

## 2012-2013 National Honey Bee Pests and Diseases Survey Report

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### Executive summary

The 2012 USDA Animal Plant and Health Inspection (APHIS) sponsored National Survey of Honey Bee Pests and Diseases was conducted in collaboration with the University of Maryland (UMD) and the USDA Agricultural Research Service (ARS) and with the cooperation of 32 states covering the timeframe from June 2012 through September of 2013. This survey is in its fourth year. The National Survey expanded from a 3 state Pilot Survey in 2009 to a Limited National survey of 13 states in 2010, and then a more extensive survey in 2011 that included 34 states. This expansion has allowed us to augment and extend the baseline pest and pathogen data collected from the previous two limited surveys. The primary focus of repeating the extended survey in 2012 was to verify the absence of exotic threats such as the parasitic mite, *Tropilaelaps* spp., *Apis cerana*, and Slow Paralysis Virus. Under current international trade agreements, the U.S. cannot deny import permits from other nations unless the exporting nation has a disease, parasite, or pest of honey bees that is not found in the U.S. Establishing the absence of threats to honey bee populations not thought to be present in the U.S. was the primary objective of this effort.

With exotic species as the primary target, a secondary objective was set to make use of the sampling by determining existing levels of other honey bee diseases and parasites known to be present in the U.S. This was also performed in the previous three surveys. The survey results are used to gauge the overall health of colonies, to create a baseline disease level, and to facilitate interpretation of ongoing and future epidemiological studies. This baseline data, including historic data from research institutions such as the ARS Bee Research Laboratory (BRL) and other ongoing field sampling and management surveys, have been incorporated into a single database as part of the Bee Informed Partnership ([www.beeinformed.org](http://www.beeinformed.org)), a 5 year grant funded by USDA NIFA (National Institute of Food and Agriculture). The 2012-2013 National Survey effort included collection of samples from 32 states: Alabama, Arkansas, California, Colorado, Florida, Georgia, Hawaii, Idaho, Illinois, Indiana, Iowa, Louisiana, Massachusetts, Maryland, Michigan, Minnesota, Montana, New York, , New Jersey, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, West Virginia and Wisconsin. California is sampled twice as

much (48 samples instead of the standard 24 samples per state) with the goal to sample 24 beekeepers who stay in California year round and 24 beekeepers who migrate to California during pollination season. New Mexico was originally scheduled to participate but had a staffing change and it was decided to hold off on their sampling until the following survey year as they received their materials late. Their participation will be included in the 2013 survey.

This end of year report is delayed because of several changes in our molecular lab that held up the data for several months. The shutdown of the government during Sequestration negatively impacted those lab changes, specifically staff changes, and ordering new equipment. We have now redesigned the molecular lab to be high-throughput to avoid large backlogs in the future. We also went to the auto generation of reports last year to help distribute them more quickly.

A total of 792 (32 statesx24 samples/state, plus the extra 24 samples for California) samples representing 6,336 colonies are expected at the completion of this survey. To date, approximately 94% of the live bee samples (for virus analysis) have been collected; however, only 92% were analyzed due to either the live bees dying in transit, loss of sample in long term storage or bad quality RNA due to insufficient nucleic acid extraction. We are creating a standard operating protocol (SOP) from the arrival of the live bee sample to analysis to minimize loss/damage of live bee samples in the future.

94% of the alcohol samples (for Varroa mite, *Nosema* and *Tropilaelaps* determination) have been received and processed. Also at this time, 100% of received samples have been analyzed and reports have been distributed to apiary specialists and beekeepers. A second full National Survey for 2013 was initiated early this summer with 32 participating states. Samples for this fourth survey have already begun to arrive.

The survey samples were analyzed for 11 known honey bee viruses, pests and pathogens. Slow Bee Paralysis Virus (SBPV), the only virus included in this year's testing that is not currently found in the U.S., was examined in all samples and no detection was made. The accuracy of the SBPV detection method was further verified using an additional set of primers. No diseases or parasites of bees not already known to exist in the country were discovered. Two common honey bee viruses, Deformed Wing Virus (DWV) and Black Queen Cell Virus (BQCV), were found in all 34 states. In this 2012 survey, 5 samples out of 727 tested positive for *Nosema apis*, or about 0.7% of the samples, and 503 samples out of 727 that were tested, tested positive for *Nosema cerana*, or about 69%. *Nosema apis* was not detected in the first two survey years (2009 and 2010) but was detected in 1.3% of samples analyzed last year (2011). For the fourth year, we saw no evidence of *Tropilaelaps* mites, or the Asiatic honey bee, *Apis cerana*. Since honey bee tracheal mites (*Acarapis woodi*) were not detected in samples in 2009 nor in 2010, samples were not analyzed for the mite last year or this year, but subsamples are saved to examine in the future. Honey bee tracheal mites are known to exist in the country and our failure to find them may be the result of our sampling; samples are generally collected during the productive seasons and mites are more prevalent in the winter

months. Varroa mites continued to be observed in all states with the exception of the Hawaiian Islands of Maui, Kauai and Molokai.

