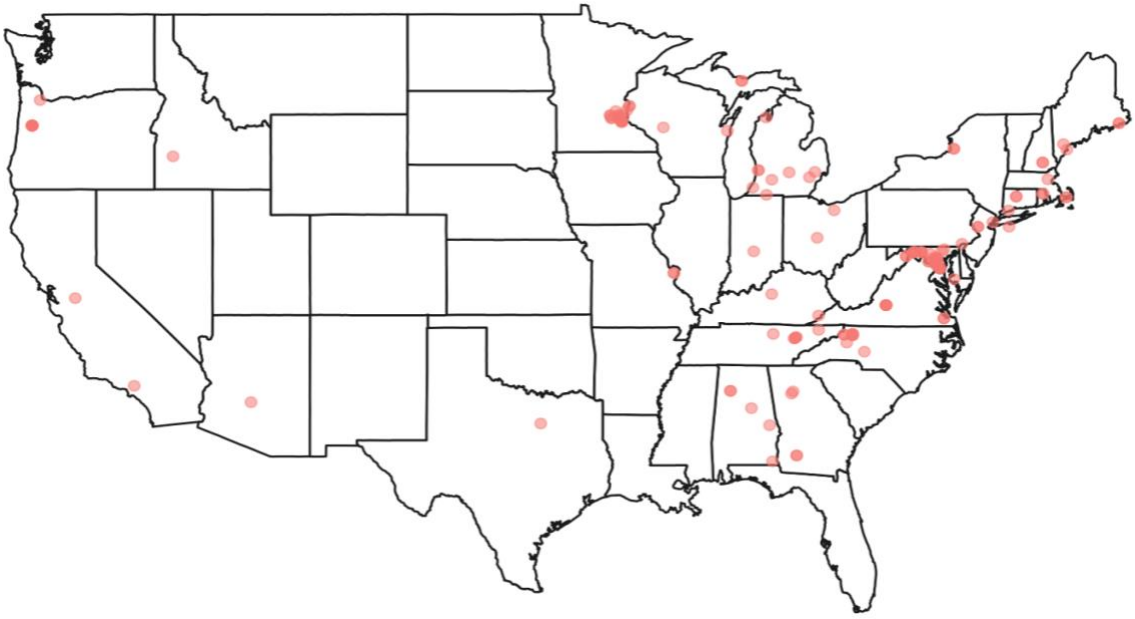


Figure 3.1. Map of 2017 and 2018 Sentinel Apiary locations. A total of 155 Sentinel Apiaries were present in 30 states.

Varroa population growth – all participants:

Mean *Varroa* load for all participants combined was lowest in May (1.34 ± 0.11 mites/100 bees) and highest in October (5.65 ± 0.25 mites/100 bees). The all participant mean exceeded the recommended treatment threshold in August, and remained above threshold in September and October (Figure 3.2). The all participant mean percent change in mite load per month was lowest between May and June (63.90 ± 10.59%) and highest between September and October (234.63 ± 33.5%). Percent change in mite load exceeded the expected value of 100% in all months except May-June (Figure 3.3). The proportion of apiaries above threshold was highest in October (65.8%, Figure 3.4).

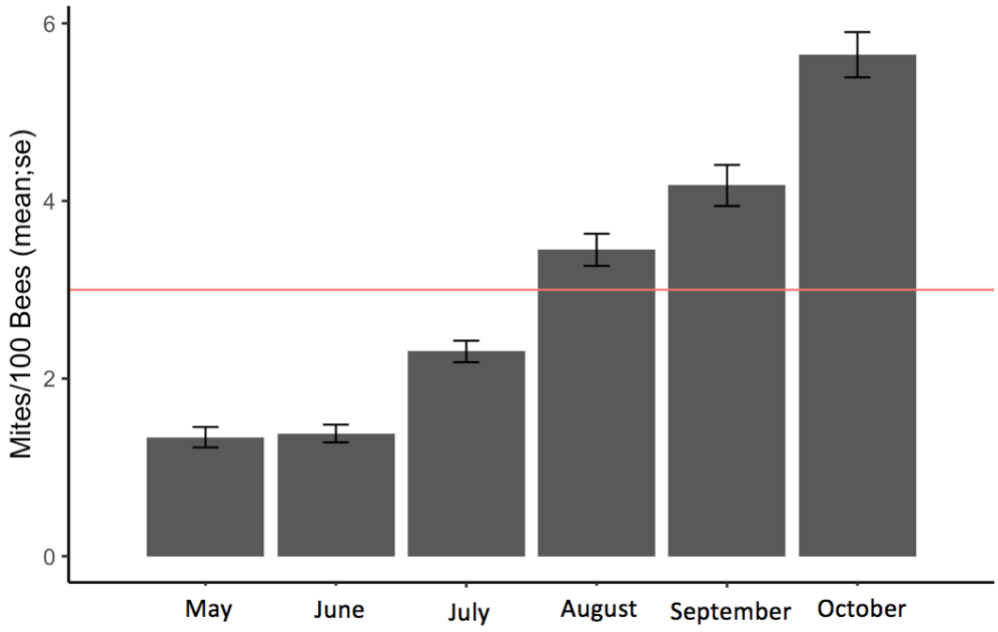


Figure 3.2. All participant apiary mean Varroa load +/- standard error in each sampling month. Mean Varroa loads exceed the treatment threshold of 3.0 mites/100 bees (red line) from August through October.

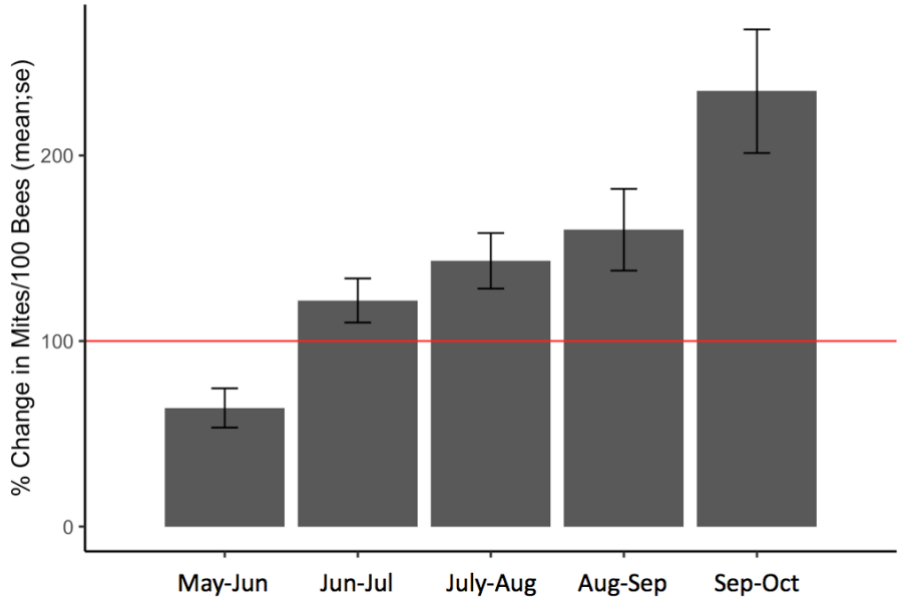


Figure 3.3. All participant mean percent change +/- standard error in Varroa load per month. Percent change exceeds the expected rate of 100% (red line) in all months except between May-June.

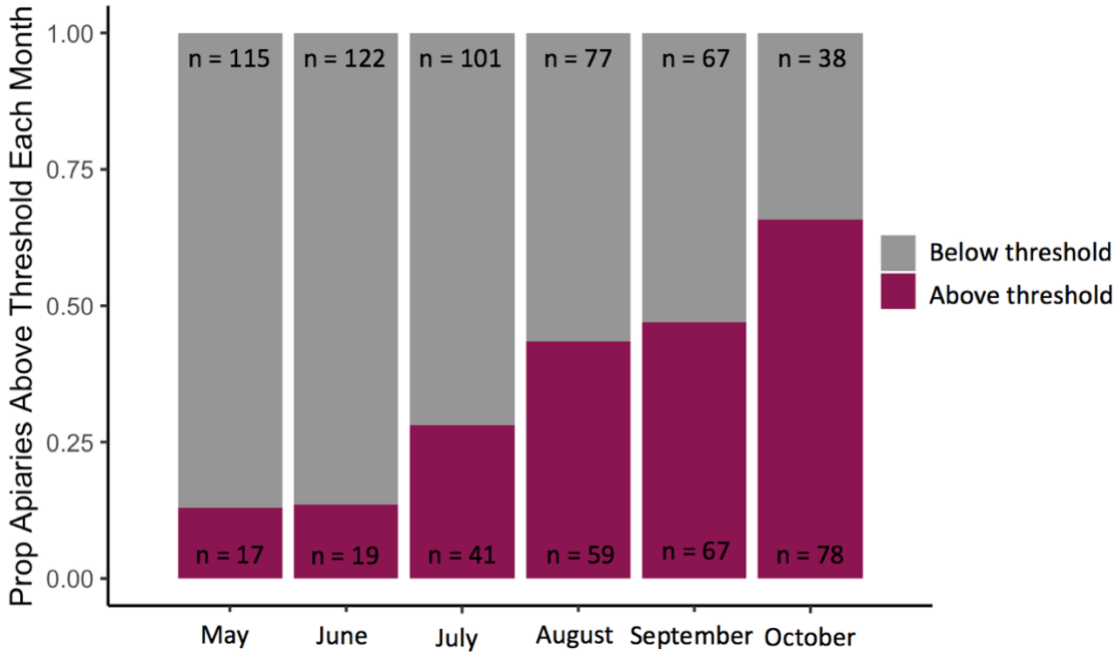


Figure 3.4. Proportion of apiaries with mean Varroa load over recommended treatment threshold of 3.0 mites/100 bees (maroon).

Treatment expectations and outcomes:

Out of all 155 apiaries, 28 were never treated and 127 were treated at least once between May and October. In the 127 apiaries that were treated, a total of 192 treatments were performed. Only 22.7% (n = 45) of treatments resulted in a decrease in *Varroa* load. The frequency of treatments resulting in a decrease in *Varroa* load was uniformly low across all sampling months (Figure 3.5, $F_4 = 0.49$, $p = 0.68$).

Varroa load in recently treated apiaries ranged from 1.16 ± 0.42 mites/100 bees in May to 4.89 ± 0.50 mites/100 bees in October, and in recently untreated apiaries from 1.68 ± 0.18 mites/100 bees in May to 7.21 ± 0.80 mites/100 bees in October (Figure 3.6). *Varroa* loads differed between recently treated and recently

untreated apiaries across all months (Figure 3.6, $F_1 = 4.55$, $p = 0.002$). When analyzing individual months, *Varroa* loads in recently treated apiaries were only significantly lower in October ($F_1 = 6.37$, $p = 0.01$). Both recently treated and untreated apiaries were still above threshold on average in October.

The monthly *Varroa* population growth rate (percent change) in recently treated apiaries ranged from 53.21 ± 6.83% between May and June to 112.54 ± 44.41% between September and October, and in recently untreated apiaries from 25.67 ± 6.83% between May and June to 213.88 ± 55.64% between September and October. *Varroa* population growth differed between recently treated and recently untreated apiaries across all months (Figure 3.7, $F_1 = 1.94$, $p < 0.001$). When analyzing individual months, *Varroa* population growth in recently treated apiaries was significantly lower than in recently untreated apiaries between August and September ($F_1 = 4.18$, $p = 0.04$) and September and October ($F_1 = 6.16$, $p = 0.01$). Mite population growth in both groups was still above the model-predicted rate of 100% per month between September and October (Figure 3.7).

Linear regressions were performed to assess the difference in change in mite load between recently treated and recently untreated apiaries. Across all months, recently treated apiaries exhibited reduced increases in mite load compared to recently untreated apiaries (treated $\beta = 0.30$, untreated $\beta = 1.01$, $F_1 = 65.81$, $p < 0.001$). When analyzing each month individually, recently treated apiaries exhibited reduced increases in mite load compared to untreated apiaries between May and June (Figure 3.8, treated $\beta = 0.24$, untreated $\beta = 0.56$, $F_1 =$

7.49, p = 0.007), July and August (treated β = 0.19, untreated β = 1.07, F_1 = 7.49, p = 0.004), and October and September (treated β = 0.22, untreated β = 1.03, F_1 = 7.49, p < 0.001).

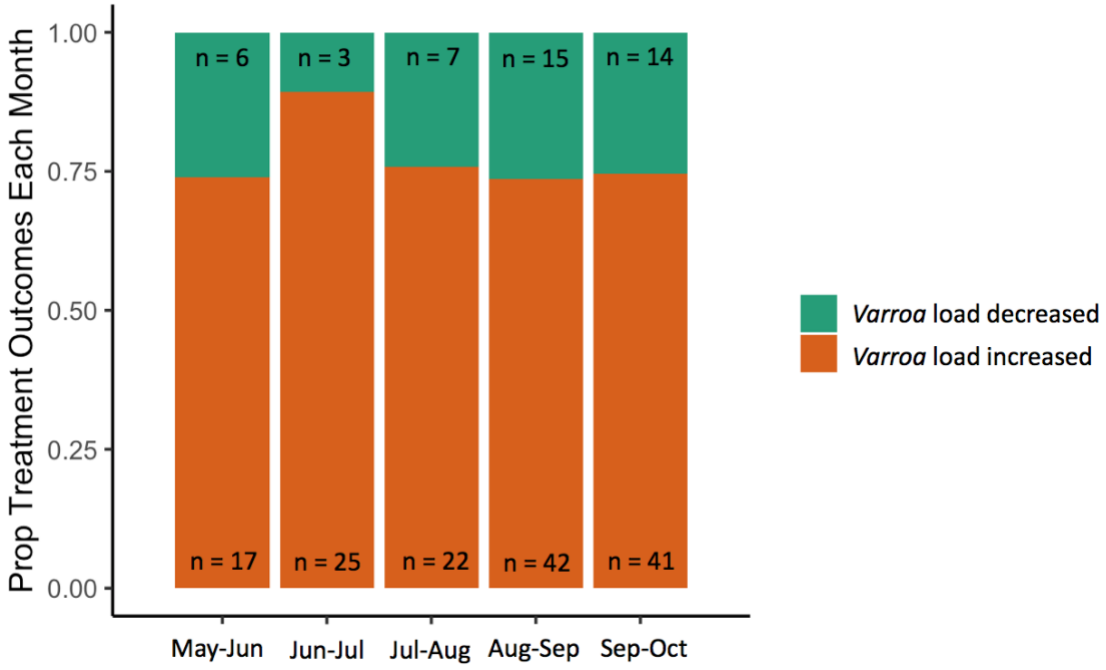


Figure 3.5. The proportion of treatments that resulted in either a decrease (green) or increase (orange) in Varroa load each month. The proportion of treatment outcome did not differ across months (glm p = 0.68).

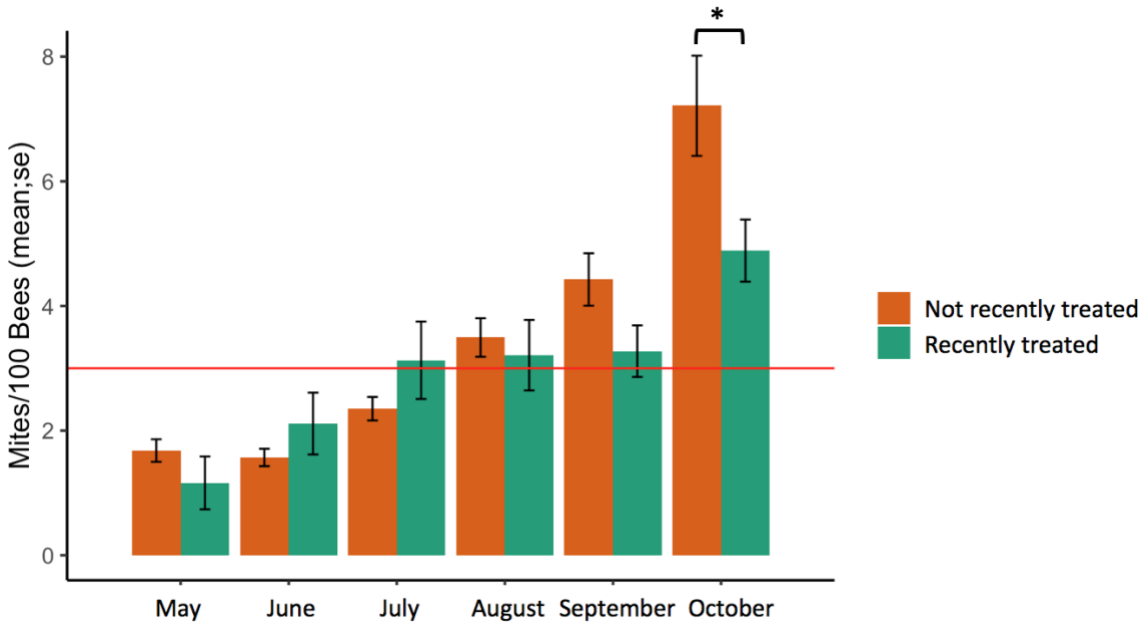


Figure 3.6. Average monthly Varroa loads +/- standard error in recently treated (green) and recently untreated (orange) apiaries. Recently treated apiaries had a lower mite load in October (glm p = 0.01). Both groups of apiaries were still above threshold (red line, 3.0 mites/100 bees) in October.*

