### 2016 – 2017 National Honey Bee Disease Survey Report

Rachel Fahey<sup>1</sup>, Karen Rennich<sup>1</sup>, Ashrafun Nessa<sup>1</sup>, Nathan Swan<sup>1</sup>, Nathalie Steinhauer<sup>1</sup>, Heather Eversole<sup>1</sup>, Daniel Reynolds<sup>1</sup>, Jay Evans<sup>2</sup>, Dennis vanEngelsdorp<sup>1</sup>, and Robyn Rose<sup>3</sup>

<sup>1</sup>University of Maryland, College Park, <sup>2</sup>USDA Agricultural Research Service, <sup>3</sup>USDA Animal and Plant Health Inspection Service

## **Executive Summary**

The 2016-2017 USDA Animal Plant and Health Inspection Service (APHIS) sponsored National Survey of Honey Bee Pests and Diseases was conducted in collaboration with the University of Maryland (UMD), the USDA Agricultural Research Service (ARS) and the cooperation of 38 states, Guam and Puerto Rico. The National Survey began as a pilot survey of 3 states in 2009 to address the emerging concern about the diminishing health of honey bee colonies. After a successful pilot, the survey expanded the following year to include 13 states in a Limited National Survey. In subsequent years, funding for the National Survey increased, and the survey expanded to 34 states in 2011, 32 states in 2012 and 2013, 28 states in 2014, and 37 states in 2015. This expansion has allowed us to augment and extend the national database of honey bee disease and pathogen information.

The primary focus of the APHIS National Survey is to verify the absence of potentially harmful exotic threats to honey bee (Apis mellifera) populations such as the parasitic mite, Tropilaelaps spp., and exotic honey bee species such as the Asian honey bee (Apis cerana), and Slow Bee Paralysis Virus (SBPV). Tropilaelaps spp. is a parasitic mite native to Asia which, like Varroa, feeds on honey bee brood and vectors viruses (Chantawannakul et al., 2018). Because of its faster reproduction cycle, Tropilaelaps dominates in regions where it coexists with Varroa (Guzman et al., 2017). Apis cerana is a species of honey bee found in south and southeastern Asia, which resemble the western honey bee in that they both build nests in cavities. This similar lifestyle might explain why pathogens and parasites affecting Apis cerana have the potential to jump host to the western honey bee in their shared geographical area. Apis cerana was the original host of Varroa destructor and Nosema ceranae (Fries, 1993; Rosenkranz et al., 2010). Slow Bee Paralysis Virus (SBPV) is one of the viruses that can be transmitted by Varroa destructor. The virus is present throughout Europe, though at a low (<2%) prevalence (de Miranda et al., 2010). When associated with high Varroa loads, the virus can result in increased bee and colony mortality (Carreck et al., 2010). After surveying for SBPV for 7 years, it was determined that it is not present in the U.S. and resources were shifted to focus on the distribution of other viruses such as Varroa Destructor Virus (VDV).

If exotic honey bee pests like *Tropilaelaps* spp. were to be introduced to the United States it would threaten managed honey bee colonies which are already facing unsustainably high colony loss rates (Kulhanek et al., 2017). With honey bees contributing approximately \$15 billion in U.S. crop production, ensuring the continued absence of those honey bee pests and disease is an issue of agricultural economics and national food security. The APHIS National Survey confirms the absence of certain exotic honey bee pests, and allows USDA to deny importation of honey bees from other nations unless the exporting nation can confirm absence of *Tropilaelaps* spp., *Apis cerana*, and Slow Bee Paralysis Virus (SBPV).

The secondary objective of the APHIS National Survey is to determine the incidence of known and established honey bee diseases and pests in the U.S, i.e. *Varroa destructor, Nosema* spp. and a series of viruses. Disease and pest information collected from the APHIS National Survey has been used to create a baseline level of reference, and to facilitate interpretation of ongoing and future epidemiological studies. All of the data collected from the survey, including historic data from research institutions such as USDA ARS and other ongoing field sampling and management surveys are incorporated into a single database, the nationwide Bee Informed Partnership (BIP) database. BIP is a non-profit 501(c)(3) and originally funded as a 5 year USDA National Institute of Food and Agriculture (NIFA) grant. Results from the APHIS National Survey are available to the public on the BIP website (programmatic details here: <a href="https://beeinformed.org/aphis/">https://beeinformed.org/aphis/</a>, diagnostic data provided here:

https://bip2.beeinformed.org/state\_reports/viruses/).

### Introduction

The 2016–2017 survey included sample collection in 38 states and two U.S. territories, Guam and Puerto Rico. The participating states were: Alabama, California, Colorado, Connecticut, Delaware, Florida, Georgia, Hawaii, Iowa, Idaho, Illinois, Indiana, Kentucky, Louisiana, Massachusetts, Maryland, Michigan, Minnesota, Mississippi, Montana, North Carolina, North Dakota, Nebraska, New Jersey, New Mexico, Nevada, New York, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, Vermont, Wisconsin, and West Virginia.

The objective of the survey is to establish a network of surveillance that maximizes the chances of detecting the arrival of the exotic pests while being representative of the managed honey bee colonies of the US. The survey was open to any state wishing to participate. In cooperating states, the state department of Agriculture (where applicable) collected samples from beekeeping operations with an effort to sample from each quadrant of their state, with particular attention to queen breeders and those areas considered higher risks of invasion (such as ports). When possible, half of the samples were collected from migratory operations and half from stationary, both commercial and small-scale operations. Beekeeper participation within the states was voluntary and any identifiable information is confidential in any resulting report and publication. With sampling occurring throughout the majority of the US, this stratified random survey offers one of the most systematic and comprehensive representation of the pests and disease levels in US managed honey bee colonies and allowed for the establishment of baselines of disease prevalence and loads. Results from the first 6 years of this survey (survey years 2009-10 till 2014-15) were published in that effect in Traynor et al. (2016).

## Milestones and Project Timelines

The survey design has evolved over time to reflect the recommendations of scientific experts and to fit the objectives of the program based on current information. These protocols or targets are likely to continue to change as new threats to honey bees are identified. In particular, the protocols updated have concerned the following:

- In 2011, Tracheal mites, *Acarapis woodi*, were removed from the list of pests analyzed, as there were no detections in 2009 or 2010.
- A pilot pollen pesticide survey was conducted in 2011, in which 11 states collected 3 samples of bee bread for pesticides analysis (conducted by the USDA Agricultural Marketing Service (AMS)

in Gastonia, NC). From 2012-2016 (except 2014), all participating states sent in 10 bee bread samples for pesticide detection and quantification.