For a second time this survey collected and analyzed bee bread (pollen stored in the colony by bees) for pesticides. A ~3 grams of pollen were collected from brood frames and tested for 174 known pesticides. The pollen was collected from the same composite 8 colonies undergoing the standard survey sampling and sent to the USDA Agricultural Marketing Service (AMS) in Gastonia, NC for analysis. All states were funded to participate in the pollen study, and each state was asked to send in composite samples of pollen from 10 of the 24 apiaries this year. We received 312 samples from these states (out of a possible 340) and of the 174 possible pesticides, 80 were detected, up from 34 pesticides detected last year (Figure 19). 82 percent of samples were positive for at least one product and on average had 3 products.

This survey was designed to be representative of the managed honey bees across the broad geography of the United States. The survey was open to any state wishing to participate and beekeeper participation was completely voluntary and the beekeeper did not have to be present or assist with the sampling. The results can be considered as representative of the distribution of pests and pathogens present in the U.S.

## **Introduction**

This 32 state survey of honey bee pests and pathogens began in 2012 and was completed in late summer 2013. This survey was delayed in completion because of 1) the variability in climates when samples can be collected from active brood areas in northern states 2) the time frame the states received their respective agreements, and 3) bottlenecks in sample processing and reporting. Funding was provided by the USDA Animal and Plant Health Inspection Service (APHIS) and the survey was conducted in collaboration with the USDA Agricultural Research Service (ARS), and the University of Maryland (UMD). Participating states were the following: Alabama, Arkansas, California, Colorado, , Florida, Georgia, Hawaii, Idaho, Illinois, Indiana, Iowa, Louisiana, Massachusetts, Maryland, Michigan, Minnesota, Montana, New York, New Jersey, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, West Virginia and Wisconsin. Kits required to sample 24 apiaries were provided to each participating state, with the exception of California, where kits required for 48 kits were provided. A total of 744 alcohol samples, 731 Tropilaelaps samples, and 743 live bee boxes were returned representing the sampling of 5928 colonies.

## **Survey Description**

Survey kits were distributed to the participating states' Apiary Specialist in June 2012. This later than ideal date was due to delayed approval to fund the survey which did not allow for some states to complete sampling until the spring of 2013. In some cases sampling

continued well into late summer of 2013 due to various weather events across the United States and other isolated circumstances.

Apiary specialists conducted an aggregate sampling from previously identified commercial, migratory, and sideliner beekeepers with at least 8 colonies per apiary. In most cases, apiaries consisted of at least 10 colonies. A single aggregate sample was collected from 8 randomly selected colonies per apiary per operation ([APHIS US Honey Bee Survey Sampling Protocol](#)). In each state, apiaries were chosen on a case by case basis with an attempt to give as close to an equal representation of the entire state as possible. Ideally, a state was sectioned into 4 quadrants with apiaries randomly chosen within a quadrant. When possible, ten queen producers were sampled. Of the remaining sampled apiaries, 1/2 were from migratory operations (move out of the state and return prior to sampling) and 1/2 were from stationary operations (only move within the state or do not move at all). Additional apiaries occurring near ports or other areas that could be considered high risk were also considered for sampling ([APHIS US Honey Bee Survey Project Plan](#)). The pollen samples were collected ([APHIS US Honey Bee Survey Pollen Sampling Protocol](#)) concurrently and from the same colonies in the apiary being sampled for the disease and pest survey.

Four distinct collection methods were used to sample each apiary. The first sample was a collection of live adult bees composed of ¼ cup of bees (~ 150 bees) that were shaken off brood frames from each of the 8 sampled colonies. The 2 cups of (~600 bees) live bees were deposited in a ventilated shipping box containing a water source and hard sugar candy (fondant). This box was shipped the same day to the USDA/ARS in Beltsville, MD where it was immediately frozen at -80C until molecular testing could be performed. The molecular tests were performed with quantitative-PCR techniques, outlined by Dr. Jay Evans at the USDA/ARS Bee Research Laboratory, to look for molecular evidence of known viruses and other pests (2006 and [Honey Bee PCR Diagnostics](#)). We have initiated a new, high-performance chemistry and a more effective and stream-lined sample processing in our molecular analysis. The goal is to achieve greater sensitivity, faster, more accurate and more cost effective diagnostic analysis. Moreover, using new PCR chemistry and automated nucleic acid extractions, the molecular viral assays need to be re-evaluated, and validated. For example, there are indications that the IAPV primers presently used may actually underestimate the number of samples positive for the virus. Ongoing work will produce new reports on IAPV prevalence for this and previous years.