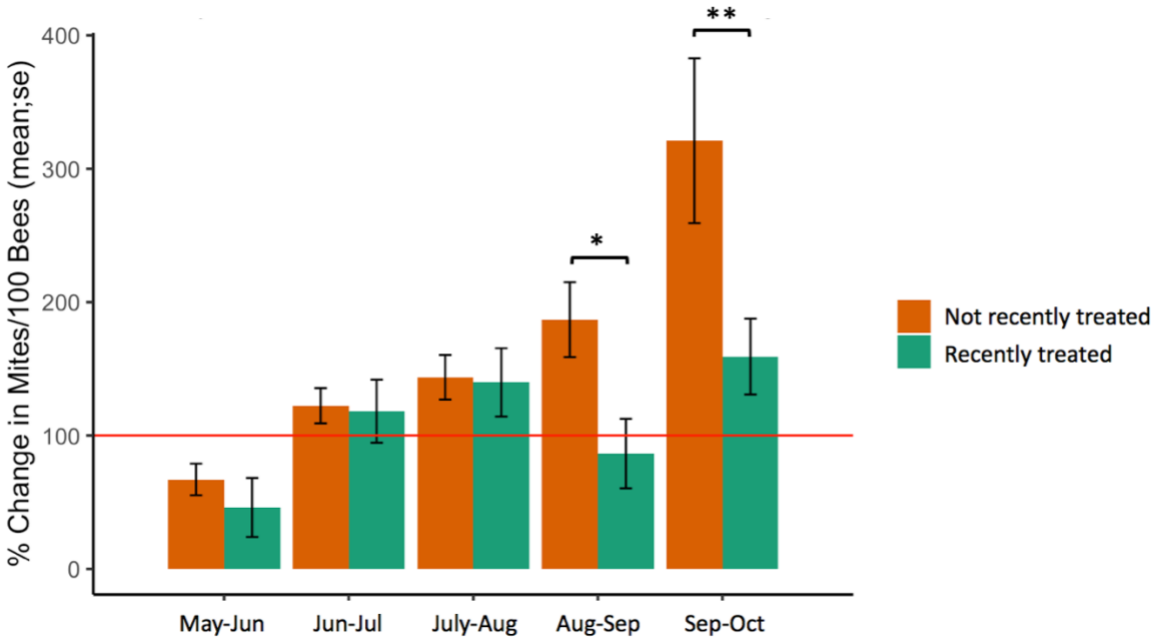


Figure 3.7. Mean percent change in Varroa load +/- standard error in recently treated (green) and recently untreated (orange) apiaries. Recently treated apiaries had significantly lower Varroa population growth between August and September (glm p =0.04) and September and October (glm p = 0.01*). Both groups population growth were above the expected 100% increase (red line) between September and October.*

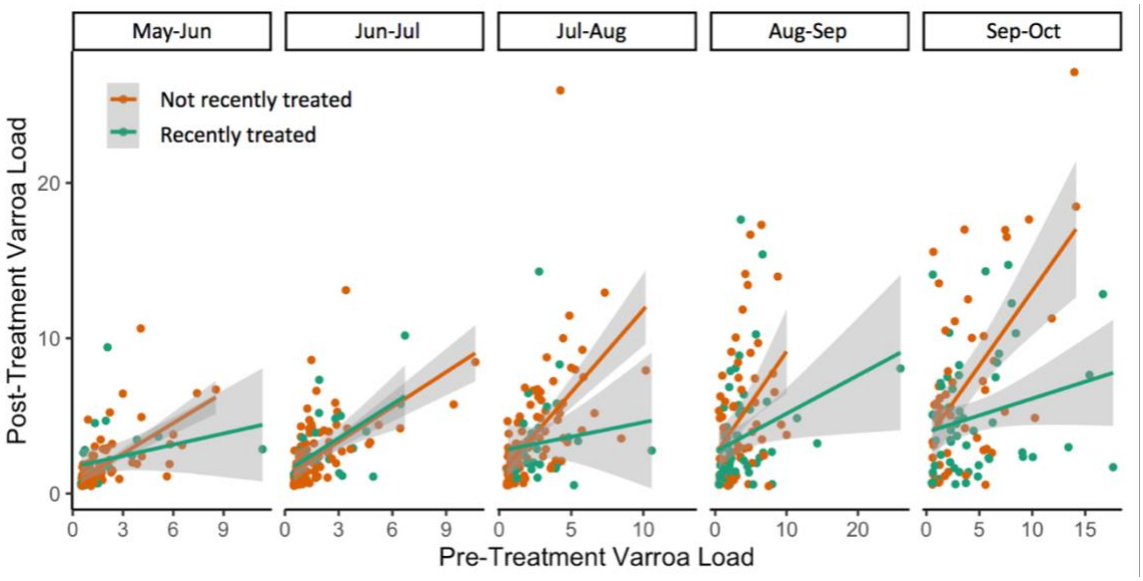


Figure 3.8. Linear regressions (with 95% CI) of the change in mite load in recently treated (green) and untreated (orange) apiaries. Recently treated apiaries exhibited reduced increases in mite load between May and June, July and August, and September and October.

Possible factors affecting changes in Varroa load:

Linear regressions were used to assess possible factors affecting changes in *Varroa* load between monthly samples. Monthly change in *Varroa* load was the response variable, with percent change in brood pattern, percent of treated colonies in the apiary, and treatment method as explanatory variables. An apiary's mean monthly percent change in brood pattern was associated with the monthly change in mite load (β = -0.29, F_1 = 6.00, p = 0.01), and this association did not differ between recently treated and untreated apiaries (Figure 3.9, treated β = -0.24, untreated β = -0.51, F_1 = 1.05, p = 0.31).

Out of the 192 treatments, 130 (67.7%) were applied to 100% of colonies in the apiary. There was a significant interaction between change in *Varroa* load and untreated apiaries (β = 1.01), 100% treated apiaries (β = 0.31), or less than

100% treated apiaries (Figure 3.10, β = 0.21, F_2 = 33.45, p < 0.001). However, there was no difference in change in mite load between 100% treated and less than 100% treated apiaries (p = 0.77).

Finally, there was no difference in the change in mite load between any treatment method used (Figure 3.11, F_8 = 0.71, p = 0.27). Although no treatment method appeared more effective than any other method, it was also useful to compare each treatment method to the control group of untreated apiaries. Compared to the recently untreated group (β = 1.01), the following treatment methods exhibited reduced increases in mite load: amitraz (Figure 3.11, β = -0.03, F_1 = 33.81, p < 0.001), combination (β = 0.20, F_1 = 6.45, p = 0.01), formic acid (β = 0.33, F_1 = 43.14, p < 0.001), oxalic acid (β = 0.51, F_1 = 7.60, p = 0.006), and thymol (β = 0.14, F_1 = 13.65, p < 0.001). For these 5 treatment methods, linear regressions were also performed for each sampling month (Supplemental Material).

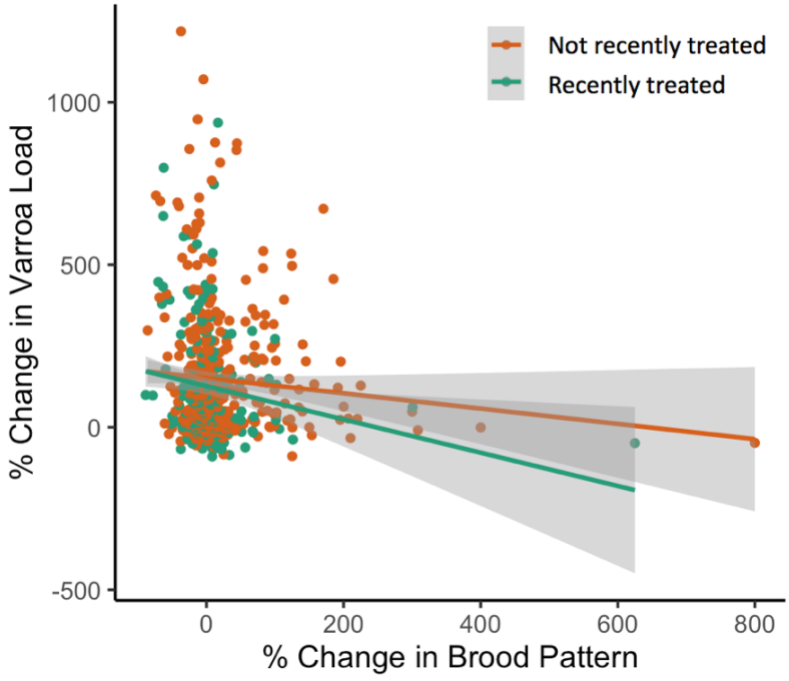


Figure 3.9. Linear regression (with 95% CI) of % change in brood pattern to % change in Varroa load in recently treated (green) and untreated (orange) apiaries.

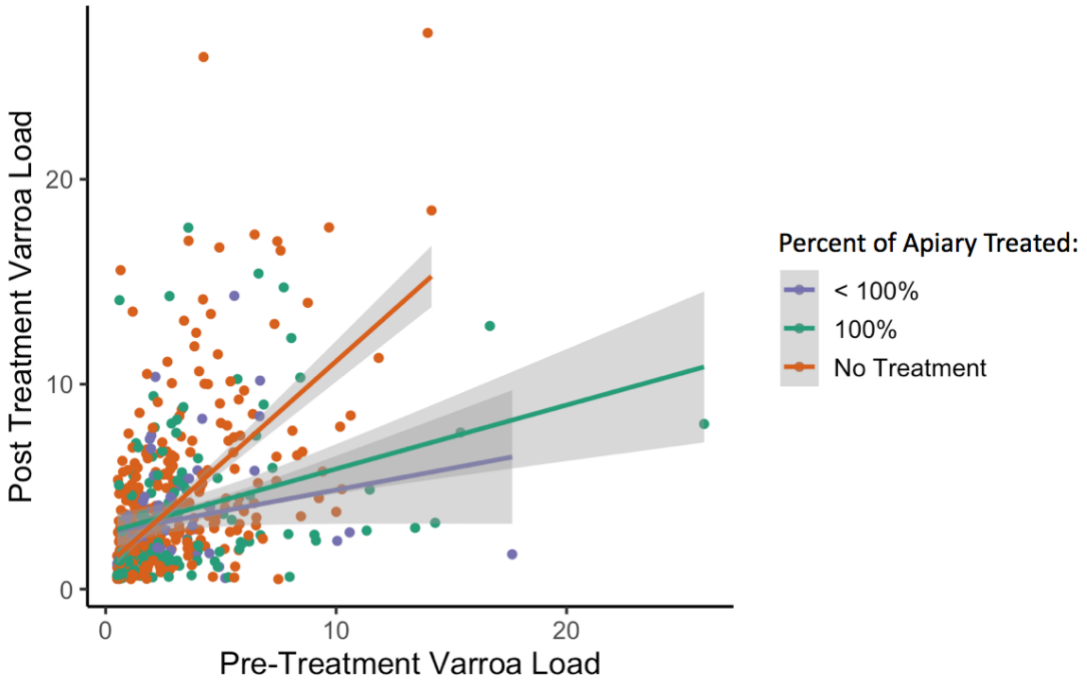


Figure 3.10. Linear regression (with 95% CI) of pre-treatment Varroa load to post-treatment Varroa load in recently untreated apiaries (orange) compared to recently treated apiaries with 100% of colonies treated (green), or recently treated apiaries with less than 100% of colonies treated (purple). Both groups of treated apiaries exhibited reduced Varroa load increases compared to untreated apiaries, but treating 100% of colonies was not different from treating less than 100% of colonies.

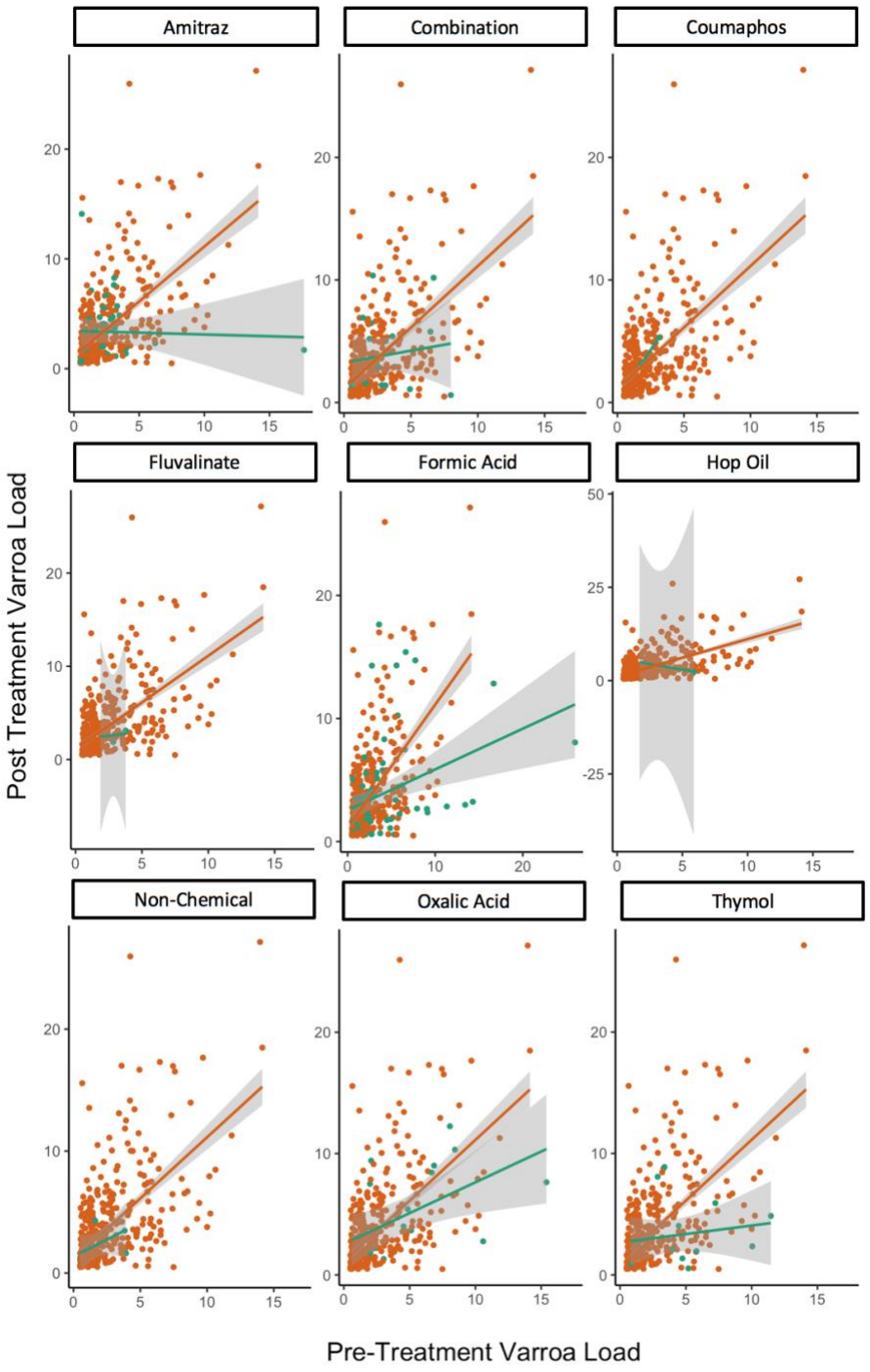


Figure 3.11. Linear regressions (with 95% CI) of each treatment method compared to each other, and to untreated apiaries. Apiaries treated with amitraz, a combination of treatments, formic acid, oxalic acid, and thymol (green) exhibited reduced Varroa load increases compared to untreated apiaries (orange).

Discussion:

The first objective of this study was to characterize seasonal *Varroa* load changes in US apiaries. As expected, *Varroa* loads were high in fall, exceeding the recommended treatment threshold of 3.0 mites/100 bees from August through October. Further, *Varroa* population growth exceeded model-predicted rates of 100% monthly increases from June through October. Most apiaries (65.8%) were above treatment threshold at the end of the season. The second objective of this study was to assess the effect of applying *Varroa* treatments. Unexpectedly, *Varroa* loads increased in 77.3% of recently treated apiaries. Recently treated apiaries exhibited lower mite loads in October and lower percent changes in mite loads from August through October than untreated apiaries. However, recently treated apiaries were still above threshold and exceeding model-predicted mite population growth at the end of the season. Linear regressions demonstrated that *Varroa* treatments do not in fact decrease *Varroa* loads, but rather slow the increase in *Varroa* loads compared to untreated apiaries.

Due to the unexpected outcome of treatments not yielding the expected level of *Varroa* control, potential factors affecting treatment outcome were investigated. Two possible explanations for unsatisfying treatment effectiveness are related to the treatment application. Applying a treatment to less than 100% of the colonies in an apiary was hypothesized to be less effective than treating 100% of colonies in an apiary. Often one or a few colonies in an apiary have higher mite loads than the rest, so beekeepers only treat the problematic colonies. Recommended best practice, however, is to treat all the colonies in an

apiary at the same time to prevent spillover of mites from heavily infested colonies [65, 107]. In this study, however, apiaries with 100% of colonies treated did not exhibit *Varroa* load increases different from apiaries with less than 100% of colonies treated. Another possible factor affecting treatment outcome was the treatment method used. Different *Varroa* control products have slightly different levels of effectiveness depending on temperature, presence of brood, and other factors. Here, the difference in change in mite load was not different among treatment methods. However, this does not mean that all treatments were ineffective. When comparing each treatment method to untreated apiaries, apiaries treated with amitraz, a combination of methods, formic acid, oxalic acid, and thymol exhibited lower increases in mites than untreated apiaries.

Most of the treatment methods used in this study have well demonstrated efficacy in the lab and no reports of resistance, thus it is unlikely that increases in *Varroa* loads after treatment are due to treatment failure [96, 108, 109]. Rather, it probable that rapid increases in *Varroa* loads in the fall made treatments appear ineffective. A hypothesized source of additional mites was a decrease in the percent of capped brood. On average, about 50% of the mite population exists underneath brood cappings at any given time [110]. When colonies start producing less brood in fall and less capped brood is present, a larger proportion of the mite population is forced to live on adult bees. Because the main *Varroa* sampling methods use adult bees only, it is possible the *Varroa* population in late fall is overestimated when brood production dwindles. This study did find a negative association between percent change in capped brood and percent

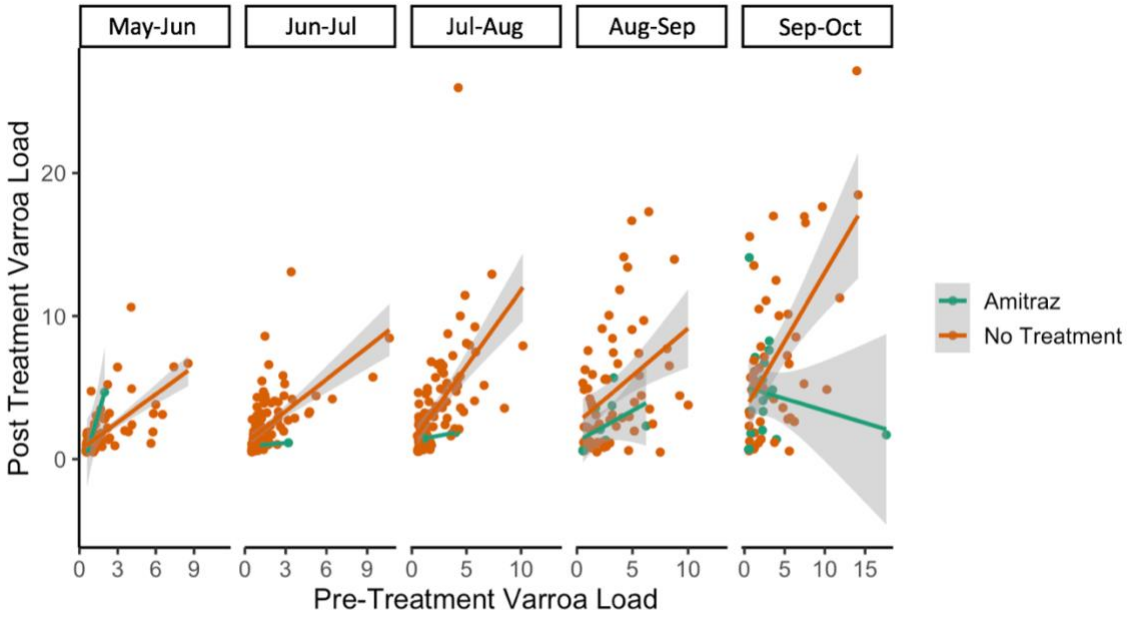
change in mite load. It is thus possible that reduction in brood area was contributing additional mites to the phoretic population. However, the association between percent change in capped brood and percent change in mite load was relatively small. So while it is possible mite loads are increasing in fall in part due to emergence from capped brood, this does not appear to explain the rapid increases in mite loads in the fall.