- Speciation identification between *Nosema apis* and *Nosema ceranae* was discontinued in 2013 after finding no detections of *Nosema apis* from 2009-2010, detections of 1.3% in 2011, and 0.7% in 2012.
- Black queen cell virus (BQCV) was replaced with Lake Sinai virus-2 (LSV-2) in 2013, as the ubiquity of BQCV became known and the concern about LSV-2 became elevated.
- Absolute quantification of viral targets via Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was adopted in favor of previous viral analysis methods in 2013, enabling direct comparison to standardized European protocols.
- All viral primers, excluding Kashmir bee virus (KBV), were updated in 2013 for increased sensitivity and specificity.

## **Survey Description**

All states participating in the survey received kits to sample 24 apiaries within their state with the exception of California, which received 48 sample kits. Half of the 48 kits in California were used to sample 24 apiaries that remain in the state year-round and the remaining were used to sample 24 migratory beekeepers who travel to CA for the annual almond pollination.

Apiary Specialists conducted an aggregate sampling from previously identified commercial, migratory, side-liner and backyard beekeepers with at least 8 colonies per apiary. In most cases, apiaries included at least 10 colonies. Under guidelines provided to the Apiary Specialists, they selected apiaries to be sampled with an attempt to give as close to an equal representation of the state as possible. Ideally, a state was divided into 4 quadrants with apiaries randomly chosen within a quadrant. When possible, ten queen producers were sampled. Of the remaining apiaries to be sampled, half were from migratory operations (apiaries that move out of the state and return prior to sampling), and half were from stationary operations (operations that only move within the state or not at all). Additional apiaries occurring near deep water shipping ports or other areas that could be at risk of exotic pest or disease invasion were also considered for sampling.

In each selected apiary, a single aggregate sample was collected from 8 randomly selected colonies. Three distinctive collection methods were used to sample each apiary: 1. Live bee sampling, 2. Alcohol preserved sampling of bees, and 3. Brood frame bump sampling.

Each colony is also fully inspected to characterize their queen status and the presence of any overt disease symptoms. Information from the inspection, sample collector, beekeeper and their operation are recorded on a datasheet (see appendix) and these data are entered and archived in the BIP database.

The live bee sample was collected from a brood frame with both capped and uncapped brood. ¼ cup of nurse bees were taken from each of the 8 colonies and collected in an aggregate sample in a live bee shipping box. Using the U.S. Postal Service (USPS), live bee shipments were mailed to USDA/ARS where they were promptly frozen at -80°C. The frozen bees were tested with qRT-PCR techniques, outlined by Dr. Jay Evans at the USDA ARS Bee Research Laboratory. These molecular procedures were updated in 2013 by Dr. Eva Forsgren from the Swedish University of Agricultural Sciences (SLU) to include absolute

quantification of the viral targets. As a result, the absolute quantification of viral loads (viral copies per bee) can be determined in addition to the presence or absence of a virus.

The alcohol preserved sample was collected from the same brood frame as the live bee sample. An additional ¼ cup of nurse bees were taken from each of the 8 colonies that were sampled in the apiary. These bees were collected into a bottle of 70% ethanol solution for preservation and sent to the University of Maryland to determine the incidence of *Varroa destructor*, *Nosema* spp. spores, and *Apis cerana*.

The brood bump sample was taken from debris dislodged by 'bumping' sampled brood frames over a collection pan. The brood frame debris was collected in a filter cloth and placed in a bottle filled with 70% ethanol solution for preservation. The brood bump sample is focused on monitoring for *Tropilaelaps* spp., but also any mites, beetles or other hive debris are observed for interest by the University of Maryland.

Bee bread was also collected in a subsample of 10 apiaries from each state. Bee bread is pollen honey bees have gathered from flowers, fermented and stored within the hive. A minimum of 3 grams of bee bread was collected from the same 8 colonies, preferably in the same brood area, from the other three samples described above. These samples were shipped to University of Maryland where they were catalogued by UMD personnel and sent to the USDA AMS Lab in Gastonia, NC for pesticide analysis.

In the 2015-2016 survey, live bee samples were analyzed for the following viruses:

- 1. Acute bee paralysis virus (ABPV)
- 2. Chronic bee paralysis virus (CBPV)
- 3. Deformed wing virus (DWV)
- 4. Israeli acute bee paralysis virus (IAPV)
- 5. Kashmir bee virus (KBV)
- 6. Lake Sinai virus-2 (LSV-2)
- 7. Varroa destructor virus (also known as deformed wing virus-B) (VDV)

The alcohol preserved bee samples were analyzed for the following:

- 1. Nosema spp. spore loads (in millions of spores per bee)
- 2. Varroa destructor loads (in mites per 100 bees)
- 3. Apis cerana presence or absence

The brood bump samples were analyzed for:

1. Tropilaelaps spp. presence or absence

The bee bread samples were analyzed for:

1. 174 different pesticides measured in parts per billion (ppb) which included varroacides, insecticides, herbicides, and fungicides (list of analytes is determined by the USDA AMS lab and is depicted in Figure 14)

All participating beekeepers, as well as Apiary Specialists, State Survey Coordinators, State Plant Regulatory Officials, and APHIS State Plant Health Directors, receive a report for each sample taken. The report provides detailed results for *Varroa* load, *Nosema* load, and presence of viruses. The reports also noted the presence or absence of *Apis cerana* and *Tropilaelaps* spp. Reports also detail the national prevalence for viruses as well as specific beekeeper percentile rankings of *Varroa* load, *Nosema* spore load, and viral copy load. Reports are sent within 4-8 months of receipt of the samples.

## Results

The APHIS National Survey has confirmed the absence, as of 2017, of *Tropilaelaps* spp., *Apis cerana*, and SBPV. The absence of these exotic pests and pathogens in the 2016 – 2017 Survey suggest that the current policies to prevent their introduction into the United States have been successful.

At the start of this survey year, a total of 984 sampling kits were sent out (38 states at 24 kits per state, plus 48 for Guam and Puerto Rico and an extra 24 for California). At the conclusion of the survey year, 940 live bee boxes were returned (95.5% return rate), 940 alcohol samples (95.5% return rate) and 940 Tropilaelaps bump samples (95.5% return rate).