This years molecular assays were designed to detect the presence of the following:

1. Acute Bee Paralysis Virus (ABPV)
2. Deformed Wing Virus (DWV)
3. Israeli Acute Paralysis Virus (IAPV)
4. Kashmir Bee Virus (KBV)
5. Chronic Bee Paralysis Virus (CBPV)
6. Black Queen Cell Virus (BQCV)
7. Slow Bee Paralysis Virus (SBPV)

8. *Nosema ceranae*

9. *Nosema apis*

The second sample of bees, consisting of ¼ cup of bees from each of the 8 sampled colonies for a total of 2 cups of bees per apiary, originated from the same brood frames as the live bee sample. These bees were put into a bottle of alcohol for preservation. This alcohol sample was shipped to USDA/BRL for microscopic analysis by University of Maryland technicians to visually quantify the following:

1. *Nosema* spp. spores
2. Varroa Mite loads
3. *A. cerana*

The third sample was taken from anything dislodged from ‘bumping’ sampled brood frames over a collection pan. This technique was developed by Dr. Jeff Pettis and Dr. Dennis vanEngelsdorp and funded by APHIS as a quick and cost effective way to detect for the *Tropilaelaps* mite (Pettis et al. 2013). The sample, also preserved in alcohol, included any mites, beetles and other hive debris filtered from bumping the brood frames. This sample was shipped to USDA/ARS Beltsville, MD and analyzed microscopically at the University of Maryland for the presence of the *Tropilaelaps* mite.

Finally, the fourth sample included a minimum of 3 grams of fresh pollen from within the hive from the same colonies, preferably in the same brood area, from the other three samples described above. These samples were placed in a tube, labeled and sent to USDA/ARS Bee Research Laboratory where they catalogued by UMD personnel and sent to AMS for pesticide analysis.

All participating beekeepers, as well as State Apiarist/Inspectors, received a single report for each sample taken. The reports detail the analysis results for Varroa mite load, *Nosema* load, all seven viruses and the species identification of *Nosema* (*N. apis* or *N. ceranae*) and notes the presence or absence of *A. cerana* and *Tropilaelaps* spp. This report was usually sent within 6 months of collection, although some reports took up to 12 months to complete from date of sampling due to the redesign of our molecular laboratory. It also included the national prevalence for viruses at the date of the report as well as specific beekeeper percentile rankings of Varroa mite load, *Nosema* spore load and viral loads for the 2 most common viruses, DWV and BQCV. There was a break in production at the USDA/ARS molecular lab and the government shutdown in the fall of 2013 also affected the viral analysis. Due to this, approximately 26% of beekeepers received a partial report with just Varroa and *Nosema* data and another later report with molecular data when it became available. Reporting is complete with the latest batch of molecular data having been completed in December of 2013.

Using the U.S. Postal Service, live bee shipments were made to USDA/ARS and percent survivability was tracked for all live bee shipments. The results of this analysis, previously

proven to be a robust and suitable alternative for shipping bees on dry ice by the Pilot and Limited Survey, continued to work well. In some states, a small number of live bee samples were degraded, such that no molecular data could be obtained for these samples.

## Results

### Pest Survey:

#### Nosema spore prevalence and load

Of the 741 alcohol samples collected in 2012-2013 and analyzed for *Nosema* via microscopy, 348 (47.0%) had detectable spore loads. An examination of 95% CI surrounding the prevalence of *Nosema* infected apiaries suggest that prevalence was highest in 2009 (Figure 1). Of the samples in 2012 that tested positive for *Nosema*, 61 samples (8.2%) exceeded the threshold thought to cause damage (> 1 million spores per bee). Samples testing positive for *Nosema* infection had a mean *Nosema* spore load of 880,000 spores per bee, (Figure 2). Figures 3 and 4 illustrate *Nosema* prevalence and *Nosema* spore load from all 4 years of the survey on a monthly basis. Any month having less than 3 data points was not included in the monthly calculations. Figure 3 shows the classic seasonal decline in *Nosema* detection in the late summer and early fall in conjunction with a decrease in detectable spore load in those same months (Figure 4). Data on treatment use across the country is being compiled from a management survey that was included with the beekeeper reports this year.