Taken together, the results of this study show that while treatments do not often result in decreased *Varroa* population growth rates and loads, they do slow the rate of *Varroa* population increase compared to not treating. If a beekeeper applied a *Varroa* treatment early in the season, it is possible they would slow the rate of population increase and have to treat fewer times throughout the year. Increases in *Varroa* load were not explained by beekeepers treating less than 100% of colonies in an apiary, or the treatment method used. Unexpected treatment outcomes were likely due to rapid increases in *Varroa* load during a treatment application. Larger increases in *Varroa* load were associated with reduction in capped brood, but not to a strong enough degree to explain the high mite loads exhibited in fall. Factors explored in this study fail to completely explain the resulting increases in mite loads exhibited in unexpected treatment outcomes.

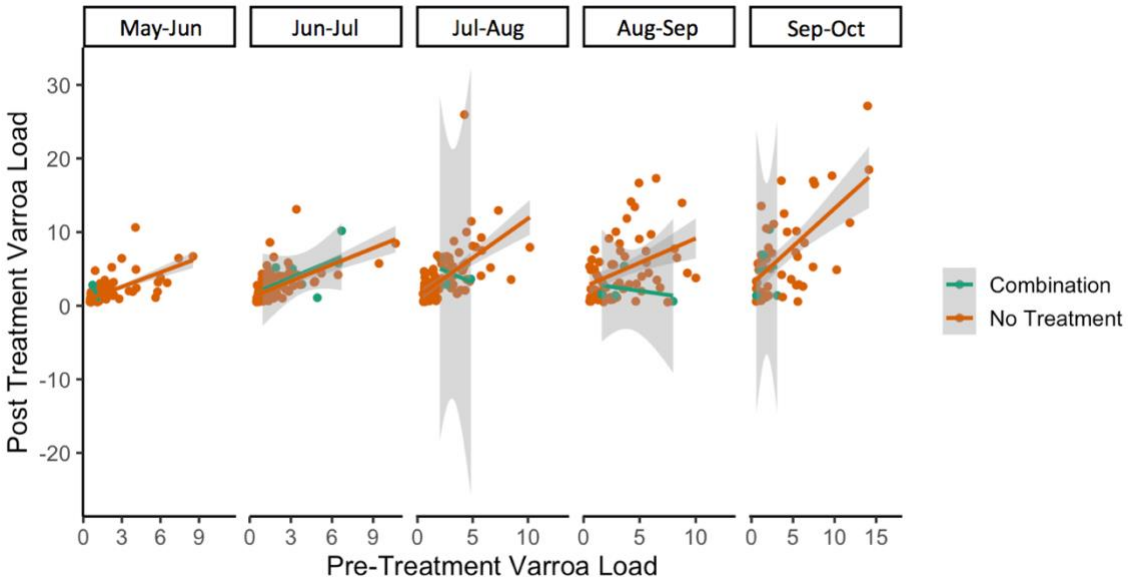
Fall increases in Varroa load exceeded the model-predicted 100% increase resulting from *Varroa* reproduction alone in every month. Because factors within these apiaries failed to explain their exhibited increases in mite load, these results strongly imply a source of mites external to the apiary. A likely

source of external mites are colonies in other apiaries nearby. Transfer of mites on bees between apiaries is well documented, and this phenomenon is known to increase in the fall [79, 104, 107, 111]. This phenomenon can explain the failure of mathematical models to accurately predict increases in *Varroa* populations in the fall; the number of foragers returning to the colony with mites from an external source help explain the gap [104]. It is therefore possible that an important factor contributing to treatment failure is the immigration of mites from other apiaries. This hypothesis is explored in Chapter 4.

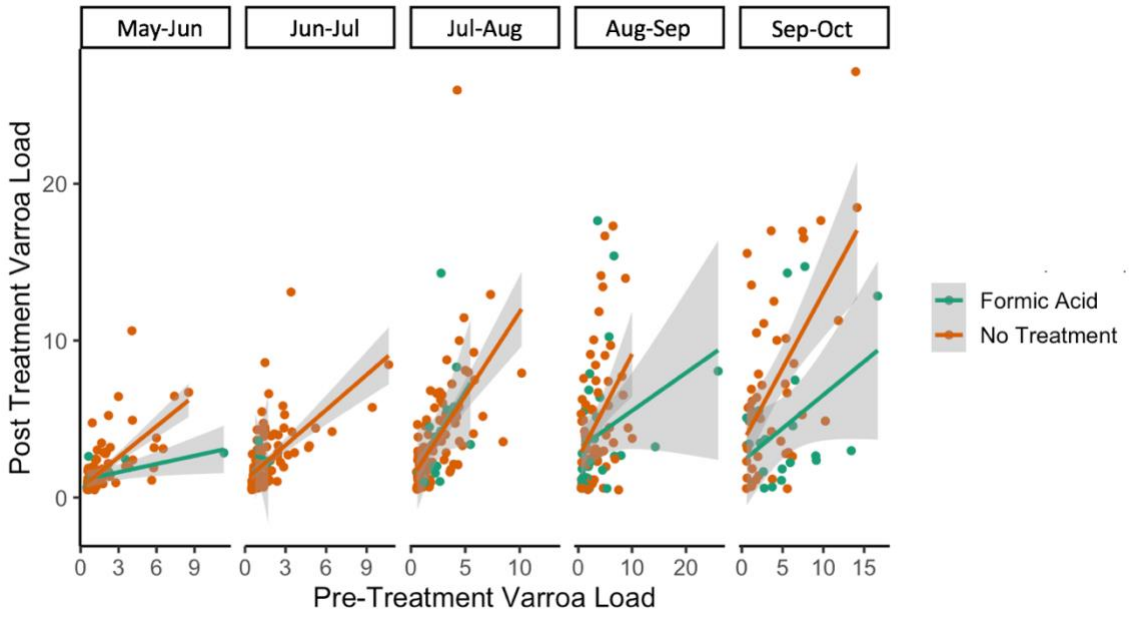
Supplemental Figures:



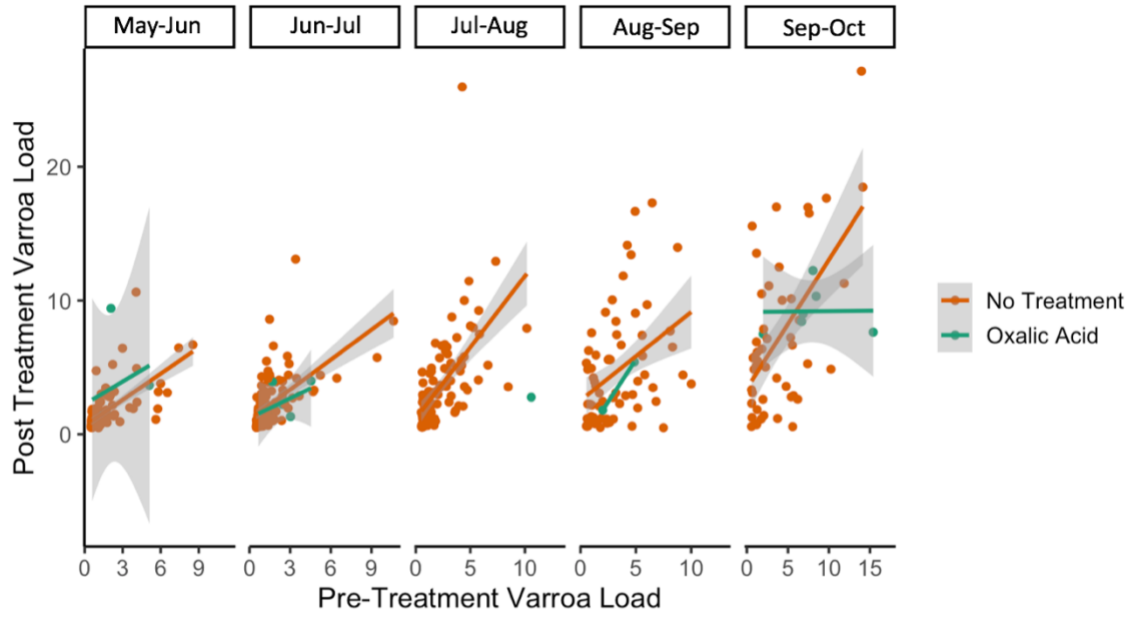
Supplemental Figure 3.1. Linear regressions for apiaries treated with amitraz compared to untreated apiaries in each sampling month.



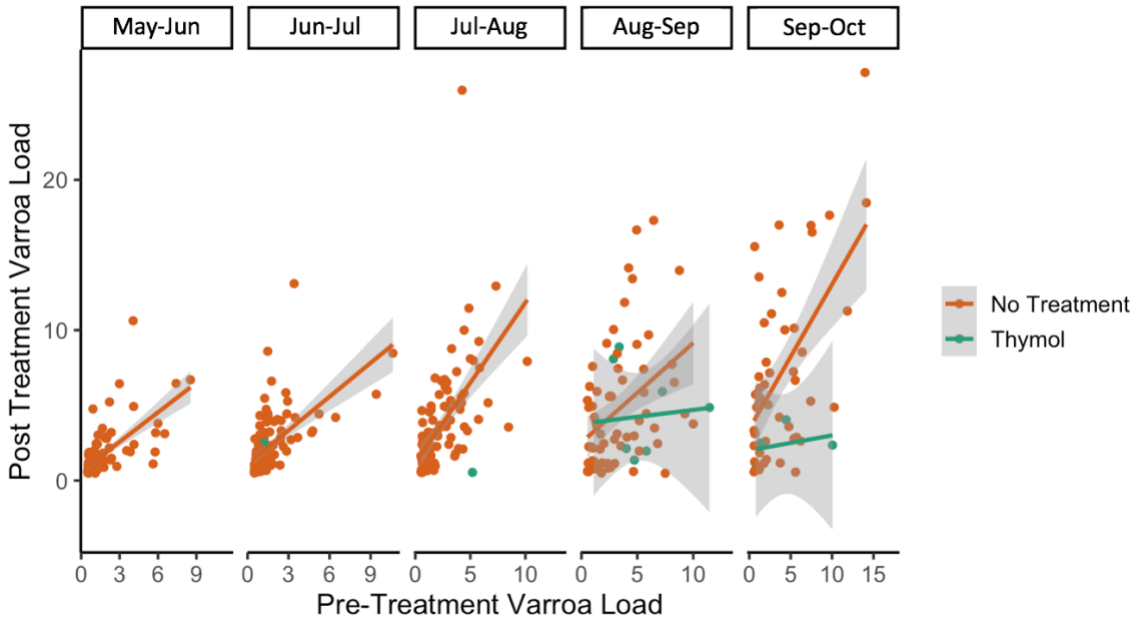
Supplemental Figure 3.2. Linear regressions for apiaries treated with a combination of treatments compared to untreated apiaries in each sampling month.



Supplemental Figure 3.3. Linear regressions for apiaries treated with formic acid compared to untreated apiaries in each sampling month.



Supplemental Figure 3.4. Linear regressions for apiaries treated with oxalic acid compared to untreated apiaries in each sampling month.



Supplemental Figure 3.5. Linear regressions for apiaries treated with thymol compared to untreated apiaries in each sampling month.

Chapter 4: A honey bee (*Apis mellifera*) colony's *Varroa destructor* population increases not because it robs, but because it is visited

Abstract:

A leading cause of honey bee colony mortality, *Varroa destructor* population growth rates exceed what is expected from *Varroa* reproduction alone, particularly in the fall. One possible explanation for rapid population increases is immigration of mite-carrying bees from other colonies. Here, the degree to which bees move between apiaries from high and low mite donor colonies, and resulting *Varroa* population changes in visited colonies were monitored. More bees from low mite colonies (n = 37) were detected in receiver apiaries than bees from high mite colonies (n = 10, $p < 0.001$). A receiver colony's *Varroa* population growth was associated with visitation by non-natal bees ($p = 0.03$). Finally, unscreened colonies experienced significantly faster *Varroa* population growth than their screened neighbors ($p = 0.01$). This data indicates that colonies were exposed to mites on visiting non-natal bees, not due to direct contact via robbing high mite colonies or visitation from high mite bees. This is a new possible route of horizontal transmission, suggesting that non-natal bees visit multiple colonies in one foraging trip, spreading mites to any colonies they visit. This study supports the notion that any untreated colony in the landscape can spread mites to its neighbors, and that landscape scale *Varroa* management is crucial for colony health and survival.

Introduction:

Honey bee provided pollination services to US crops are valued at over $14 billion [1]. Crop yields are influenced by the density and quality of honey bee colonies placed in fields and orchards [112-116]. However, high honey bee colony mortality rates threaten efficient pollinator dependent crop production [8, 51]. Though many colony health stressors have been identified over the past decade [5, 8, 26], the parasitic mite *Varroa destructor* has garnered special attention from researchers and beekeepers [12, 55, 117]. *Varroa* is particularly detrimental to colony health because it causes direct damage from feeding [53, 118], and indirect damage by vectoring viruses that weaken the colony [13, 93]. On average, beekeepers attribute only 20% of colony losses to *Varroa* in self-reporting surveys [65]. However, sampling of colonies for the USDA-APHIS National Honey Bee Disease Survey shows that over 50% of samples collected in the critical months of August-November have mite levels well above the recommended treatment threshold of 3 mites/ 100 bees [119]. This discrepancy is indicative of two larger issues: beekeepers underestimating their *Varroa* infestations and trying to manage infestations with repeated failure.

Management survey results show that between 2010 and 2018, 53% of backyard beekeepers (beekeepers with 1-50 colonies) did not treat for *Varroa*. This number has decreased each year (23% not treating in 2018), but treatment-free beekeepers historically experience a winter losses 12.5 percentage points higher than their treating counterparts (51.3% compared to 38.8%, respectively) [5, 65]. Lower colony loss rates are correlated with use of a common Varroacide product, which is expected considering the robust modeling and real world trials

that support this strategy as superior to not treating [12, 13, 15, 38]. Sociological surveys of this non-treatment group revealed that they believe honey bees perform best when left alone [67]. However, untreated colonies in a landscape crowded with beekeepers can represent a real risk of horizontal transmission, or the spreading of mites from heavily infested colonies to nearby apiaries.

Even among beekeepers who do monitor and treat for *Varroa*, infestation loads in the fall are often difficult to control. Long term studies on *Varroa* population growth over time, and mathematical models of *Varroa* population growth suggest that colonies can survive for three years with no *Varroa* control [105, 106, 120]. In reality, most beekeepers are required to use multiple *Varroa* treatments per year to keep levels below damaging thresholds [15, 65]. Further, longitudinal monitoring of *Varroa* loads in multiple apiaries across the US found that even after treatments, *Varroa* population growth rates often far exceed predicted rates from *Varroa* reproduction alone, and this discrepancy is not explained by within colony or apiary factors (see Chapter 3, [105]). This indicates that there is some probable immigration of mites from an outside source, most likely other colonies nearby.

Bees often drift between colonies, representing a potential route for *Varroa* transmission [121, 122]. Crowding of colonies within apiaries and in the landscape results in increased *Varroa* infestations as bees are more likely to move between colonies [84, 107]. *Varroa*-free colonies can be invaded by robbing and/or drifting bees from up to 1.5 km away, increasing the *Varroa* loads in affected colonies [79, 80]. It is possible bees drift into non-natal colonies due to

a demonstrated inhibition of homing abilities resulting from *Varroa* infestation, which makes it less likely for them to return to their natal colony [123]. Bees can also enter non-natal colonies intentionally in a phenomenon called robbing, when bees rob honey from other colonies when food resources are scarce [107]. Robbing can be especially detrimental in the late fall when colonies with unchecked *Varroa* infestations start to collapse. These weakened colonies with inflated *Varroa* loads are robbed by nearby healthy colonies, and mites are picked up by healthy colonies in the process [111, 124]. Late fall is a critical time period for beekeepers as they prepare for winter, ensuring food stores are adequate, mite loads are low, and colonies are healthy. Re-infestation of *Varroa* from non-natal bees during this period can undo the effects of a successful treatment. It is thus critical to understand the underlying mechanism of late fall inter-apiary mite transmission, so that effective management interventions can be developed.

To address this objective, the degree to which bees moved between apiaries from high and low mite donor colonies was monitored. Visitation to receiver colonies, and the effect of bee visitation to receiver colonies on mite levels was assessed. The effectiveness of robbing screens on minimizing *Varroa* population growth was also tested. It was hypothesized that more bees from high mite donor colonies would visit receiver colonies, colonies visited by high mite bees would exhibit accelerated *Varroa* population growth, and that colonies with robbing screens would experience slower *Varroa* population growths and visitation rates than unscreened colonies.

Methods:

Apiaries:

Two types of apiaries were established for this project: eight receiver apiaries and one donor apiary. Receiver apiaries consisted of four colonies each, housed in either a single deep brood box (n = 28) or one deep and one medium brood box (n = 4). All receiver colonies were established from splits with new queens in August 2019 to equalize colony strength and facilitate movement into the experimental location. Receiver colonies were moved into the experimental location on August 30th, and received a *Varroa* treatment (Mite Away Quick Strips, NOD Apiary Products, Alberta, CAN) from September 18th to September 24th to ensure low initial mite loads.