All trends discussed below are numerical only and have not been tested for potential confounding of sampling bias over time.

## Nosema spp. Spore Load and Prevalence

Of the 940 alcohol samples that were analyzed for *Nosema* spp. spore load, 433 (46.1%) tested positive (Figure 1). The average *Nosema* spore load was 0.54 million spores per bee for samples that tested positive (Figure 2). Of all samples that were processed for *Nosema* spp. spores, 6% (55) exceeded the threshold thought to cause damage (more than 1 million spores per bee). Figures 1 and 2 illustrate *Nosema* spp. prevalence, and *Nosema* spp. spore load from 2010 to 2017. Average *Nosema* spp. spore load (Figure 3) varies throughout the year, with the highest loads occurring in the winter and early spring periods followed by a sharp decline in summer months when most of the samples were collected.

### Varroa Load and Prevalence

Of the 940 alcohol samples that were analyzed for *Varroa*, 809 (86.1%) were positive for mites (Figure 4). While the economic threshold for *Varroa* is seasonally and regionally specific, an average load of over 3 mites per 100 bees is the general threshold thought to cause damage to a colony of honey bees. This threshold was exceeded in 32.1% (302) of all samples analyzed. The average *Varroa* load was found to be 3.27 mites per 100 bees for samples that tested positive (Figure 5). Figure 6 illustrates the dynamic nature and seasonality of mite populations across all years of the APHIS National Honey Bee Survey. Generally, *Varroa* increases exponentially in the late summer and peaks in the fall.

### Viral Load and Prevalence

Of the 940 live bee boxes that were received, 901 (95.9%) of all samples were analyzed for viruses. The other 39 live bee samples were insufficient for analyses. Reasons for a sample to be insufficient can include live bees dying in transit, loss of sample in long-term storage or low quality RNA due to insufficient nucleic acid extraction. Figure 7 illustrates the viral prevalence of all targets that were tested from 2010 to 2017 (ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV-2 and VDV). The most prevalent virus detected in the 2016 – 2017 survey was DWV found in 83.6% (753) of all samples. This is an decrease from the previous year of the survey (2015-2016) in which the average for the virus was 91.7%. *Varroa destructor* is known to be a vector of DWV, transferring the virus from one bee to another (Bowen-

Walker et al., 1999). Support of this can been found in the APHIS National Survey by the association between prevalence of DWV and *Varroa* (Figure 8).

The least prevalent virus in the 2016-2017 survey was Kashmir bee virus (KBV) detected in 5.4% of all samples tested. Although KBV does not appear to be problematic for the U.S. honey bee population, the rising prevalence of CBPV may become concerning. When the survey first began in 2010, the incidence of CBPV was quite low, occurring in only 9% of all samples tested. However in recent years (2016-2017 survey year), prevalence of CBPV has risen to 14.2% (Figure 7). The APHIS National Survey will continue to monitor changes in CBPV incidence.

Another subject of growing concern is Lake Sinai virus (LSV-2). Lake Sinai virus was first detected in 2011 near Lake Sinai in South Dakota and was added to the APHIS National Survey list of viruses tested for in the 2013-2014 survey year. Prevalence of LSV-2 displays a strong seasonality across all years of the survey (Figure 10). Incidence of the virus is higher in the spring, peaking in February at 81% in the 2016-2017 survey year. These levels gradually decreased into the fall, and were at their lowest in September at 16%. A positive correlation between the prevalence of LSV-2 and Nosema spores has also been observed (Traynor et. al., 2016) and is referenced in Figure 11.

Acute Bee Paralysis Virus (ABPV) seasonality can also be seen across all survey years (Figure 12). Incidence of ABPV was at its highest in the winter months, decreasing throughout the spring and was at its lowest in the summer months. Average prevalence of ABPV has varied since the beginning of the APHIS National Survey, hovering around 20% detection in all samples tested each survey year (Figure 7).

### **Pesticide Detections in Bee Bread**

This year, 34 states (Alabama, California, Colorado, Connecticut, Delaware, Florida, Georgia, Idaho, Illinois, Indiana, Kentucky, Louisiana, Massachusetts, Maryland, Michigan, Minnesota, Montana, North Carolina, North Dakota, Nebraska, New Jersey, New Mexico, New York, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, Vermont, Wisconsin and West Virginia) submitted composite bee bread samples (311 total samples). These samples were tested by USDA AMS in Gastonia, NC through their Apiculture Pesticide Residue Screen, which includes testing for 162 different compounds.

The most prevalent pesticides in bee bread are miticides applied by beekeepers to control infestations of *Varroa destructor*. These miticides, also known as varroacides, include the Amitraz metabolite 2,4 Dimethylphenyl formamide (detected in 48.2% of samples), Coumaphos (detected in 32.5% of samples), Thymol (detected in 29.3% of samples), and Fluvalinate (detected in 17.7% of samples). The most prevalent insecticide detected was Methoxyfenozide, found in 9.6% of samples, followed by Chlorpyrifos, found in 9.3% of samples. The fungicide with the highest number of detections was Pyraclostrobin, found in 16.7% of samples. The most prevalent herbicide was Atrazine, detected in 22.8% of samples.

On average, each sample had 3 different compounds detected with as many as 11 compounds detected in a single sample. The full set of results, grouped by their classification as a varroacide, insecticide, fungicide or herbicide is in Figure 17. The level of detection (LOD), or minimum amount that can be detected, the prevalence (%) within this survey year, the average quantity detected (ppb), and the range of detection (ppb) are provided for each pesticide tested. If a pesticide was detected only once, a single

value is given for the range and is marked with an asterisk. The breakdown in classification of the pesticides detected for the 2016-2017 survey can be found in the pie chart, Figure 18.

## Conclusions

*Nosema* spp. spore prevalence has been historically consistent since the origin of the APHIS National Survey. On average, *Nosema* has been detected in 50% of all samples taken. Although prevalence has remained about the same, the average load of *Nosema* spores appears to be decreasing over time. The average *Nosema* spore load for this survey (2016-2017) was 0.54 million spores per bee, which is slightly lower than the previous 5 years of the survey where the average *Nosema* spore load was 0.66 million spores per bee. This trend will continue to be monitored in subsequent years of the National Survey to determine if this decrease in Nosema disease load is significant.