The donor apiary consisted of two high mite colonies (*Varroa* load > 3 mites/100 bees) and two low mite colonies (*Varroa* load < 1 mite/100 bees). These colonies were overwintered and selected based on results of alcohol washes performed in August. Low mite colonies received a formic acid treatment before the experiment began (September 18th to September 24th) to ensure a low initial mite load. The donor apiary was established at the experimental location on September 27th. Low mite colonies received a second formic acid treatment on October 3rd, to combat any *Varroa* increases they had incurred from their close proximity to the high mite colonies, as substantial drift within the donor apiary was likely. This additional mite treatment means that low mite donor colony mite population growth rates cannot be compared to receiver colony mite population growth rates.

All apiaries were placed at the Central Maryland Research and Education Center located at Clarksville, Maryland. The donor apiary was placed near the geographic center of the farm. Four receiver apiaries were placed approximately 0.8 km (0.5 mi) from the donor apiary. Four additional receiver apiaries were placed approximately 1.6 km (1 mile) from the donor apiary (Figure 4.1).

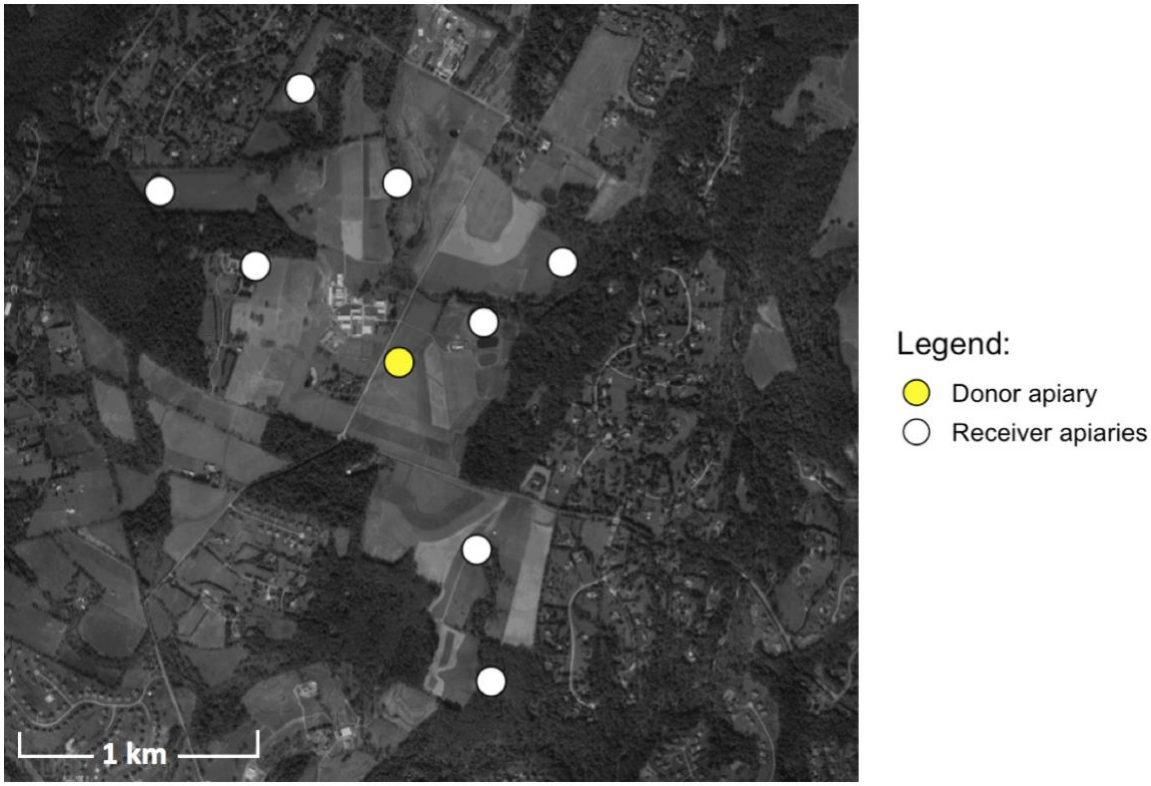


Figure 4.1. Map of donor and receiver apiary locations.

Painting bees:

To achieve maximum possible detection of bee movement between apiaries, as many bees in the donor apiary were painted as possible. A painting method that would not interrupt the bee or *Varroa* brood cycles was necessary, so the common method of painting emerging bees in the lab was rejected. Most methods of painting bees in the field involve placing a marker over the colony

entrance, but this method paints both foragers native to that colony and any robbing or non-natal bees who pass through the entrance. To ensure only bees originating from each donor colony were painted, the following method was developed.

All frames of adult bees were shaken into a plastic tub one at a time, covering the tub with its lid in between each frame. This resulted in containing the majority (~90%) of the adult bee population in the tub. The lid of the tub was then lifted just enough to scoop ~500 bees into a small plastic cylinder (Figure 4.2, CO_2 *Varroa* Tester: Logar Beekeeping Equipment, logar-trade.com). These *Varroa* testers have a small hole where CO_2 can be injected into the cylinder. The bees received CO_2 until they became unconscious, and were then poured out onto a flat surface for painting (Figure 4.3). The bees regained consciousness after about 15 seconds, but were disoriented and remained still enough to paint for up to 10 minutes. High mite colony bees were painted red, and low mite colony bees were painted blue (Sharpie Oil-Based Paint Marker). This process was repeated eight times for each donor colony, resulting in ~2,000 painted bees per colony. Painting occurred the day the colonies were moved to

Figure 4.2. Plastic cylinder with ~500 bees before CO_2 was injected.

the experimental location on September 27th, and again three weeks later on October 18th as an entire new brood cycle had emerged and the proportion of painted bees in the colony had decreased.

Figure 4.3. A batch of freshly painted bees on a flat surface. Here IPM sticky boards were used.

Camera sensors:

In preliminary trials of this experiment, it became evident that manually searching receiver colonies for painted bees was impractical. To overcome this hurdle, a camera sensor was developed to capture painted bees entering receiver colonies. A simple computer (Raspberry Pi 3B+) fitted with a camera module (Pi Camera 2) was programmed with OpenCV (Python 3) to detect user-specified colors. For this experiment, the RBG values associated with blue and red paint colors were used. A generous range of RBG values was used to

Figure 4.4. Receiver colony mounted with camera sensor.

account for variation in colors due to time of day, shade, or clouds. The cameras were programmed to capture a photo at 3 frames/second when they detected red or blue. Photos were saved with time and date stamps to help identify unique individuals. Colony entrances were reduced to limit the bees path of entry to within the camera's field of view. White cardboard was mounted under cameras to provide a neutral backdrop. All 36 colonies in the experiment (donor and receiver) were mounted with a camera sensor from September 28th through November 10th (Figure 4.4). Since many colonies were shaded for many hours per day, cameras were powered with 20,000 mAh high capacity power banks instead of solar panels. These batteries were changed and recharged daily for the duration of the experiment.

While cameras were mounted on all colonies, the robbing screens resulted in glare and interfered with the cameras' field of vision, so the data presented here is from cameras mounted on colonies without robbing screens only. Additionally, many cameras took an exorbitant number of photos (between 20,000-60,000). Because the cameras were programmed with a generous range

of RBG values to not miss any detections of painted bees in varying light, occasionally other colored objects in a camera's field of vision (*e.g.* grass, fallen persimmons, etc.) appeared blue or red and triggered photo capture. To eliminate irrelevant photos, if a camera contained a set of over 1,000 photos taken in a short period of time, this was deemed an unlikely true detection and ignored. Sets of photos that contained fewer than 1,000 photos were checked for true detections of painted bees. Unique individuals could be discerned with reasonable confidence because their paint marks were typically distinctive. This allowed the counting of the actual number of separate individual donor bees visiting receiver colonies.

Robbing screens:

To test the effectiveness of robbing screens as a preventative measure, screens were placed on 50% of receiver colonies. In each receiver apiary, one colony in the middle and one on the end of the row received a robbing screen (Mann Lake, Hackensack, MN). Robbing screens are metal mesh that block the regular colony entrance, and have a separate hidden entrance at the top of the screen. Only bees that live in the screened colony learn the new entrance, so non-natal bees are deterred from entering. Whether the left most or right most colony was screened was chosen randomly, but screening both end colonies was avoided, as unpublished data suggests that end colonies are more susceptible to receiving visiting bees. After the first screened colony was randomly chosen, a second colony not adjacent to the screened colony received a robbing screen.

Monitoring and sampling:

Donor and receiver colonies were monitored throughout the experiment for changes in *Varroa* load. An alcohol wash was performed at the beginning (September 24th) and the end (November 10th) of the experiment. Sticky boards placed under each receiver colony were changed and counted approximately every three days, dependent on weather. Each receiver colony was also manually checked for painted bees at the middle (October 23rd) and end (November 10th) of the experiment. Manual checks consisted of removing and visually inspecting every frame in each colony for painted bees.

Donor colonies were checked once a week to monitor for paint retention and for colony size. The proportion of the population that was still painted was visually assessed as a percentage of the total adult bee population. When ~50% of the bees in a colony were unpainted (3 weeks and one brood cycle later), a second round of painting was performed. Colony size was assessed by the standard method of a frames of bees estimate by observing the top bars of each colony [69]. The goal of this study was to continue until either the high mite donor colonies collapsed (and the movement of bees from collapsing colonies could be tracked) or until the weather became so cold that bees no longer foraged regularly. Under these guidelines, the experiment was conducted from September 18th-November 10th.

Statistics:

All statistical analyses were performed in R (version 3.3.3). Summary statistics are reported as mean ± SE unless otherwise stated. Student's t tests

were used to check if *Varroa* loads and colony size were the same between treatment groups at the starting point of the experiment. Pearson's Chi-squared test was used to check for differences in mite loads between colonies within the donor apiary, as well as in painted bee detections of each color in receiver colonies. Generalized mixed effects models with apiary as random effects were used to compare between groups at different time points (start and end or number of experimental weeks). Models were eliminated in a stepwise fashion using ANOVAs until the simplest best fit model was identified. Spearman's correlations were performed to check for correlations between the number of painted bees detected and the starting *Varroa* load.

Results:

Donor colony adult bee populations:

The experiment began on Sept 28th, 2019 with all donor colonies at the same population size (Figure 4.5, 12.5 ± 0.5 frames of bees, $t = -1$, $df = 1$, $p = 0.5$), and while high mite donor colonies did lose more population than low mite donor colonies, they did not collapse before Nov 10th, when weather no longer warranted continued observation. At the end of the experiment, high mite colonies were functionally dead (less than 1 frame of bees) and were significantly smaller than the low mite colonies (high mite 1.75 ± 1.25 vs. low mite 6.0 ± 0.35 frames of bees, $F_1 = 12.49$, $p < 0.001$). All 32 receiver colonies survived the entire length of the experiment.

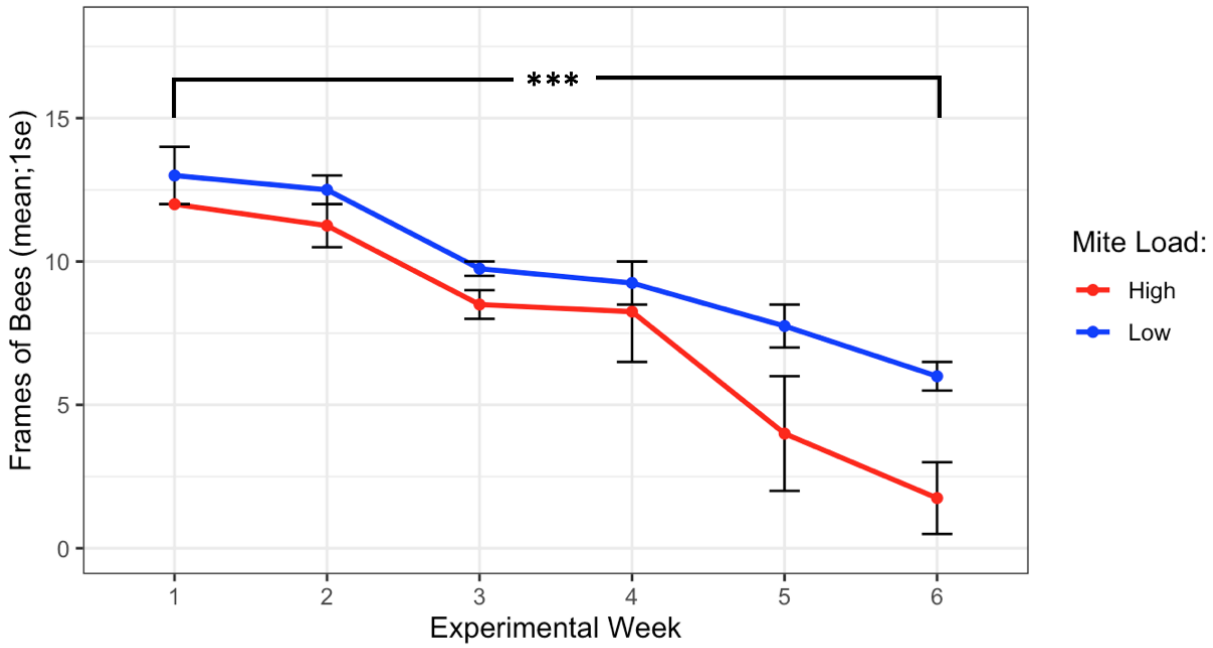


*Figure 4.5. Colony population sizes in high and low mite donor colonies over each experimental week. Low mite colonies were significantly larger than high mite colonies over the duration of the study (glm ***p < 0.001).*

Detections of donor bees in receiver apiaries:

In total, 47 unique painted bees were detected by the 16 camera sensors on unscreened colonies. Considering ~2,000 bees were painted in each of the 4 donor colonies at two time points, a total of 16,000 bees were painted. Thus the 47 bees detected equal a 0.29% recovery rate. Despite the fact that high mite colonies lost more population than low mite colonies, of the 47 detections, more low mite bees were detected (n = 37) than high mite bees (Figure 4.6, n = 10, χ_2= 15.5, df = 1, p < 0.001). Painted bees were detected in 62.5% (n = 5) of receiver apiaries and at 56.3% (n = 9) of non-screened receiver colonies.

There was substantial drift of bees between colonies within the donor apiary. Donor colonies were mounted with cameras, and the number of non-natal

bees detected was higher than could be quantified (hundreds in each donor colony camera). The two manual checks performed of receiver colonies for painted bees did not result in any detections, indicating painted bees did not permanently remain in non-natal colonies. With only one detection in apiaries placed at the further 1.6 km radius, donor bees were much more likely to visit closer apiaries then further apiaries (χ_2= 43.1, *df* = 1, *p* < 0.001). The visited apiary at the further radius only received one visitor to one unscreened colony. In all other apiaries that received donor bee visitors, both unscreened colonies were visited.

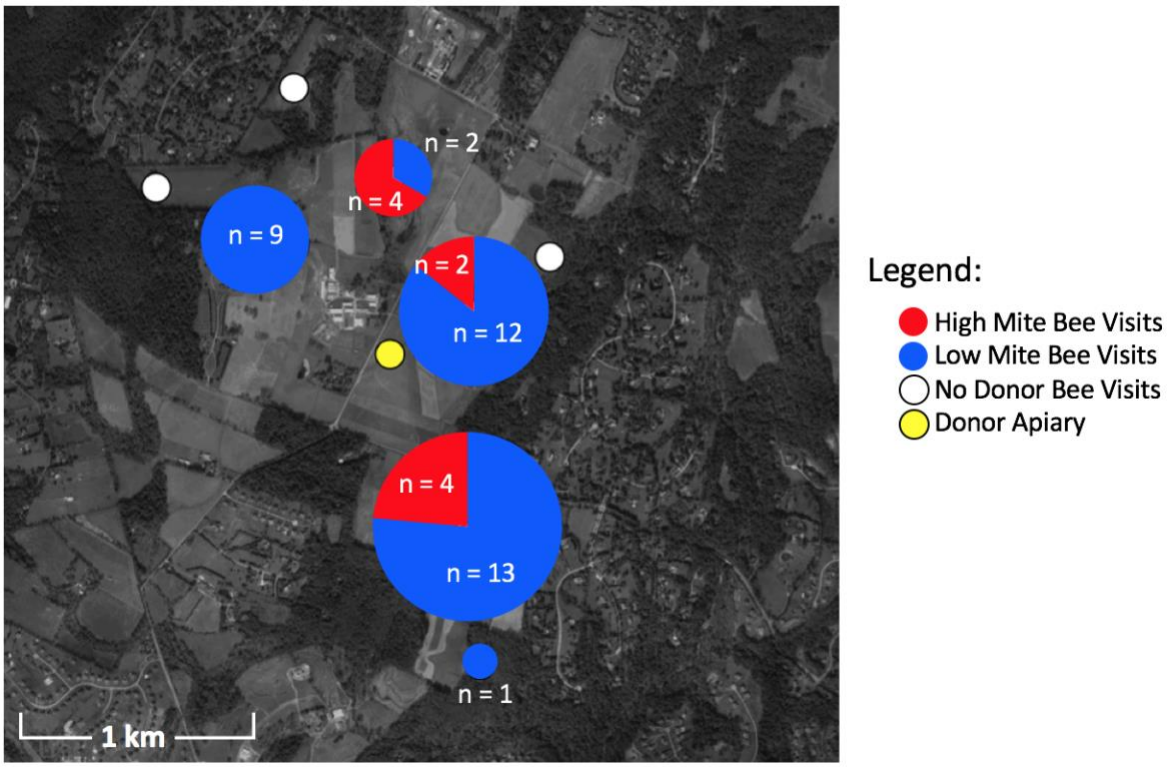


Figure 4.6. Location, number, and color of painted bee detections. Pie charts represent the number of high and low mite bees detected in each receiver apiary. White circles are receiver apiaries where no painted bees were found, the yellow circle indicates the donor apiary.

Varroa loads:

Varroa loads in low mite donor colonies remained low throughout the study (from 0.17 ± 0.17 to 1.95 ± 0.10), while *Varroa* loads in high mite donor colonies grew substantially (from 9.57 ± 5.12 to 34.8 ± 29.41). The two high mite colonies started with significantly different mite infestations (Supplemental Table 4.1, (χ_2= 5.5, df = 1, p= 0.02), but their mite loads were always higher than in low mite colonies (Figure 4.7, χ_2= 29.4, df = 1, p < 0.001). *Varroa* loads in all receiver colonies increased over the duration of the study (from 0.91 ± 0.22 to 1.94 ± SE 0.32, p < 0.001).