The prevalence of *Varroa destructor* in APHIS National Survey samples has remained relatively the same since 2010, and has been detected in 90% of samples each year on average. In a similar trend as *Nosema, Varroa* load has decreased over time despite little to no change in prevalence. Average *Varroa* load was at its highest during the 2012-2013 survey year averaging at 5.5 mites per 100 bees and has gradually decreased until this year's survey with an average of 3.3 mites per 100 bees. An explanation could be that nationwide outreach and extension efforts towards beekeepers about monitoring and treatment of *Varroa* has been successful. An alternative explanation is that the viruses that *Varroa destructor* transmits have become more virulent, resulting in higher colony loss and therefore a drop in mite populations.

Results from the 2016-2017 APHIS National Survey provide strong evidence for the absence of *Tropilaelaps* spp., *Apis cerana* and SBPV. The absence of these species suggest that the current methods of preventing potentially harmful honey bee pests from entering the United States have been successful.

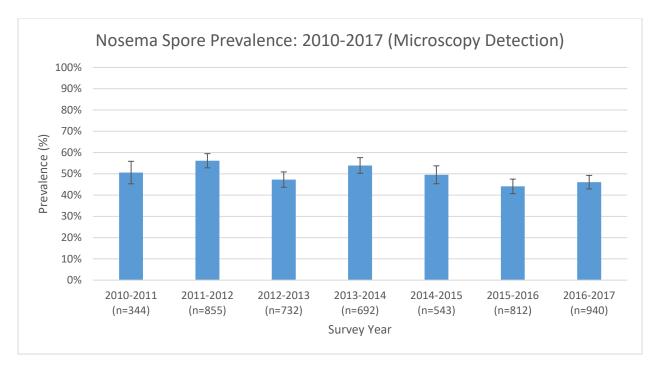


Figure 1: Nosema prevalence by survey year (95% confidence intervals shown)

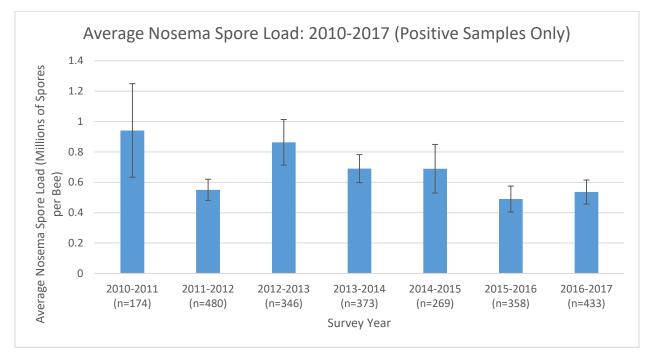


Figure 2: Nosema spore load by survey year (95% confidence intervals shown)

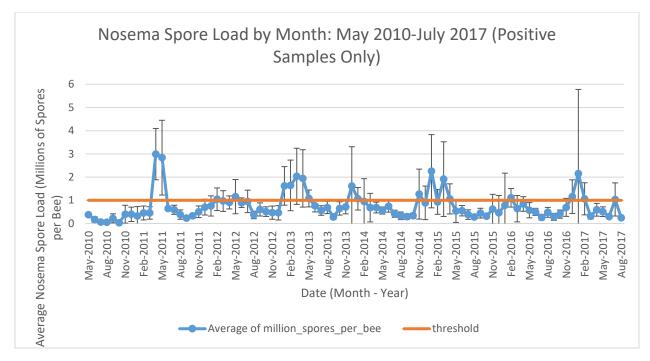


Figure 3: Nosema spore load by month from May 2010 to August 2016 (95% confidence intervals shown)

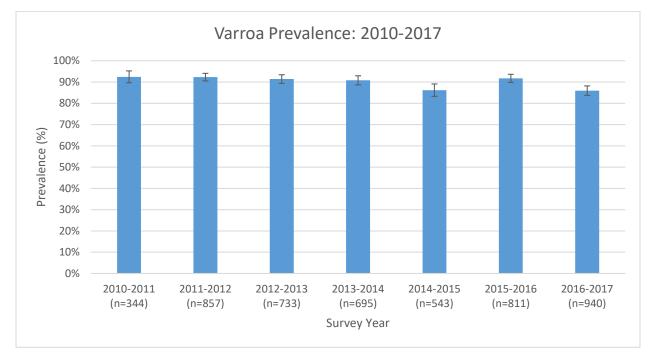


Figure 4: Varroa prevalence by survey year (95% confidence intervals shown)

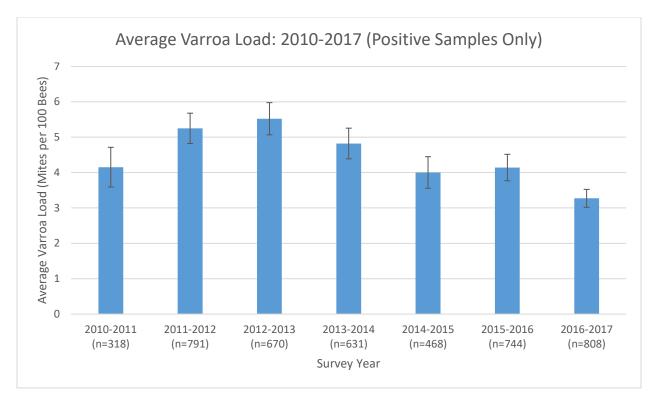


Figure 5: Varroa load by survey year (95% confidence intervals shown)

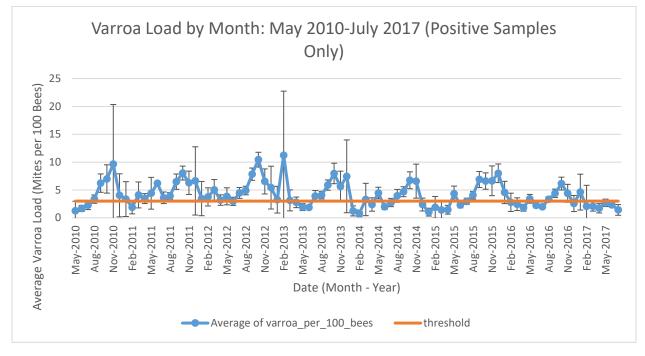
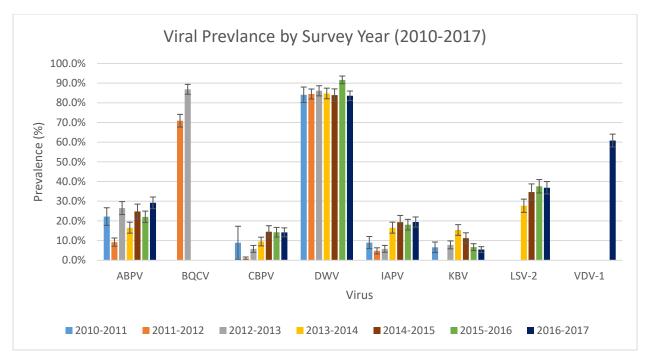