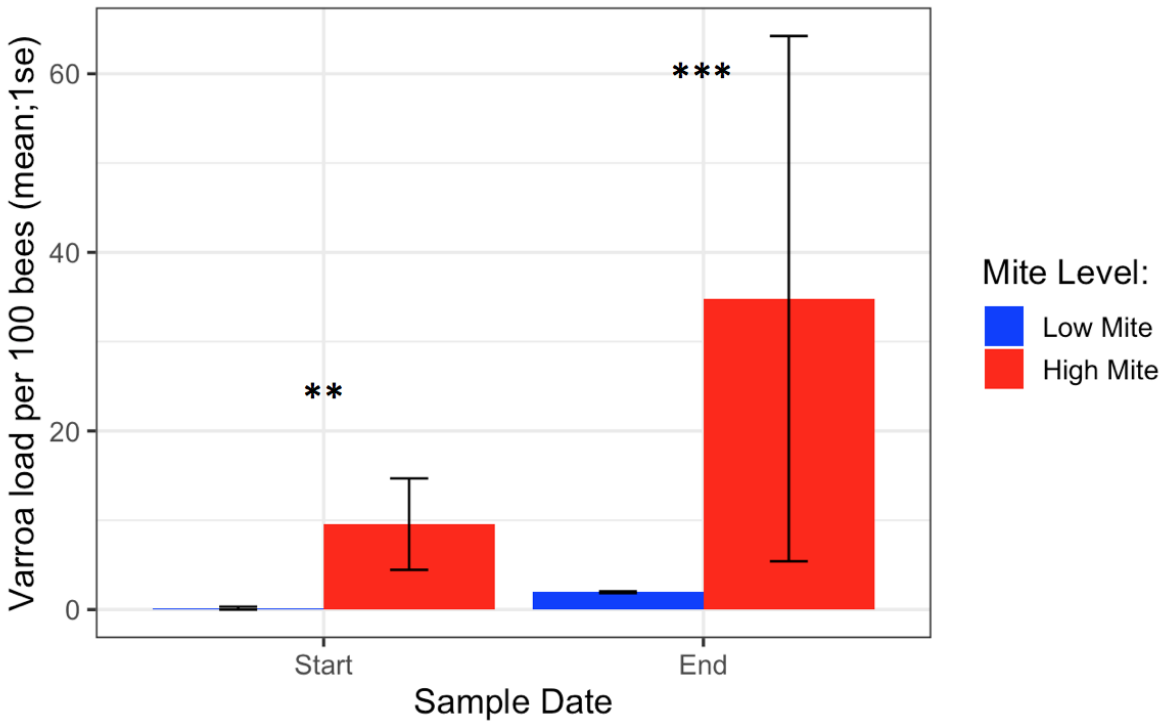


*Figure 4.7. Varroa loads in low vs. high mite donor colonies at the start and end of the experiment. High mite colonies had significantly more mites than low mite colonies throughout the study (χ_2 **p < 0.01, ***p < 0.001).*

Receiver colonies that were visited by high mite donor bees started the study with similar *Varroa* loads to colonies that were not visited by high mite donor bees (t = 0.80, df = 9.4, p = 0.45). Whether a high mite donor bee visited a receiver colony did not affect the receiver colony's mite population increase over the duration of the study (Figure 4.8, F_1 = 1.42, p = 0.19).

Receiver colonies that were visited by any donor bee (from high or low mite donor colonies) also started the study with similar mite loads to unvisited receiver colonies (t = 1.34, df = 9.33, p = 0.21). However, *Varroa* loads in colonies that were visited by any donor bee increased significantly faster than colonies not visited by donor bees (Figure 4.9, F_1 = 4.57, p = 0.03). Within apiaries that were visited by donor bees, there was a positive correlation between a colony's starting mite load and the number of non-natal bees it received (Figure 4.10, Spearman's r = 0.62, p = 0.05). However, an increased number of visitors did not result in accelerated *Varroa* population growth (Spearman's r = -0.14, p = 0.71). *Varroa* population growth was only associated with whether a colony was visited, and not the total number of visitations.

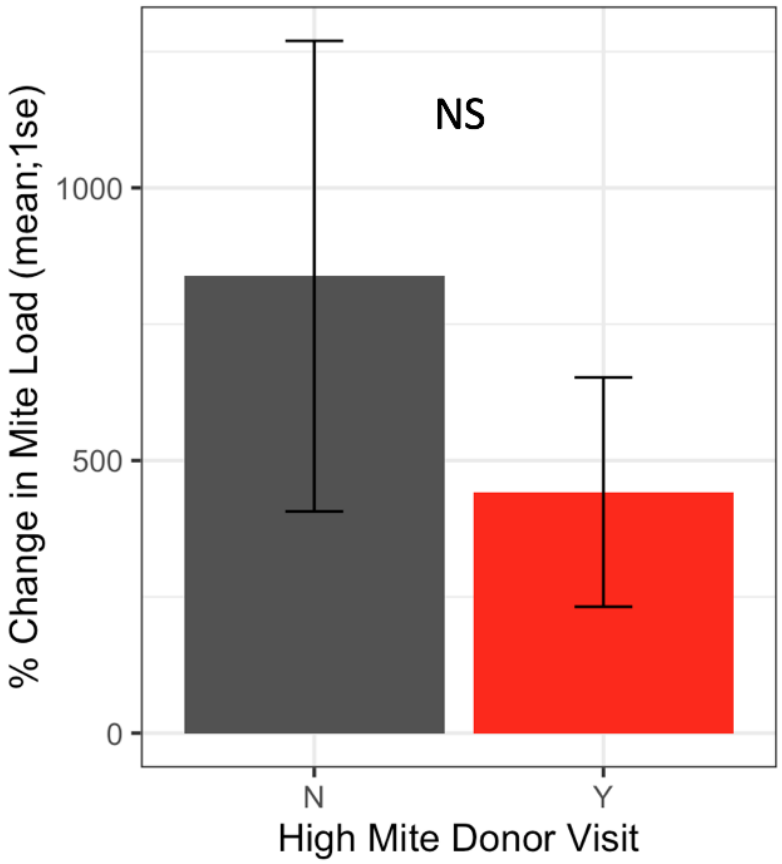


Figure 4.8. Percent change in Varroa loads in colonies that received red bees compared to colonies that did not receive red bees. There was no difference in percent change between colonies visited by high mite bees and unvisited colonies at the start or end of the study (glm p = 0.19).

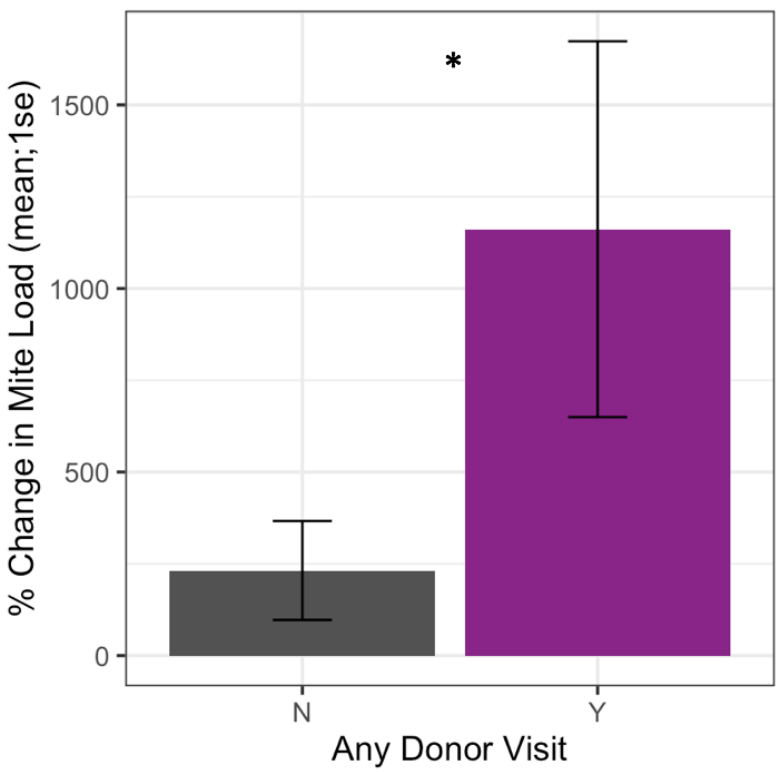


*Figure 4.9. Percent change in Varroa load in colonies that received any painted bee (blue or red) compared to colonies who did not receive any painted bee. Colonies that were visited by painted bees experienced significantly faster Varroa population growth than unvisited colonies (glm **p = 0.03).*

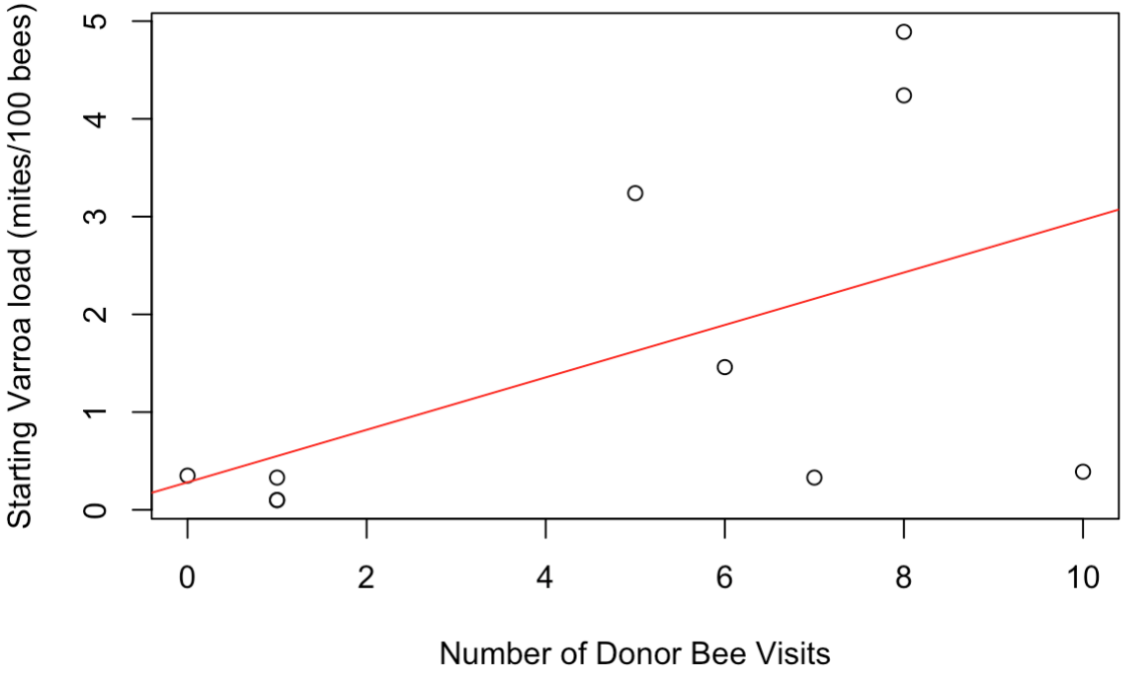


Figure 4.10. Correlation between a colony's starting Varroa load and the number of donor bee visitors it received. Colonies with higher starting Varroa loads received more visitors than colonies with lower starting Varroa loads. Spearman's r = 0.62, p = 0.05.

Robbing screens:

Alcohol wash *Varroa* counts from receiver colonies with and without robbing screens were not different at the start of the experiment (t = -1.61, df = 22.2, p = 0.12). However, colonies with robbing screens had significantly lower increases in *Varroa* population (Figure 4.11, F_1 = 6.16, p = 0.02). Sticky board *Varroa* counts show a similar trend, with starting counts not differing between colonies with or without screens (Figure 4.12, t = -0.99, df = 29.8, p = 0.33). Sticky board counts over the whole experiment show that colonies with robbing screens had consistently lower *Varroa* loads than colonies without robbing screens (F_1 = 14.31, p < 0.001). Sticky board mite counts in the first experimental week were significantly higher than any other week (first week 6.35 ± 0.98 vs. other weeks 1.71 ± 0.12, t = 4.69, df = 31.9, p < 0.001). This is likely due to residual mite drop from the formic acid treatment that ended one day before sticky boards were placed on colonies.

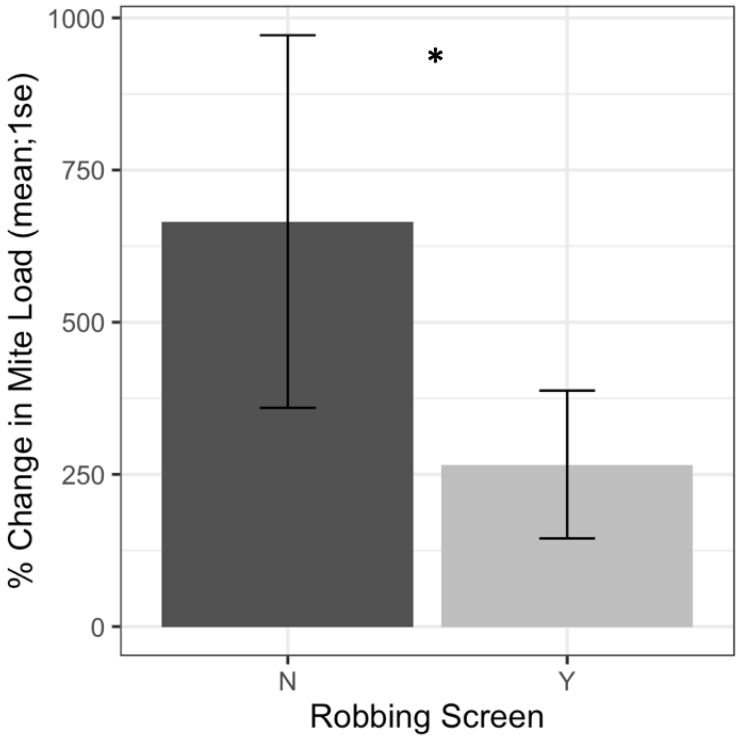


*Figure 4.11. Percent change in Varroa loads in colonies with and without robbing screens. Colonies with robbing screens had reduced increases in Varroa population compared to unscreened colonies. (glm *p = 0.02).*

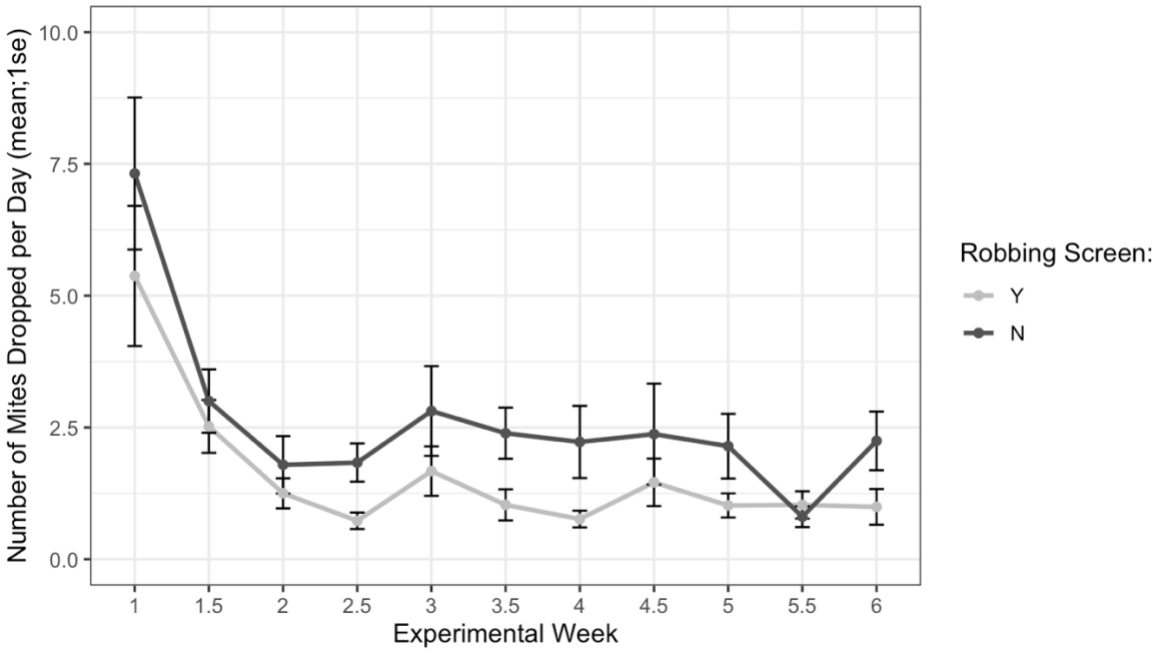


*Figure 4.12. Sticky board Varroa loads in colonies with and without robbing screens over each experimental week. Colonies with robbing screens had fewer mites on sticky boards than colonies without screens (glm ***p < 0.001).*

Discussion:

This study hypothesized that more bees from high mite donor colonies would visit receiver colonies. However, the majority of bee visitations were from low mite donor colonies. Despite the finding that high mite donor bee visitation was low, visited receiver colonies experienced accelerated *Varroa* population growth compared to unvisited colonies. Additionally, screened colonies exhibited reduced *Varroa* population growth compared to unscreened colonies. Bees visiting other colonies transfer mites in the process whether their own colony's mite load is high or not.

Past studies have implicated robbing or drifting as important contributors to *Varroa* population growth, however, these studies failed to track bees [79, 80]. Studies which have tracked bees have done so at small distances and/or with fewer colonies [111, 122, 124]. This study is the first to track bee movement and resulting mite population changes over large distances within several apiaries and colonies. Closer apiaries were more subject to visitation by bees from the donor apiary, with only one detection occurring at the 1.6 km radius. This can be explained by energetic optimization of foraging, when foragers expend as little energy as possible seeking resources as nectar and pollen become scarce in the late fall [125, 126]. Colonies that were visited experienced more significant *Varroa* population growth, indicating that visiting bees may vector mites to receiver colonies. The increase in *Varroa* loads was due to visitation by non-natal bee.

The finding that increases in *Varroa* load were not due to contact with bees from high mite colonies suggests that a high mite colony does not need to

crash and send bees into the landscape to impact *Varroa* loads of other colonies. This conclusion is supported by prior work which found that high mite colonies were not more likely to produce drifted bees than low mite colonies, but rather high mite colonies were more likely to receive non-natal bees than low mite colonies [124]. Further, a study which tracked the movement of bees between high and low mite apiaries and the resulting change in mite populations found that large numbers of mites spread to low mite colonies via their own bees that were robbing the crashing high mite colonies [111]. This study builds on this prior work by testing these hypotheses at a larger scale and with more colonies, and agrees with the finding that *Varroa* horizontal transmission is not primarily occurring from a “mite bomb” phenomenon where crashing colonies send bees and mites to neighbors. However, the present study disagrees with prior work, indicating that *Varroa* is transmitted to colonies via healthy bee visitation, and not as a result of a colony’s own robbing of high mite donors.