Figure 6: Varroa load by month from May 2010 to August 2016 (95% confidence intervals shown)



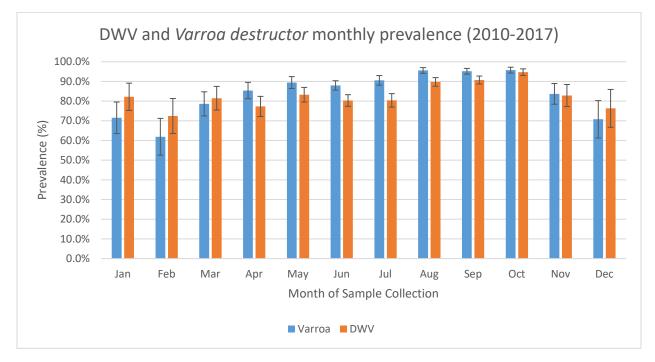
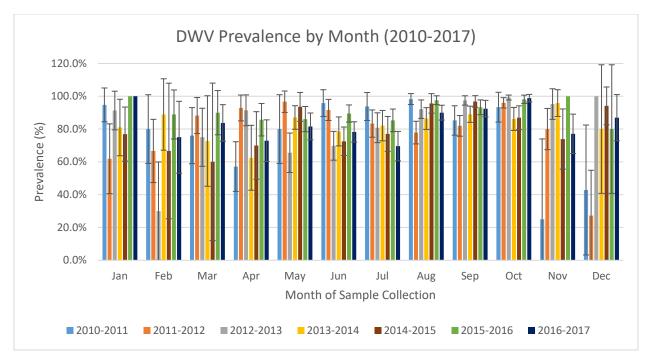


Figure 7: Yearly changes in viral prevalence from 2010 to 2016 (95% confidence intervals shown)

Figure 8: Comparison of deformed wing virus (DWV) and Varroa destructor prevalence by month (95% confidence intervals shown)



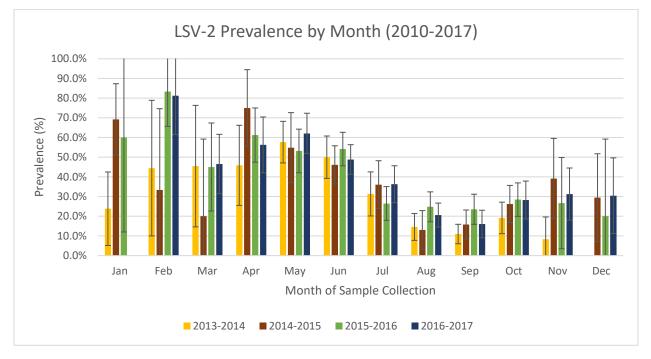


Figure 9: Prevalence of deformed wing virus (DWV) by month (95% confidence intervals shown)

Figure 10: Prevalence of Lake Sinai virus 2 by month (95% confidence intervals shown)

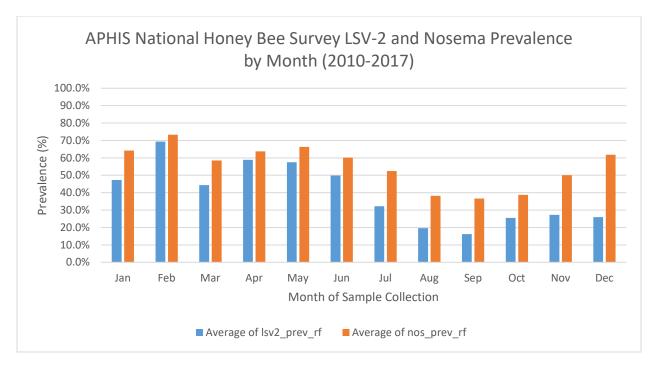


Figure 11: Prevalence of Lake Sinai virus 2 and Nosema by month (95% confidence intervals shown)

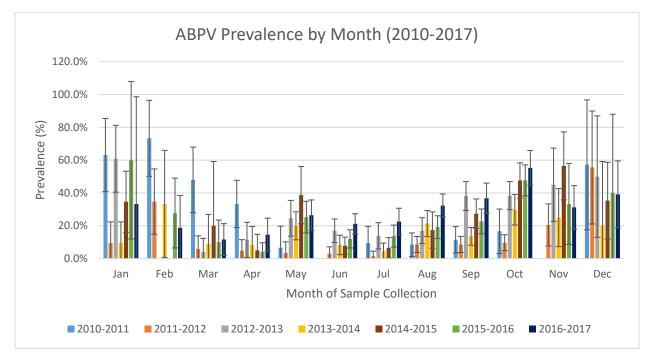
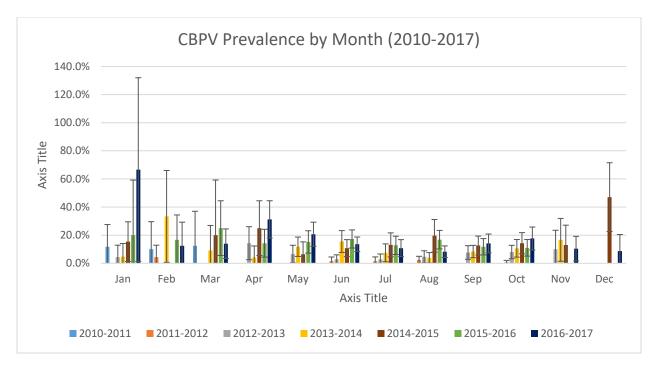


Figure 12: Prevalence of acute bee paralysis virus by month (95% confidence intervals shown)



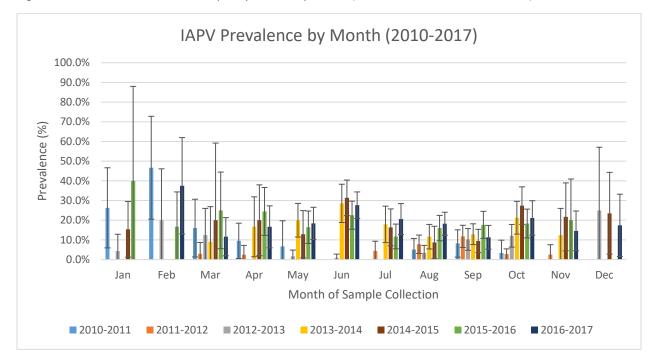
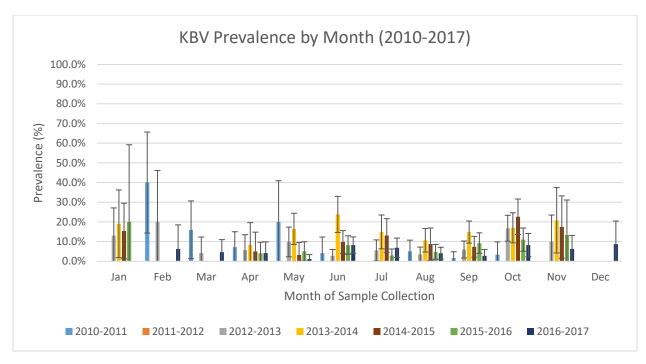


Figure 13: Prevalence of chronic bee paralysis virus by month (95% confidence intervals shown)

Figure 14: Prevalence of Israeli acute paralysis virus by month (95% confidence intervals shown)



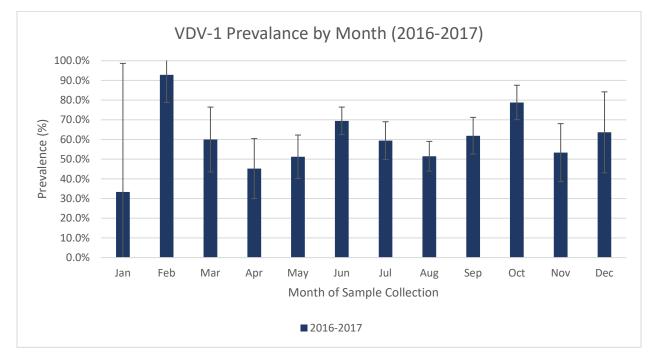


Figure 15: Prevalence of Kashmir bee virus by month (95% confidence intervals shown)

Figure 16: Prevalence of Varroa destructor virus by month (95% confidence intervals shown)

Pesticide	Туре			Average detection if	Range if
Pesticide	Туре			detection if	Range if
Pesticide	Туре			nositive for	-
resticide	Type	LOD	Prevalence	positive for target	positive for target
		(ppb)	%	(ppb)	(ppb)
1-Naphthol	Insecticide	(ppb) 50	0.64%	(pp5) 125	(ppb) 100 - 150
	Varroacide	250	0.00%	N/A	N/A
2,4 Dimethylphenyl	variouciae	250	0.0070	14/7	
	Varroacide	5	48.23%	165.9	5 - 1800
3-Hydroxycarbofuran	Insecticide	10	0.00%	N/A	N/A
4-				·	Trace -
Hydroxychlorothalonil	Fungicide	10	0.64%	Trace	Trace
Acephate	Insecticide	50	0.00%	N/A	N/A
Acetamiprid	Insecticide	4	2.25%	11	Trace - 23
Acetochlor	Herbicide	15	0.96%	70.3	16 - 103
Alachlor	Herbicide	15	1.93%	79.7	42 - 131
Aldicarb	Insecticide	25	0.00%	N/A	N/A
Aldicarb sulfone	Insecticide	15	0.00%	N/A	N/A
Aldicarb sulfoxide	Insecticide	25	0.00%	N/A	N/A
Aldrin	Insecticide	30	0.32%	99	99*
Allethrin	Insecticide	10	0.00%	N/A	N/A
Amicarbazone	Insecticide	15	0.00%	N/A	N/A
					Trace -
	Herbicide	4	22.83%	193.7	8600
Azinphos methyl	Insecticide	15	0.00%	N/A	N/A
Azoxystrobin	Fungicide	5	4.82%	96.3	Trace - 450
	Insecticide	10	0.32%	15	15*
Benoxacor	Herbicide	15	0.32%	Trace	Trace*
•	Insecticide	15	0.00%	N/A	N/A
	Insecticide	15	0.00%	N/A	N/A
Bifenthrin	Insecticide	10	2.25%	16	11 - 22
Boscalid	Fungicide	10	11.25%	67	Trace - 500
	Fungicide	50	0.00%	N/A	N/A
Buprofezin	Insecticide	60	0.32%	155	155*
Conton	Funcioida	50		770.0	Trace -
Captan	Fungicide	50	4.50%	778.9	3500 Trace -
Carbaryl	Insecticide	2	7.72%	678.8	6600
Carbendazim	Fungicide	5	12.22%	77.6	Trace - 780
	Insecticide	10	0.00%	N/A	N/A
	Fungicide	15	0.00%	N/A	, N/A
	Herbicide	5	0.00%	N/A	, N/A

					Trace -
Chlorantraniliprole	Insecticide	15	4.82%	4.82% 238	
Chlorfenopyr	Insecticide	5	0.00%	N/A	N/A
Chlorfenvinphos	Insecticide	10	0.00%	N/A	N/A
					Trace -
Chlorothalonil	Fungicide	100	7.72%	2414.3	15400
Chlorpropham (CIPC)	Insecticide	10	0.00%	N/A	N/A
Chlorpyrifos	Insecticide	5	9.32%	48.7	Trace - 150
Chlorpyrifos methyl	Insecticide	5	0.00%	N/A	N/A
Clofentezine	Insecticide	6	0.00%	N/A	N/A
Clothianidin	Insecticide	15	0.64%	Trace	Trace - Trace
Coumaphos	Varroacide	3	32.48%	76.9	Trace - 2000
Coumaphos oxon	Varroacide	2	6.43%	10.6	Trace - 27
Cyfluthrin	Insecticide	10	0.64%	20	Trace - 20
Cyhalothrin total	Insecticide	5	2.89%	26.9	5 - 110
Cypermethrin	Insecticide	10	0.00%	N/A	N/A
Cyprodinil	Fungicide	10	9.00%	198.5	Trace - 3530
DDD p,p'	Insecticide	5	0.00%	N/A	N/A
DDT p,p'	Insecticide	5	0.00%	N/A	N/A
Deltamethrin	Insecticide	50	0.00%	N/A	N/A
Diazinon	Insecticide	15	0.00%	N/A	N/A
Dichlorvos (DDVP)	Insecticide	15	0.00%	N/A	N/A
Dicloran	Fungicide	15	0.00%	N/A	N/A
Dicofol	Insecticide	5	0.64%	34.5	7 - 62
Dieldrin	Insecticide	10	0.00%	N/A	N/A
Difenoconazole	Fungicide	10	6.11%	64.4	Trace - 150
Diflubenzuron	Insecticide	5	5.79%	22.2	Trace - 64
Dimethenamid	Herbicide	10	0.64%	28	24 - 32
Dimethoate	Insecticide	15	0.00%	N/A	N/A
Dimethomorph	Fungicide	25	0.32%	Trace	Trace*
Dinotefuran	Insecticide	10	0.00%	N/A	N/A
Diphenamid	Herbicide	3	0.00%	N/A	N/A
Endosulfan I	Insecticide	10	0.32%	17	17*
					Trace -
Endosulfan II	Insecticide	10	0.96%	Trace	Trace
Endosulfan sulfate	Insecticide	10	1.61%	Trace	Trace - 19
Endrin	Insecticide	25	0.00%	N/A	N/A
Epoxiconazole	Fungicide	5	0.00%	N/A	N/A
Esfenvalerate	Insecticide	5	0.96%	30.5	Trace - 52
Ethion	Insecticide	15	3.22%	32.1	19 - 48