The robbing screen results support the assertion that increases in mite populations were not a result of colonies bringing home mites after robbing. Colonies with robbing screens experienced reduced *Varroa* population growth, which would not have occurred if natal bees were bringing home mites. Natal bees are not deterred by screens, indicating that non-natal visiting bees are a more likely source of immigrating mites. This is possible if non-natal bees are visiting multiple colonies, as mites can switch phoretic hosts or enter a brood cell within seconds [12, 127]. Because of their close proximity to the high mite colonies, bees from low mite colonies were almost certainly visiting their weak

neighbors, and much mixing between donor colonies was observed [80, 107]. However, the *Varroa* load in low mite colonies remained low throughout the experiment, indicating that visiting low mite donor bees did not bring home a significant number of mites. Thus, bees from low mite colonies may have visited high mite colonies, and then visited receiver colonies before returning home, transmitting mites from high mite colonies to other apiaries in the process.

It also appears that non-natal bees visit the most vulnerable colonies, and a colony's *Varroa* population growth is related to its attractiveness to visitors. The number of visitations to screened colonies is unknown, but their reduced *Varroa* population growth indicates that they were visited less often because they were less accessible. If all colonies in an apiary were screened and no unscreened, easily accessible colony was nearby, visiting bees may be more persistent and find ways to enter screened colonies. Additionally, the number of visitors a colony received was positively associated to its starting mite load, indicating that elevated *Varroa* loads can make colonies more susceptible to non-natal bee visitation. This could be a result of reduced colony size or strength either caused by or resulting in elevated mite loads, which could affect a colony's ability to defend itself against intruding bees. Regardless of a colony's initial mite load, visitation by non-natal bees resulted in accelerated *Varroa* population growth. It appears that colonies with high *Varroa* loads are more susceptible to visitation, which results in further accelerated *Varroa* population growth.

This experiment confirmed prior observations of bees moving between apiaries, and resulting increases in *Varroa* loads. However, a unique

phenomenon was observed of mite loads increasing in colonies that were visited by non-natal bees, not as a result of a colony directly picking up mites by visiting other colonies. There were also promising results that robbing screens can help interrupt horizontal transmission of mites, which may be an effective management option. Regardless of how the horizontal transmission of mites occurs, the outcome of increased *Varroa* loads in the late fall is detrimental to a beekeeper's attempts to manage *Varroa*. An untreated colony in the landscape represents a significant risk to beekeepers in the area. In the future, cooperative *Varroa* management will likely become increasingly important to improving colony health and survival. As such, beekeeping communities should work together for active *Varroa* management and coordinate the timing of treatments for maximum effectiveness.

Supplemental Figure:

Supplemental Table 4.1. Mite loads in each donor colony at the start and end of the study. These mite loads were counted from samples of 300 adult bees by alcohol wash.

Colony	***Starting Varroa load (mites/100 bees)***	***Ending Varroa load (mites/100 bees)***
Low Mite Colony 1	0.00	2.04
Low Mite Colony 2	0.33	1.85
High Mite Colony 1	4.45	14.69
High Mite Colony 2	14.69	64.22

General Conclusion

This dissertation aimed to characterize effectiveness of US beekeeping management practices, and identify obstacles to successful *Varroa* management. This objective was approached in four steps. The first step, Chapter 1: A national survey of managed honey bee 2015–2016 annual colony losses in the USA, established the level of colony losses challenging US beekeepers, as well as leading causes of colony mortality. Beekeepers lost an average of 37.7% of their colonies over the winter, almost double their acceptable loss rate of 19.0%. *Varroa* was the most commonly reported cause of colony loss, followed by queen failure and colonies being weak in the fall. High rates of colony mortality, often due to *Varroa* infestations, were of express concern to US beekeepers.

The next step after identifying colony losses and prevalent colony health stressors was to test potential preventive strategies. Empirical best management practices derived from four years of survey data were tested for 3 years compared to average beekeeping practices. Apiaries treated according to best practices exhibited reduced *Varroa* loads from May-October, and exceeded the treatment threshold of 3.0 mites/100 bees one month later than apiaries treated according to average practices. The benefits of reduced *Varroa* infestation were apparent in the fall, when best apiaries exhibited significant reductions in the intensity of viral infections. After 3 years, best apiaries produced more honey and splits, and experienced a 30 percentage point reduction in winter mortality. The cumulative effects of colony health stress and management compounded over

time, demonstrating that beekeepers should be patient when implementing new practices. This study validated the importance of proactive *Varroa* management to improve colony health and reduce mortality.

After validating that active *Varroa* management was necessary for colony health, Sentinel Apiary Program data was used to characterize current *Varroa* population growth and associated management practices among US beekeepers (Chapter 3). *Varroa* loads fluctuated over the season, exceeding treatment threshold in August, September, and October. The population growth rates observed well exceeded the model-predicted rate of a 100% monthly increase in all months after May. Alarmingly, recently treated apiaries only exhibit lower *Varroa* loads and *Varroa* population growth than untreated apiaries in the fall months. Even then, recently treated apiaries were still above threshold on average, and experienced population growth rates of over 100%. Treatments were slowing the rate of increase in *Varroa* load, but were not providing the expected level of control.

Evidence suggested that the treatments themselves were not failing, but rather rapid increases in *Varroa* loads during treatment applications made treatments appear unsuccessful. Potential sources of these rapid *Varroa* increases were explored. Treating less than all colonies in an apiary, and the treatment method used had no effect on treatment outcome. The reduction in capped brood explained some but not all of the increased in *Varroa* load. It is likely that excessive mites were immigrating from an outside source: highly infested colonies in the landscape.

The final step in investigating obstacles to successful *Varroa* management was to assess the level of mite immigration between apiaries (Chapter 4). Bees frequently visited colonies in other apiaries, and visited colonies experienced increased *Varroa* population growth. These increases were not due to direct contact with high mite colonies (via robbing or visitation by high mite bees), but were associated with visitation by any non-natal bee. Robbing screens reduced the rate of *Varroa* population growth in visited apiaries. These results suggest that healthy colonies visiting high mite colonies can vector mites to subsequent colonies they visit, and that any high mite colony in the landscape represents risk to beekeepers nearby.

This dissertation demonstrates that beekeepers have the power to mitigate some of their colony losses through the application of good management practices. This process requires patience, but it is possible. However, even with best management practices, some level of colony loss is inevitable. Here, *Varroa* treatments did not yield expected results, leaving many apiaries above treatment threshold in the fall. Lack of treatment success due to landscape level *Varroa* transmission represents a threat to any beekeeper within the flight radius of their neighbor's apiary. Active, creative, and cooperative *Varroa* management is likely to become increasingly vital to beekeeper success in the future.

Appendix

The Bee Informed Partnership

Using beekeepers' real world experience to solve beekeepers' real world problems
Be Included, Be Involved, Bee Informed

Protocol for Real Time Disease Load Monitoring

(Sentinel Apiary Program)

Overview:

By monitoring disease levels over time you, the beekeeper, will be able to make better decisions about when to treat colonies and if treatments are effective. Participating beekeepers will be asked to collect samples from 8 colonies once a month over the sampling season. These samples will be sent to the University of Maryland and processed to determine Varroa and Nosema levels. Each sampling involves opening the eight colonies (the same eight colonies are sampled each period) and removing one frame that contains young, developing brood. Adult bees from this frame are then collected following the standardized method described in this document and placed into sample bottles containing a salt water solution. You will collect two, ¼ cup scoops of bees from each hive. You will pour these two scoops of bees into the provided sample bottle and cap them. You will repeat this procedure for each of the 8 hives. In summary, you should leave the apiary with eight sample bottles full of bees and one data sampling sheet. You will finally send the 8 samples to the University of Maryland Diagnostic Lab for analysis.

More details about the Sentinel Pilot is available at
http://beeinformed.org/programs/sentinel-hive-scale-program/
This sampling protocol is based off of the USDA AHPIS National Honey Bee Survey. For additional information on this effort please visit http://beeinformed.org/aphis/

Please read this protocol carefully prior to initiating sampling. For additional information, email danrbrl@umd.edu or kkulhane@umd.edu or leave us a message on voice mailbox at 301-405-3799 and we will return your message promptly.

Materials:

You received 6 months (or 6 sampling periods) worth of sampling material. These kits include:

Material (Figure 1):	**Quantity:**	**Checklist:**
Hive tags	10	☐
¼ cup measuring cup	1	☐
Funnel	1	☐
Shipping boxes	6	☐
Pre-addressed mailing labels (to UMD Bee Lab)	6	☐
Sampling Data Sheets	6	☐
125 mL bottles with salt water	48	☐
Gallon zip lock bags	6	☐

Figure 1: Sampling supplies that will be mailed to you

You will also need:

- ☐ A staple gun (to affix the hive tag) or nails
- ☐ Postage to return the sample kits (estimated cost: $12/month)
- ☐ Washtub (optional)

STEP 1: Select 8 colonies

1. Select 8 random colonies located in the same apiary to start the sampling survey. It is important you select colonies of differing strengths, so we can obtain an accurate representation of disease levels in your apiary.

2. Affix the unique colony tag to each hive (see Figure 2). It is vital to sample the same 8 colonies throughout the duration of the season. Note that you have received 10 tags. Use only 8 initially and save the spare two tags in the event one or two of the 8 colony dies and you need to tag another colony.

NOTE: IF A COLONY DIES DURING THE SAMPLING PERIOD, REMOVE THE SAMPLE TAG AND USE ONE OF THE SPARE SAMPLE TAGS FOR A NEW COLONY IN THE SAME APIARY. If you use the last sample tag, request more. Please do not reuse sample tags.

Figure 2: Colony ID Tag

3. Fill out the required apiary location information on the data collection sheet.

Sampling Steps:

You will need to repeat the following steps every month, around the 15th of the month ± 1 week. Try to sample around the same time each month.

STEP 2: Sampling in the apiary

1. As you normally would, open the selected colony to the brood nest and examine for disease and queen status/condition. Record any disease/queen status, or unusual conditions present on the data information sheet.
2. Remove the lid from one of the sample bottles and place the funnel in the 125 mL bottle filled with the salt water solution (Figure 3).

Figure 3: Sample bottle with funnel

3. Find a frame containing young, developing brood.
4. Carefully examine the frame to ensure the queen is not on this frame. You don't want to collect her!
5. Gently scrape two, ¼ cup scoops of adult bees (about 300) from the brood frame (Figure 4) and place them into the funnel (Figure 5). Gently knock the bottle and funnel to get the bees to fall through the funnel and into the solution. 2, ¼ cups of bees should fill more than half of the bottle. Alternatively, if you have a wash tub, shake the bees from the frame into the washtub, gently knock the tub so the bees collect in the corner of the tub and scoop the bees from the tub (Figure 6). Then place the bees into the funnel as described above.

Figure 4: Scooping bees off the brood frame

Figure 5: Moving bees from measuring cup to sample bottle

Figure 6: Scooping bees from wash tub

6. Close the bottle tightly; shaking it to make sure the bees are fully dampened with solution. Please note that this colony number MUST match the colony number listed on the data collection sheet you have filled out.
7. Repeat steps one through six until eight colonies have been sampled.

STEP 3: Sending the samples

1. Double check that all the lids on the bottles are tightly in place and all bottles are labeled.
2. Place the 8 sample bottles (containing bees) into a large Ziploc bag to contain any leaks from the solution before placing them into the shipping box (Figure 7)**.**

Figure7: Packaging the 8 sample bottles for return shipment to UMD

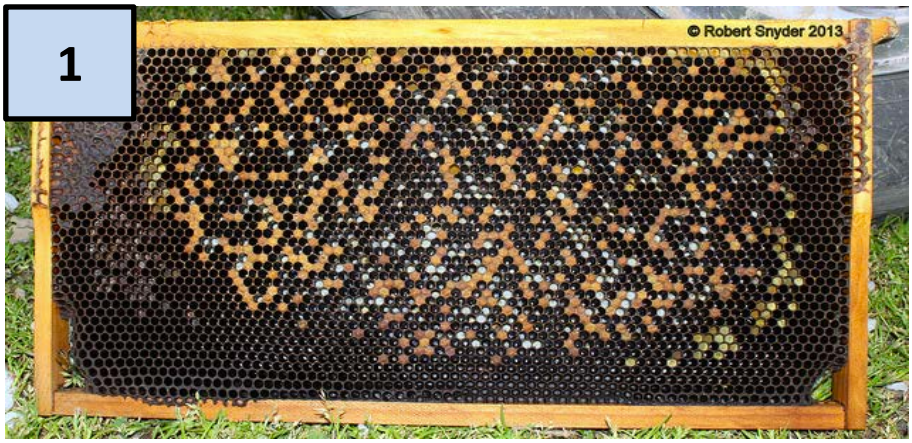
3. Ensure data collection sheets are completely filled out and legible and place in the shipping box.
4. Place the mailing label (Daniel Reynolds, University of Maryland, 4291 Fieldhouse Dr. College Park, MD 20742) on the shipping box. Write FROM and your return address on the upper left corner of the box.
5. Email us at askbeeinformed@gmail.com within 24 hours of shipment to notify personnel that a shipment is expected.

You should receive a report on the disease levels within 2 weeks from the day the UMD lab receives your sample. We will email electronic reports to you each month.

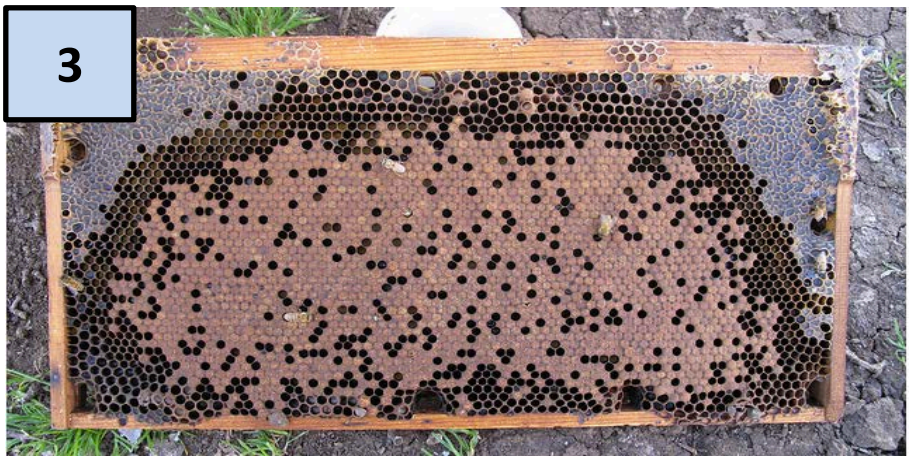
Sentinel Apiary Program – Determining Brood Pattern

Brood pattern ranges from 1-5, 1 being the poorest/most spotty and 5 being the most solid or with the fewest open cells. Often a rating of 1 indicates a brood disease or queen issue. Rating of the brood pattern is only relevant during the brood production season.

1



3



5

Sentinel Apiary Program – Determining Queen Status

Queen status can be confirmed siting the queen or eggs that appear normal (i.e. not multiple eggs per cell or eggs on the side of a cell, indicating laying workers). While a visual of the queen before sampling is ideal, it is not necessary as it will slow sampling down considerably. Check your frame to be sampled until you are confident the queen is not on it, shaking off some bees if necessary. If there are too many bees on the frame you risk missing the queen.

Abbreviation	Queen Status	Description
QS	Queen Seen	Queen seen while sampling
QR	Queen Right	Queen was not seen, but eggs are seen
QNS	Queen Not Seen	Queen and eggs not seen, but looks queen right
VQ	Virgin Queen	Queen seen, but appears new, may not be laying yet
DL	Drone Layer	Lots of drone brood interspersed with worker brood
QL	Queen Less	Queen and young brood not found
LW	Laying Worker	Queen dead; Multiple eggs per cell, often on the side walls

Sentinel Apiary Program – Frames of Bees (FOB)

This is the first colony-grading measurement that should be taken. After initially gently smoking the colony, (let's assume it is two deeps), hinge the top box up and gauge how many full frames of bees are in the bottom box from the appearance of the top-bars and how many full frames of bees are in the top box from the appearance of the bottom-bars. You may adjust their FOB estimate as they work the colony.

Look to see how completely the bees are covering the frames from end to end, how far down the bees go, and how crowded the bees look. You need to count how many frames are filled with bees, and then subtract the frame portion with no bees. On the photo at the left, there are 9 total frames, with about 7 totally covered in bees and the outer 2 partially covered. In this case each of the outer 2 frames is counted as a half. This box has 8 frames of bees.

By only looking at the top of the frames, you cannot tell if the bees go all the way to the bottom of the frames and you may overestimate the frames of bees. Tipping up the box to view the underside shows you if the frames are really full with bees or if the bees don't go all the way to the bottom of the frames. Counting both the top and bottom of frames gives a much better estimate of the actual frames of bees. The left image (a.) has about 5.5 frames of bees and the right image (b.) has approximately 1.5 frames of bees.

Sentinel Apiary Program – Disease/Symptom and Pest Guide

Correct observation and identification of pest/symptoms, can save a considerable amount of time and headache. Below are some of the more common diseases and pests associated with Honey bee health. Feel free to use these abbreviations on your data sheets. Due to space restrictions detailed photos would not fit. A visit to beeinformed.org or a Google search will provide more detailed images.

Abbreviation	Disease/Pest		Description
AFB	American Foulbrood		Spotty brood, perforated cells, brown sunken larvae, rotting odor, larval roping, brown/black scale
CDB	Chewed Down Brood		Usually a symptom of PMS, or an indication of poor colony health.
Chalk	Chalkbrood		Spotty brood, chalk-like mummies at entrance or in open brood
EFB	European Foulbrood		Twisted/curled, whitish-yellow/brown deflated larvae, can have sour smell, can have larval roping
PMS	Parasitic Mite Syndrome		Spotty brood, Varroa on adult bees, aggressive colony behavior, mites visible in open brood cells, low adult population
SBV	Sacbrood Virus		Perforated sealed brood, pupa with underdeveloped head
SHB	Small Hive Beetles		Pest that can cause significant damage to comb, honey, and pollen.
WAX	Waxmoth		Pest that can cause significant damage to comb and stored equipment.

BIP Sentinel Apiary Program– Sample Data Sheet

Date:

Since your last sampling, did you move this entire apiary? ❍Yes ❍No
If yes, please provide new address and GPS information.