Ethofumesate	Herbicide	20	0.00%	N/A	N/A
Etoxazole	Insecticide	5	0.32%	Trace	Trace*
Etridiazole	Fungicide	5	0.00%	N/A	N/A
Famoxadone	Fungicide	25	0.00%	N/A	N/A
Fenamidone	Fungicide	30	0.00%	N/A	N/A
Fenbuconazole	Fungicide	15	4.82%	196.7	Trace - 560
Fenhexamid	Fungicide	30	0.96%	317.7	79 - 770
Fenoxaprop-ethyl	Herbicide	15	0.00%	N/A	N/A
Fenpropathrin	Insecticide	10	0.00%	N/A	N/A
Fenpyroximate	Varroacide	4	6.75%	83.7	Trace - 400
Fenthion	Insecticide	15	0.00%	N/A	N/A
Fipronil	Insecticide	50	0.00%	N/A	N/A
Flonicamid	Insecticide	15	0.96%	218	Trace - 370
Flubendiamide	Insecticide	10	0.00%	N/A	N/A
Fludioxonil	Fungicide	60	0.64%	3341.5	833 - 5850
Fluopyram	Fungicide	5	7.72%	65.1	Trace - 387
Fluoxastrobin	Fungicide	5	0.32%	Trace	Trace*
Fluridone	Herbicide	5	0.00%	N/A	N/A
Flutolanil	Fungicide	15	1.93%	42	Trace - 109
		_			Trace -
Fluvalinate	Varroacide	5	17.68%	85.9	1330
Heptachlor	Insecticide	15	0.00%	N/A	N/A
Heptachlor epoxide	Insecticide	15	0.00%	N/A	N/A
Hexachlorobenzene (HCB)	Insecticide	5	0.00%	N/A	N/A
Hexythiazox	Fungicide	15	1.29%	35	Trace - 54
Hydroprene	Insecticide	100	0.00%	N/A	N/A
Imazalil	Fungicide	20	0.00%	N/A	N/A
Imidacloprid	Insecticide	6	1.29%	25.3	Trace - 39
Imidacloprid 5-					
hydroxy	Insecticide	150	0.00%	N/A	N/A
Imidacloprid olefin	Insecticide	50	0.00%	N/A	N/A
Indoxacarb	Insecticide	30	0.32%	33	33*
Iprodione	Fungicide	50	7.07%	326.7	Trace - 1300
Lindane	Insecticide	10	0.00%	N/A	N/A
Linuron	Herbicide	15	0.00%	N/A	N/A
Malathion	Insecticide	10	0.64%	51	23 - 79
Metalaxyl	Fungicide	5	0.32%	Trace	Trace*
Metconazole	Fungicide	10	0.32%	18	18*
Methamidophos	Insecticide	40	0.00%	N/A	N/A
Methidathion	Insecticide	5	0.00%	N/A	N/A

Methomyl	Insecticide	25	0.00%	N/A	N/A
					5090 -
Methoprene	Insecticide	80	0.64%	5480	5870
Methoxyfenozide	Insecticide	5	9.65%	25.5	Trace - 100
Metolachlor	Herbicide	5	10.61%	20.2	Trace - 165
Metribuzin	Herbicide	5	0.64%	8	Trace - 8
MGK-264	Insecticide	25	0.32%	25	25*
MGK-326	Insecticide	30	0.00%	N/A	N/A
Myclobutanil	Fungicide	15	0.96%	232.7	15 - 600
Naled	Insecticide	50	0.00%	N/A	N/A
Norflurazon	Herbicide	15	0.00%	N/A	N/A
Oxamyl	Insecticide	15	0.00%	N/A	N/A
Oxyfluorfen	Herbicide	5	3.54%	11	Trace - 18
Paradichlorobenzene	Insecticide	250	0.00%	N/A	N/A
Parathion methyl	Insecticide	10	0.00%	N/A	N/A
Pendimethalin	Herbicide	15	2.89%	29.8	Trace - 52
Permethrin total	Insecticide	25	0.00%	N/A	N/A
Phenothrin	Insecticide	30	0.32%	40	40*
Phorate	Insecticide	25	0.00%	N/A	N/A
Phosalone	Insecticide	15	0.00%	N/A	N/A
Phosmet	Insecticide	50	1.29%	252	Trace - 330
Piperonyl butoxide	Insecticide	15	3.86%	140.3	Trace - 200
Pirimiphos methyl	Insecticide	15	0.00%	N/A	N/A
Prallethrin	Insecticide	20	0.00%	N/A	N/A
Profenofos	Insecticide	30	0.00%	N/A	N/A
Pronamide	Herbicide	5	0.00%	N/A	N/A
Propachlor	Herbicide	25	2.89%	1004.8	419 - 1690
Propargite	Insecticide	15	0.64%	20.5	16 - 25
Propazine	Herbicide	10	0.00%	N/A	N/A
Propetamphos	Insecticide	20	0.00%	N/A	N/A
Propham	Herbicide	15	0.00%	N/A	N/A
Propiconazole	Fungicide	15	7.40%	107.8	Trace - 415
Pymetrozine	Insecticide	30	0.00%	N/A	N/A
Pyraclostrobin	Fungicide	5	16.72%	51.1	Trace - 220
Pyrethrins	Insecticide	250	0.00%	N/A	N/A
Pyridaben	Insecticide	5	0.64%	16.5	14 - 19
Pyrimethanil	Fungicide	15	4.82%	119.1	Trace - 380
Pyriproxyfen	Insecticide	5	0.96%	27	Trace - 32
Quinoxyfen	Fungicide	15	0.96%	16	Trace - 16
Quintozene (PCNB)	Fungicide	5	0.00%	N/A	N/A
Resmethrin	Insecticide	30	0.00%	N/A	N/A
Sethoxydim	Herbicide	10	0.00%	N/A	N/A

Simazine	Herbicide	50	0.64%	50	Trace - 50
					Trace -
Spinosad	Insecticide	15	0.96%	Trace	Trace
Spirodiclofen	Insecticide	5	0.64%	28	23 - 33
Spiromesifen	Insecticide	50	0.00%	N/A	N/A
Tebuconazole	Fungicide	5	4.18%	84.6	Trace - 250
Tebufenozide	Insecticide	5	0.96%	21.7	6 - 37
Tebuthiuron	Herbicide	15	0.00%	N/A	N/A
Tefluthrin	Insecticide	5	0.00%	N/A	N/A
Tetrachlorvinphos	Insecticide	15	0.00%	N/A	N/A
Tetraconazole	Fungicide	15	0.00%	N/A	N/A
Tetradifon	Insecticide	5	0.00%	N/A	N/A
Tetramethrin	Insecticide	30	0.00%	N/A	N/A
Thiabendazole	Fungicide	5	0.64%	26	Trace - 26
Thiacloprid	Insecticide	5	0.32%	29	29*
Thiamethoxam	Insecticide	10	0.64%	10	Trace - 10
ТНРІ	Fungicide	15	2.89%	1250.3	Trace - 6600
Thymol	Varroacide	50	29.26%	2028.5	Trace - 18000
Triadimefon	Fungicide	10	0.00%	N/A	N/A
Triadimenol	Fungicide	25	0.00%	N/A	N/A
Tribufos (DEF)	Fungicide	10	0.64%	130	Trace - 130
Trifloxystrobin	Fungicide	10	0.32%	62	62*
Triflumizole	Fungicide	40	0.00%	N/A	N/A
Trifluralin	Herbicide	5	1.93%	Trace	Trace - Trace
Triticonazole	Fungicide	30	0.00%	N/A	N/A
Vinclozolin	Fungicide	5	0.00%	N/A N/A	N/A N/A
VINCIOZOIIN	-			IN/A	

Figure 17: Pesticide detection in the 2016 – 2017 survey year (311 samples) (\*denotes single detection only) (positive detections are highlighted in yellow)

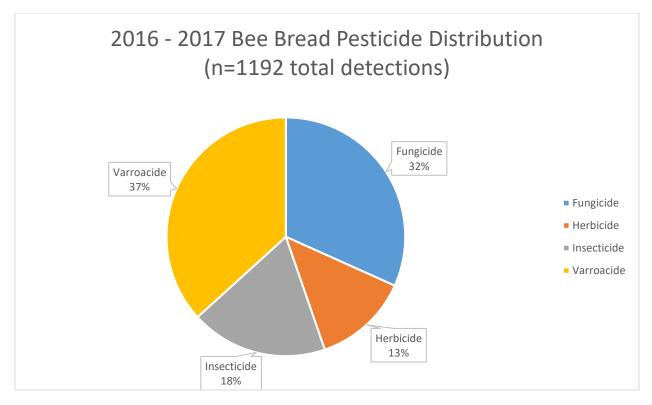


Figure 18: Classification of pesticide type detected in the 2016 – 2017 survey year. 311 total samples, with 1,192 total pesticide detections.

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# Appendix

Collection date:	-			1.					
			0						
Sampler name:		_	Samp	ler phor	ne #:				
Sampler address:		_	Beeke	eeper pl	hone #:				
Beekeeper name:		_	Beeke	eeper er	mail ado	dress:			
Beekeeper address:			GPS -	use d	ecimal	degree	es, e.g. (	dd.ddd	bbb
		_		Latitu	ude:	-			
Sampling Address:		_		Long	itude: _				
		_			ations (c n the <u>pa</u>			) where	e hive was
Sampling County:		_							
State Origin of Hive:		_	Whick	h of the	follow	ing bes	st descr	ibes tl	he primary
Is the sampled apiary part of a migratory opera []Yes[]No	ation?		[]hor	ney pro		[]pol			een produc
the disease or pest, please write a "0" in the box u completing this form.	inless a	therwis	e direc		e back		ditional	guida	nce in
						6	7	8	Total
Brood disease	1	2	3	4	5	10	/		10100
Brood disease AFB	1	2	3	4	5	0	/	-	Total
	1	2	3	4	5	0	/		Total
AFB	1	2	3	4	5	0			
AFB EFB	1	2	3	4	5				
AFB EFB Sac Brood	1	2	3	4	5				
AFB EFB Sac Brood Chalkbrood	1	2	3	4	5				
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood	1	2	3	4	5				
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease	1	2	3	4	5				
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus	1		3	4	5				
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees	1		3	4	5				
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees Pest infestation	1		3	4	5				
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees Pest infestation Small hive beetle - larvae or adult	1		3	4					
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees Pest infestation Small hive beetle - larvae or adult Wax moth – larvae or adult Queen condition Queen cells present	1		3	4					
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees Pest infestation Small hive beetle - larvae or adult Wax moth – larvae or adult Queen condition Queen cells present Drone laying queen	1		3						
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees Pest infestation Small hive beetle - larvae or adult Wax moth – larvae or adult Queen condition Queen cells present Drone laying queen Queen right (queen or eggs are viewed)	1								
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees Pest infestation Small hive beetle - larvae or adult Wax moth – larvae or adult Queen condition Queen cells present Drone laying queen	1		3						
AFB EFB Sac Brood Chalkbrood Parasitic Mite Syndrome (PMS)/Snotty brood Adult disease Deformed wing virus Black shiny bees Pest infestation Small hive beetle - larvae or adult Wax moth – larvae or adult Queen condition Queen cells present Drone laying queen Queen right (queen or eggs are viewed)	1								