Apply label

Bottle	Colony TAG Number	Queen status (*)	Brood Pattern (0 to 5)	Colony configuration: Number of Boxes	Colony configuration: # Frames	Number of frames of adult bees (**)	Particular observation? (disease, pest, brood pattern, queen cells, unusual circumstance observed…)	Mark (X) the colony with: Hive scale	Mark (X) the colony with: Pollen trap	Since **your last visit**: did you… (circle your answer) requeen this colony?	move this colony?	feed this colony?	treated this colony?	Perform management?
1		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No
2		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No
3		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No
4		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No
5		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No
6		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No
7		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No
8		❍ QS ❍ DL ❍ QR ❍ QL ❍ QNS ❍ LW ❍ VQ		super shallow medium deep	5 8 10					Yes No	Yes No	Yes No	Yes No	Yes No

(*) Queen Status: **QS** (Queen Seen) ; **QR** (Queen Right: did not see the queen but found eggs) ; **QNS** (Queen Not Seen, nor eggs, but looks queen right) ; **VQ** (Virgin Queen: queen seen but appear new) ; **DL** (Drone Layer) ; **QL** (Queenless) ; **LW** (Laying Worker) ; or other if you know.

(**) Estimation of FRAME number: Frame count includes front and back or a frame (if a frame is covered front and back, it counts as 1 frame);

Hive Provenance

Please fill this in for all 8 (or 4) colonies **on month 1** and only for **new replacement** colonies in the following months (**skip this page** if no new colony)

Colony TAG number	What is the origin of this colony? (choose one)	If this is a new colony, when did you install it? (date)	How old is the queen? (month /year)	When did you **last** treat this colony for varroa?		When did you last used antibiotics and/or Nosema treatment?		What hive type is this colony in? (*)
				Date	Product	Date	Product	
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							
	❍ overwintered colony ❍ other: ❍ new from split ❍ new from package							

(*) Hive type: Langstroth hive body 10 frames; Langstroth hive body 8 frames; Warre; Top bar hive; Nuc box; Homemade (not Langstroth dimensions); …

Visits | **In the Sentinel apiary**

Since the last sampling visit, how many times on average did you open your Sentinel colonies? (including the visits to take samples for this project)

and why? ❒ Normal seasonal management ❒ Monitor for pests / disease ❒ Feeding
❒ Applying chemical treatments ❒ Displaying other pest control techniques ❒ Honey production

Demographics | **SINCE YOUR LAST SAMPLING VISIT** | **In the Sentinel apiary**

On this date (sampling), how many living colonies* do you have in the Sentinel Apiary?
* a colony is a QUEEN RIGHT unit of bees (include full size colonies, queen right nucs but NOT mating nucs); "Living" means alive on this date, independent of future prospects

How many colonies, splits or increases did you make/buy **since your last sampling visit**?

How many colonies, splits or increases did you sell/give away **since your last sampling visit**?

How many Sentinel colonies did you lose **since your last sampling visit**?

In this Sentinel apiary, did you catch and install any **swarm**? ❍ Yes ❍ No

If you did catch a swarm, was it your own? ❍ Yes ❍ No ❍ Don't know

In this Sentinel apiary, did you install any new package or colony? ❍ Yes ❍ No

If you did, were did they came from? State: ❍ Don't know

Did you **move in** colonies from another apiary into this one? ❍ Yes: ❍ No

Did you **move out** colonies from this apiary into another one? ❍ Yes: ❍ No

If you lost colonies **in this Sentinel apiary**, how many did you lose to:

❒ No loss this month	❒ Queen failure	❒ Poor weather condition
❒ Poor nutrition	❒ Starvation	❒ Management error
❒ Varroa mites	❒ Nosema disease	❒ Small Hive Beetles
❒ Pesticides	❒ Colony Collapse Disorder (CCD)	❒ Natural disaster (ex: flood, bear…)
❒ Other, please specify:	❒ Don't know	please, specify:

Colony Numbers of the dead colonies (ex. S18-SAAA-1):

Seasonal Information

Since your last sampling visit, has the weather been… ❍ Typical ❍ Atypical

If atypical, please describe: (too dry/ too cold/ milder/…)

If atypical, did it impact your colonies? ❍ Yes, POSITIVELY ❍ Likely not ❍ Yes, NEGATIVELY ❍ I don't know

How would you quantify the **nectar** flow (if any)?

Heavy		Medium		Light	No flow
					❍

How would you quantify the **pollen** flow (if any)?

Heavy		Medium		Light	No flow
					❍

In your best knowledge, near what **forage** were your bees this month? What was blooming around your apiary and used by the bees?
Ex: alfalfa, apples, cane crops (e.g. raspberries, blackberries, etc), canola (rape), citrus, clover, corn, cranberries, cucumbers, sweet corn, cotton, lime-tree, soybeans, sunflowers, watermelons, other melons, wild flower meadow, forest environment,... or nothing, or don't know.

Apiary management **Please consider ONLY the Sentinel apiary!**

Since your last sampling visit, did you try to monitor VARROA and/or NOSEMA on your own?

❍ Yes (in addition to the samples send to BIP) ❍ No (just BIP)

If YES, please describe the technique and frequency for each (Ex. Varroa; ether roll; all colonies; 2 times in the month):

Pest	Detection technique	% of colonies sampled	Dates (of samples collected)
❒ Varroa ❒ Nosema			
❒ Varroa ❒ Nosema			
❒ Varroa ❒ Nosema			

Since your last sampling visit, did you use a TREATMENT and/or TECHNIQUE to try to control pests/parasites/diseases in your colonies?

Ex. of pests: Varroa mites, Nosema, Small Hive Beetles, Wax moths, ...
Ex. of techniques of control: traps, sticky board, drone brood removal, screened bottom board,...

❍ Yes ❍ No

If YES, please describe (Ex. Small Hive Beetle; CheckMite+; 1 strip; on bottom board ; 50%; 1/month):

Pest	Product or Technique	Dose	Delivery method	% of colonies treated	Dates applied

Since your last sampling visit, did you FEED or add a food substitute or stimulant to your colonies?

❍ Yes ❍ No

If YES, please describe (Ex. Fondant; 1 ounce; all colonies; 2 times in the month):

Type	Product	Quantity	% of colonies fed	Dates applied
❒ Protein ❒ Carbohydrate				
❒ Protein ❒ Carbohydrate				
❒ Protein ❒ Carbohydrate				

Since your last sampling visit, did you employ any other MANAGEMENT PRACTICES in your apiary?

❍ Yes ❍ No

If YES, please describe (Ex. Re-queened colonies, open bottom board, replaced brood frames, added honey supers, …):

Practice	% of colonies	Dates

Since your last sampling visit, did you harvest any honey?

❍ Yes ❍ No

If YES, please describe (Ex. 10 lbs/ colony; 25% colonies harvested):

Average per colony (lb)	% of colonies harvested	Dates of harvest

Anything else you would like to share? Any unusual circumstances or important information?

beeinformed.org

Bee Informed Partnership
Sentinel Apiary Report

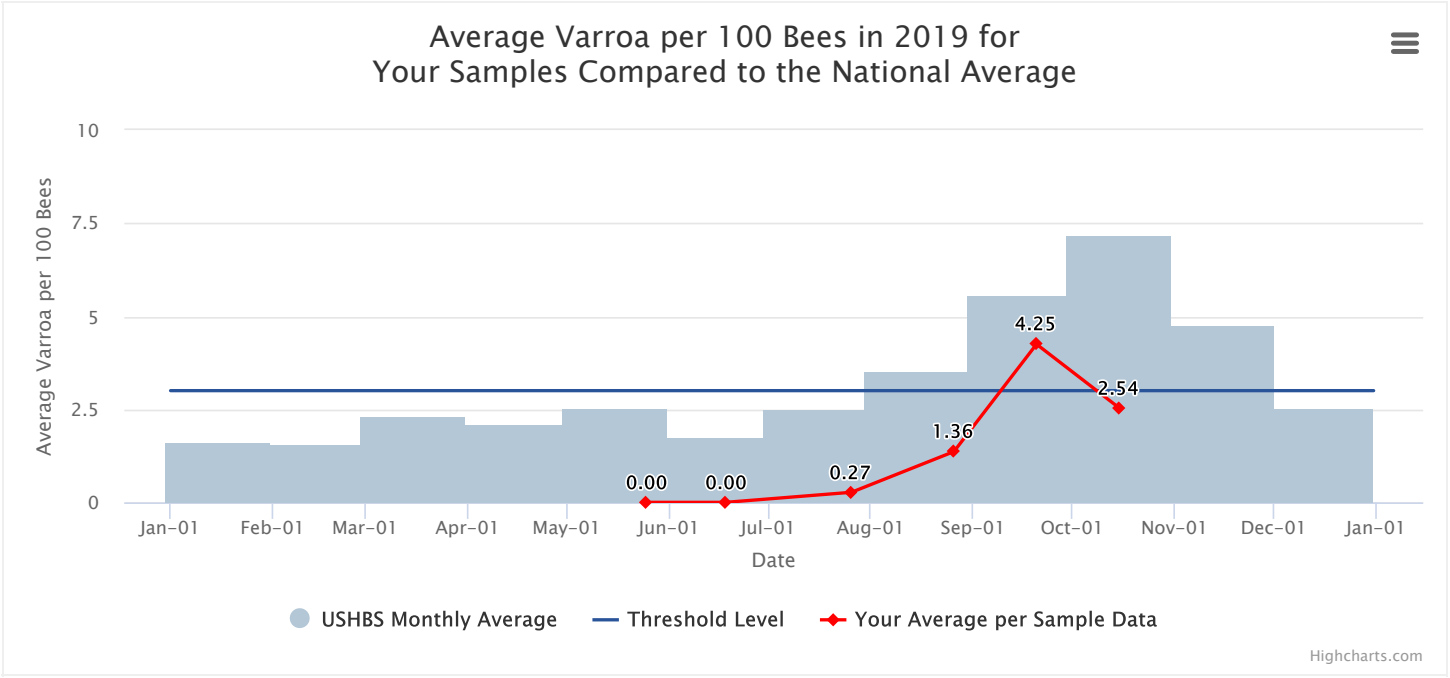
Beekeeper: Year: 2019 Report date: 10/25/19
Sample Kit Code: SAPA

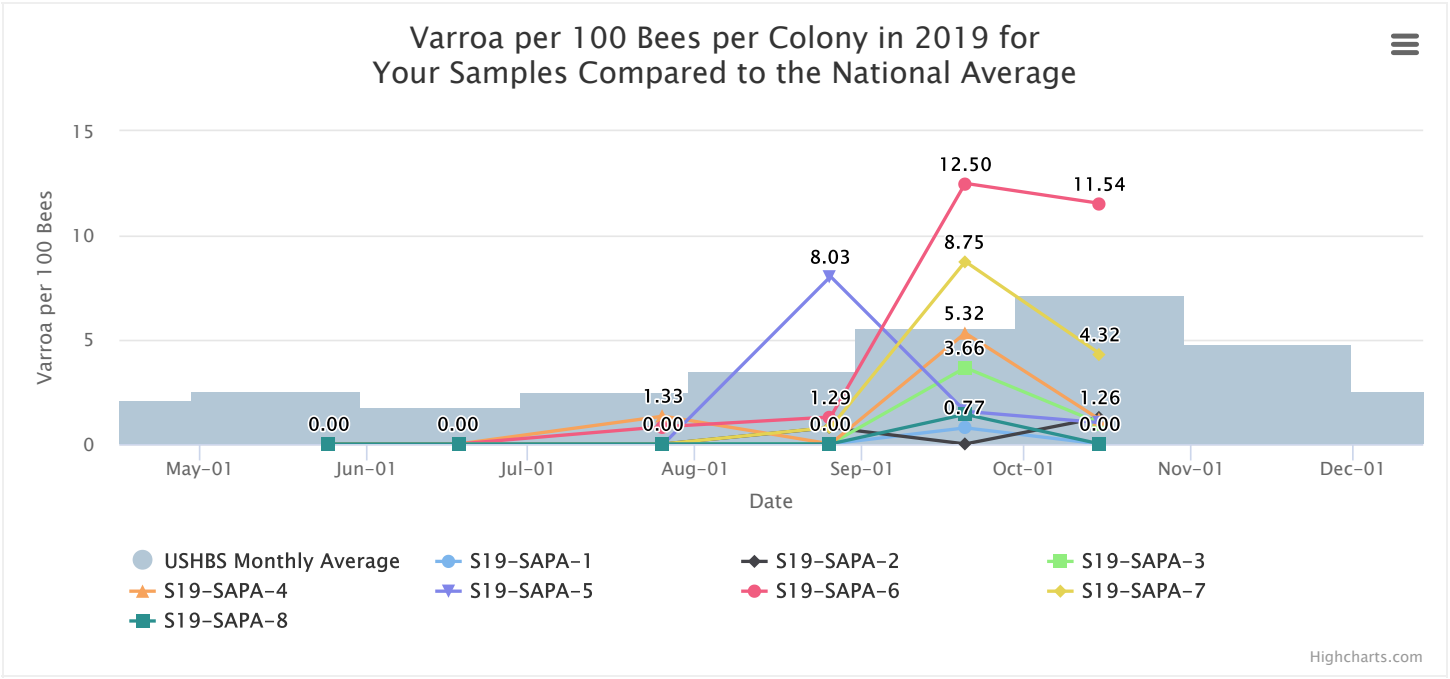
	Varroa (mites per 100 bees)					
Hive	**May**	**June**	**July**	**August**	**September**	**October**
S19-SAPA-1	0.0	0.0	0.0	0.0	0.8	0.0
S19-SAPA-2	0.0	0.0	0.0	0.8	0.0	1.3
S19-SAPA-3	0.0	0.0	0.0	0.0	3.7	1.0
S19-SAPA-4	0.0	0.0	1.3	0.0	5.3	1.2
S19-SAPA-5	0.0	0.0	0.0	8.0	1.6	1.0
S19-SAPA-6	0.0	0.0	0.8	1.3	12.5	11.5
S19-SAPA-7	0.0	0.0	0.0	0.8	8.8	4.3
S19-SAPA-8	0.0	0.0	0.0	0.0	1.4	0.0
Your Monthly Average	0.0 ±0.0 (8)	0.0 ±0.0 (8)	0.27 ±0.43 (8)	1.36 ±2.29 (8)	4.25 ±3.67 (8)	2.54 ±3.24 (8)
USHBS Average	2.55 ±0.27 (579)	1.72 ±0.13 (1052)	2.47 ±0.21 (846)	3.51 ±0.22 (1109)	5.54 ±0.36 (1133)	7.17 ±0.47 (946)
Sentinel Average	1.04 ±0.23 (379)	1.45 ±0.26 (389)	2.82 ±0.5 (398)	3.35 ±0.57 (380)	5.94 ±1.11 (328)	5.39 ±1.6 (85)
Sentinel Last Year Average	0.99 ±0.22 (458)	1.18 ±0.32 (502)	1.99 ±0.33 (495)	2.75 ±0.41 (475)	2.73 ±0.4 (420)	4.45 ±0.61 (365)

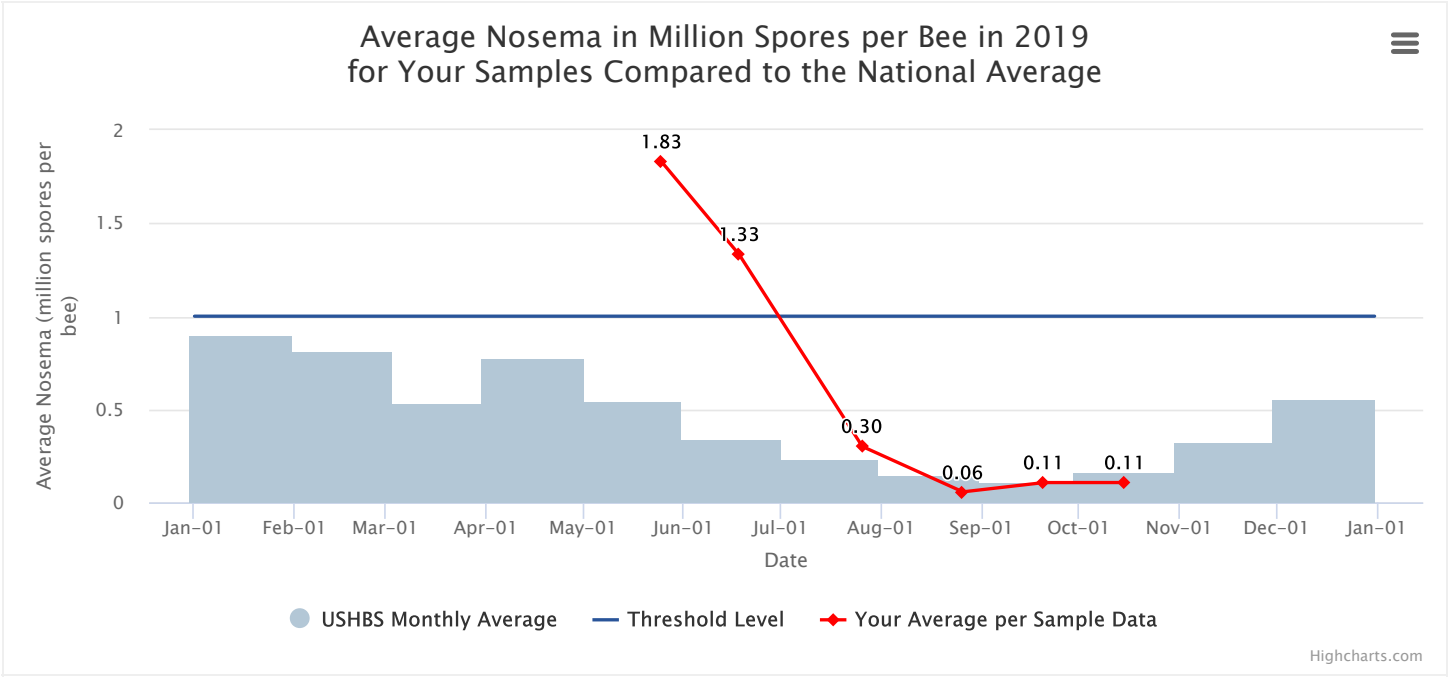
- Data presented: average ± 95% Confidence Interval (# of samples)
- The ± 95% Confidence Interval represents the range of expected values for 95% of the data. Observations outside this range may have occurred, but we consider those outliers and not representative of the majority of the data.
- Sentinel Average, Last Year includes Sentinel data starting in June 2013.
- APHIS Honey Bee Disease Survey is a national effort sponsored by USDA Animal and Plant Health Inspection Service (APHIS) in collaboration with the Agricultural Research Service (ARS) and University of Maryland (UMD). To date, the data provided for the APHIS monthly average is a composite of data from 2009 - Present.
- We consider => 5 mites per 100 bees (highlighted in red) as approaching a high threshold at or beyond where you may want to consider some varroa mite control strategy.
- If you collected two sets of samples within the same calendar month, they are reported in the two separate closest months in this table. Example, samples collected on May 30th may show up in the June column if you already have samples collected earlier in May.

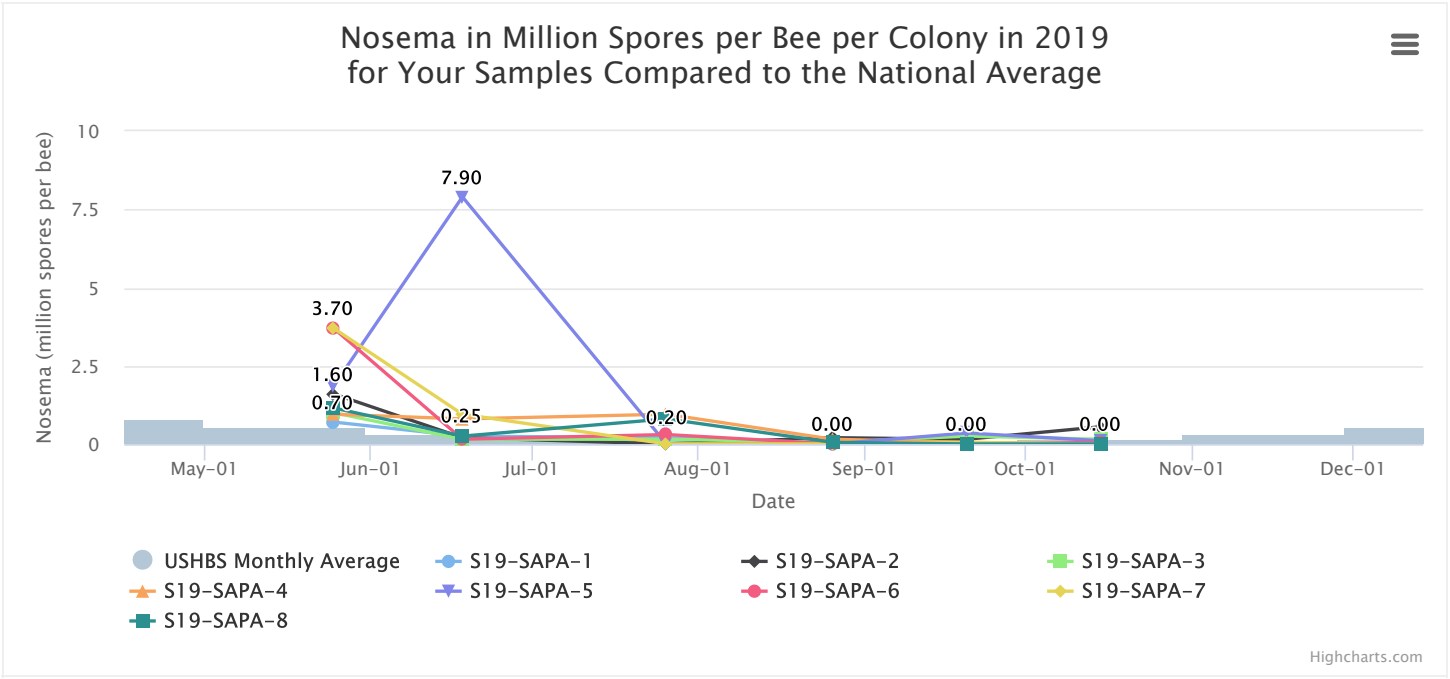
	Nosema (millions of spores per bee)					
Hive	**May**	**June**	**July**	**August**	**September**	**October**
S19-SAPA-1	0.7	0.3	0.2	0.0	0.0	0.0
S19-SAPA-2	1.6	0.2	0.0	0.2	0.1	0.6
S19-SAPA-3	1.0	0.1	0.1	0.1	0.3	0.1
S19-SAPA-4	0.9	0.8	0.9	0.1	0.1	0.0
S19-SAPA-5	1.9	7.9	0.0	0.0	0.3	0.1
S19-SAPA-6	3.7	0.1	0.3	0.0	0.0	0.1
S19-SAPA-7	3.7	0.9	0.0	0.0	0.1	0.0
S19-SAPA-8	1.1	0.3	0.8	0.1	0.0	0.0
Your Monthly Average	1.83 ±1.01 (8)	1.33 ±2.23 (8)	0.3 ±0.31 (8)	0.06 ±0.06 (8)	0.11 ±0.11 (8)	0.11 ±0.16 (8)
USHBS Average	0.54 ±0.09 (579)	0.34 ±0.04 (1052)	0.23 ±0.04 (846)	0.14 ±0.03 (1110)	0.11 ±0.02 (1133)	0.16 ±0.03 (946)
Sentinel Average	1.21 ±0.23 (379)	0.45 ±0.11 (389)	0.19 ±0.05 (398)	0.23 ±0.1 (380)	0.18 ±0.08 (324)	0.14 ±0.09 (85)
Sentinel Last Year Average	1.05 ±0.17 (459)	0.49 ±0.11 (502)	0.12 ±0.05 (495)	0.14 ±0.05 (471)	0.29 ±0.14 (421)	0.22 ±0.06 (365)

- Data presented: average ± 95% Confidence Interval (# of samples)
- The ± 95% Confidence Interval represents the range of expected values for 95% of the data. Observations outside this range may have occurred, but we consider those outliers and not representative of the majority of the data.
- Sentinel Average, Last Year includes Sentinel data starting in June 2013.
- APHIS Honey Bee Disease Survey is a national effort sponsored by USDA Animal and Plant Health Inspection Service (APHIS) in collaboration with the Agricultural Research Service (ARS) and University of Maryland (UMD). To date, the data provided for the APHIS monthly average is a composite of data from 2009 - Present.
- We consider => one million spores per bee (highlighted in red) to be the acceptable threshold in a hive. Your nosema levels will fluctuate with temperature and colonies' sun exposure every month.


Average Varroa per 100 Bees in 2019 for
Your Samples Compared to the National Average
Average Varroa per 100 Bees
10
7.5
5
2.5
0
Jan-01
Feb-01
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-01
Date
0.00
0.00
0.27
1.36
4.25
2.54
USHBS Monthly Average
Threshold Level
Your Average per Sample Data
Highcharts.com


Varroa per 100 Bees per Colony in 2019 for
Your Samples Compared to the National Average
Varroa per 100 Bees
15
10
5
0
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Date
0.00
0.00
1.33
0.00
8.03
1.29
0.00
12.50
11.54
8.75
5.32
3.66
4.32
0.77
1.26
0.00
USHBS Monthly Average
S19-SAPA-1
S19-SAPA-2
S19-SAPA-3
S19-SAPA-4
S19-SAPA-5
S19-SAPA-6
S19-SAPA-7
S19-SAPA-8
Highcharts.com


Average Nosema in Million Spores per Bee in 2019
for Your Samples Compared to the National Average
Average Nosema (million spores per bee)
2
1.5
1
0.5
0
1.83
1.33
0.30
0.06
0.11
0.11
Jan-01
Feb-01
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-01
Date
USHBS Monthly Average
Threshold Level
Your Average per Sample Data
Highcharts.com


Nosema in Million Spores per Bee per Colony in 2019
for Your Samples Compared to the National Average
Nosema (million spores per bee)
10
7.5
5
2.5
0
7.90
3.70
1.60
0.70
0.25
0.20
0.00
0.00
0.00
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Date
USHBS Monthly Average
S19-SAPA-1
S19-SAPA-2
S19-SAPA-3
S19-SAPA-4
S19-SAPA-5
S19-SAPA-6
S19-SAPA-7
S19-SAPA-8
Highcharts.com

All Samples for year									
Hive	**Sampling Date**	**Queen Status**	**Brood Pattern**	**Frames of Adults**	**Particular Observation**	**Recent Management**	**# Bees in Sample**	**# Mites / 100 Bees**	**Millions of Spores per Bee**
S19-SAPA-1	May 25, 2019	QR	3.0	9.0	a little irate/clingy, lots of bees	feeding treatment management	130	0.0	0.70
S19-SAPA-2	May 25, 2019	QL	2.0	2.0	new installed nuc Russian, deep nuc, put in 2 medium boxes, bees were very receptive to her, started to feed her, no young larvae or eggs	feeding treatment management	94	0.0	1.60
S19-SAPA-3	May 25, 2019	QR	3.0	4.0	brood on all 3 levels, need other level, added queen, very full hive, hive suprisingly gentle, considering how angry they were the last few months, lots of eggs, lots of brood,	feeding treatment management	111	0.0	1.00
S19-SAPA-4	May 25, 2019	QL	1.0	1.0	added queen, gentle; added frame of brood, survived winter, very small; brood dated by hive 3	feeding treatment management	108	0.0	0.95
S19-SAPA-5	May 25, 2019	QL	3.0	9.0	all capped brood, 4th box was added 3 weeks ago; lots of ants; full of honey, appeared to be queenless; added queen in second box	feeding treatment management	125	0.0	1.85
S19-SAPA-6	May 25, 2019	QR	3.0	5.0	bees seem crowded, add 1 more box for a total of 3; in frame feeder top box added frames of comb in brood nest; back filling brood nest with honey, added a mated queen	feeding treatment management	160	0.0	3.70
S19-SAPA-7	May 25, 2019	QR	3.0	1.0	up second level, drawing, saw eggs/small larvae; needs a level	feeding treatment management	110	0.0	3.70
S19-SAPA-8	May 25, 2019	QR	3.0	9.0	bringing lots of pollen, good brood pattern, fed sugar syrup	feeding treatment management	106	0.0	1.15
S19-SAPA-1	June 18, 2019	QR	3.0	6.0	Added 1 med box. Too much honey. No feeding. Bees in all frames	feeding treatment management	114	0.0	0.25
S19-SAPA-2	June 18, 2019	QR	2.0	3.0	From Russian nuc. Lots of capped queen cells. Swarmed?	feeding treatment management	93	0.0	0.20
S19-SAPA-3	June 18, 2019	QR	4.0	6.0	Backfilling, moved empty frames to encourage brood building. Healthy hive.	feeding treatment management	147	0.0	0.15
S19-SAPA-4	June 18, 2019	QNS	0.5	5.0	Queen still alive after several weeks (banked Qs) No brood under queen. Queen in bottom. Added bank.	requeen feeding treatment management	125	0.0	0.80
S19-SAPA-5	June 18, 2019	QNS	1.5	7.0	No queen? Lots of bees/honey. Empty brood chamber, minimal uncapped brood.	feeding treatment management	101	0.0	7.90
S19-SAPA-6	June 18, 2019	QR	2.0	6.0	Added a box. Honey bound, backfilling. Checker boarding brood area.	feeding treatment management	109	0.0	0.15
S19-SAPA-7	June 18, 2019	QR	5.0	5.0	Packaged in April/Skak bees. Hive seems healthy building up. Missing thermometer.	feeding treatment management	115	0.0	0.95

All Samples for year									
Hive	**Sampling Date**	**Queen Status**	**Brood Pattern**	**Frames of Adults**	**Particular Observation**	**Recent Management**	**# Bees in Sample**	**# Mites / 100 Bees**	**Millions of Spores per Bee**
S19-SAPA-8	June 18, 2019	QR	4.0	7.0	She hasn't refill the brood needs another box. Every frame full of bees. Very successful overwinter hive.	feeding treatment management	134	0.0	0.25
S19-SAPA-1	July 26, 2019	QR	3.0	5.0	Calm bees eventhough 3 full boxes of honey. Queen getting honey bound. Added one more level just above brood chamber.	management	114	0.0	0.20
S19-SAPA-2	July 26, 2019	QR	3.0	3.0	Top box still empty, just starting in excellent brood pattern all types of brood seen. Fed sugar water.	feeding management	94	0.0	0.00
S19-SAPA-3	July 26, 2019	QR	3.0	4.0	Eggs seen 2 empty queen cells, added 3 frames, calm bees	management	53	0.0	0.15
S19-SAPA-4	July 26, 2019	QR	3.0	3.0	Gentle queen right, lots of brood, average honey, added pollen patty	management	75	1.3	0.95
S19-SAPA-5	July 26, 2019	QR	3.0	4.0	Bees mildly agitated, back filling w/ honey, no feeding but adding patty. Brood on three levels.	movement feeding treatment management	98	0.0	0.00
S19-SAPA-6	July 26, 2019	QR	3.0	7.0	Heavy propolis , back filling brood, still plenty brood.	management	121	0.8	0.30
S19-SAPA-7	July 26, 2019	QR	4.0	4.0	Heavy propolis, patches of brood everywhere. Check in a week (add a box?)	feeding management	72	0.0	0.00
S19-SAPA-8	July 26, 2019	QL	0.0	7.0	Added a frame of uncapped brood. Lots of bees and honey.	management	92	0.0	0.80
S19-SAPA-1	Aug. 26, 2019	QNS	3.0	6.0	Colony may have been queenless. Capped queen cells. Extracted honey 4/3/19. Queen cells 3 damaged?	feeding	100	0.0	0.00
S19-SAPA-2	Aug. 26, 2019	QR	3.0	4.0	good brood pattern, queen laying well, bringing in lots of pollen. Requeened 8/3/19	requeen	127	0.8	0.20
S19-SAPA-3	Aug. 26, 2019	QR	3.0	7.0	larvae-uncapped/young larvae. Uncapped swarm cell. Eggs seen. Strong completely filled a new box of honey since August 3rd.	feeding	134	0.0	0.05
S19-SAPA-4	Aug. 26, 2019	QR	4.0	6.0	beautiful brood pattern. Lots of eggs, lots of brood. Brining lots of pollen. Hive has recovered since a weak start in the spring.	feeding	172	0.0	0.15
S19-SAPA-5	Aug. 26, 2019	QR	3.0	4.5	Mildy aggressive due to wasps. Bringin multiple colors of pollen. Didn't see queen but saw all stages of brood.		211	8.0	0.00
S19-SAPA-6	Aug. 26, 2019	QR	2.5	6.0	3 boxes full of honey including one that was empty and returned to hive after extraction. Eggs and larvae seen. Gentle trying to back fill brood with honey.	feeding	155	1.3	0.00

All Samples for year									
Hive	**Sampling Date**	**Queen Status**	**Brood Pattern**	**Frames of Adults**	**Particular Observation**	**Recent Management**	**# Bees in Sample**	**# Mites / 100 Bees**	**Millions of Spores per Bee**
S19-SAPA-7	Aug. 26, 2019	QR	3.0	4.5	Back filling brood with honey. Eggs and small larvae seen. Good honey stored/bringin in pollen. Possible SHB. Will treat anyways to be safe.		126	0.8	0.00
S19-SAPA-8	Aug. 26, 2019	QR	4.0	5.0	Good honey store/gentle. Excellent brood/good pollen/good honey. Eggs seen. Larvae all stages.	feeding	121	0.0	0.05
S19-SAPA-1	Sept. 20, 2019	QR	3.0	5.0	All stages of brood lots of honey. Still bringing in pollen, average temperament. Reducing entrance. Drones still in hive.	feeding	129	0.8	0.00
S19-SAPA-2	Sept. 20, 2019	QR	3.0	3.0	Reducing to 3 boxes from 4. Beautiful brood pattern. Worried about honey stores. Bringing in pollen.	feeding	143	0.0	0.15
S19-SAPA-3	Sept. 20, 2019	QNS	2.0	4.0	Still bringing in nectar, gentle. Lots of propolis. Backfilling brood chamber w/ honey. Appears QR due to behavior. Drones in hive.	feeding	82	3.7	0.25
S19-SAPA-4	Sept. 20, 2019	QR	2.0	5.0	Nice brood. Gentle, lots of honey, lots of pollen, still bringing in pollen.		94	5.3	0.05
S19-SAPA-5	Sept. 20, 2019	QR	2.0	5.0	So much honey, nicely kept brood, good pollen, bringin in pollen.		64	1.6	0.35
S19-SAPA-6	Sept. 20, 2019	QR	2.0	4.0	Lots of propolis, gentle lots of honey, drones seen	feeding	72	12.5	0.00
S19-SAPA-7	Sept. 20, 2019	QR	3.0	4.0	Lots of propolis, kept brood, gentle, good honey, 2 boxes	feeding	80	8.8	0.05
S19-SAPA-8	Sept. 20, 2019	QR	1.0	4.0	gentle lots of pollen, 2 boxes of honey, lots of propolis	feeding	211	1.4	0.00
S19-SAPA-1	Oct. 15, 2019	QNS	0.0	6.0	Few drones seen, good honey and pollen stores. No kept brood, back filling brood area w/ honey and pollen.	feeding treatment management	99	0.0	0.00
S19-SAPA-2	Oct. 15, 2019	QS	0.0	4.0	Gentle bees, some kept brood. Queen was seen, need honey stores. Feed, feed, feed!	feeding treatment management	158	1.3	0.55
S19-SAPA-3	Oct. 15, 2019	QNS	0.0	4.0	Very gentle, good honey stores, bees seen with yellow pollen baskets. Give some pollen patty after treatment. Queen must be around because of their attitude.	feeding treatment management	101	1.0	0.15
S19-SAPA-4	Oct. 15, 2019	QR	2.0	5.0	All stages of brood seen. Bringing in yellow popllen, gentle bees, good honey and pollen stores.	feeding treatment management	86	1.2	0.00
S19-SAPA-5	Oct. 15, 2019	QR	2.0	3.0	Larvae present brood. Gentle goodhoney and pollen stores. Will share w/#2, bringin in yellow pollen.	feeding treatment management	98	1.0	0.10
S19-SAPA-6	Oct. 15, 2019	QR	0.0	6.0	A few kept cells left (8). Gentle, back filling brood.	feeding treatment management	78	11.5	0.05

All Samples for year									
Hive	**Sampling Date**	**Queen Status**	**Brood Pattern**	**Frames of Adults**	129 **Particular Observation**	**Recent Management**	**# Bees in Sample**	**# Mites / 100 Bees**	**Millions of Spores per Bee**
S19-SAPA-7	Oct. 15, 2019	QR	2.0	4.0	Gentle, kept brood only, backk filling w/ honey. Good honey honey and pollen stores.	feeding treatment management	161	4.3	0.00
S19-SAPA-8	Oct. 15, 2019	QR	1.0	6.0	It has brood (kept brood only). Good pollen and honey. Gentle enough.	feeding treatment management	126	0.0	0.00

- Hive # highlighted in blue indicates hive scale installed. Yellow indicates pollen trap installed.

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