#### Varroa mite prevalence and load

Varroa mite prevalence for 2012 is similar to that observed in 2011 (91.8%), 2010 (92.4%) and 2009 (87.1%) with 90.7% of all 741 alcohol samples collected had at least one Varroa mite (Figure 5). While prevalence did not change over the four years of survey, the overall mite load in infested apiaries has increased steadily since 2009 until 2011 and seems to have stayed steady between 2011 and 2012 (Figure 6). Again, caution should be used when interpreting this data as the 2009 sampling time frame was not the same as in subsequent years. While the economic threshold for Varroa mites is seasonally and regionally specific, generally an average load of over 3 mites per 100 bees is of concern. An alarming results shows that, in 2012, 52.8% of the samples that tested positive for Varroa (354 out of 671) exceeded the actionable threshold .

Figures 7 and 8 illustrate the dynamic nature of Varroa mite populations over the course of the year. Varroa mite levels were highest in the late summer and fall months .

#### Viral prevalence

Figures 9, 10, 11, and 12 illustrate the viral prevalence profiles for the survey years 2009, 2010 2011, and 2012 respectively. Four viruses were consistently tested for all 4 years

and include Israeli Acute Paralysis Virus, Deformed Wing Virus, Acute Bee Paralysis Virus and Slow Bee Paralysis Virus (SBPV). The survey in 2009 reported the highest incidence of IAPV but 2010 saw the highest incidence of DWV and ABPV. In this survey year, DWV and BQCV, the two most ubiquitous viruses among honey bees, were detected in every state sampled.

The monthly prevalence of four commonly found viruses (IAPV, DWV, ABPV and BQCV) is provided in Figures 13, 14, 15 and 16. IAPV (Figure 13) and ABPV (Figure 14) illustrate seasonality in these viruses. In contrast, DWV (Figure 15) and BQCV (Figure 16) exhibit a more constant presence with a slight peak for BQCV seen in the late spring/early summer months. No monthly prevalence graphs are provided for SBPV as this virus was not detected

Finally, this study found no evidence of *Tropilaelaps* or *Apis cerana*. Visual analysis of samples collected in alcohol did not detect a presence of this exotic *Apis* species and *A. mellifera* sub-races.

Molecular detection of *Nosema sp.*:

The molecular techniques employed in this survey are based on analysis of the RNA extracted from each sample. Therefore, our molecular identification focuses on detection of actively reproducing *Nosema* (vegetative stage), not dormant (spore stage) *Nosema*. Subsequently, it is possible that the samples examined by microscopy had detectable levels of *Nosema* (spores) while the molecular analysis quantifies active infection. This accounts for the difference in the PCR and microscopic detection of *Nosema* in these samples (Figure 17 and 18).

It should also be noted that *N. apis* was detected in 1.25% of samples this survey year, similar to last year where it was found in 1.3% of samples. *N. apis* was detected for the first time in the 2011 survey, possibly because of more sensitive primers used in PCR. This year, 36.91% samples tested positive for *N. cerana*, which still appears to have largely replaced *N. apis* in the European honey bee (*Apis mellifera*) after migrating from its original host, *A. cerana*.

### **Pesticide Survey:**

This year each participating state submitted ten composite pollen samples from 10 of their 24 apiaries. To date the most prevalent pesticides are miticides applied directly to hives to control Varroa mites and include Coumaphos and its metabolites (detected in 37.7% of the samples), Fluvalinate (detected in 50% of the samples), and Thymol and 2, 4 Dimethylphenyl (a metabolite of Amitraz), detected in 21.1% and 21.3% of samples respectively. Chlorpyrifos (detected in 20.4% of the samples) was the most prevalent pesticide found not used for Varroa control.

On average each sample had 3 different products and or metabolites with as many as 15 products and or metabolites found in a single sample. The full set of results, grouped by their

classification as an insecticide, herbicide or fungicide, is given in Figure 20. The level of detection (LOD), or the minimum amount that can be reliably detected, the national prevalence (%) seen by this limited survey, the average level detected (parts per billion or ppb) and the range of detection (ppb) are provided for those samples that tested positive for that specific pesticide. If a pesticide was detected once, a single value is given for the range and it is marked with an asterisk. With the additional information of pollen collected concurrently with the live bee and *Nosema* and Varroa mite samples, it may be possible to correlate colony health to in hive pesticide residue.

## Conclusions

The increased sample size this year allows for the expansion of our database of pests and pathogens and places the collected data into a temporal context. This National Survey, which took place between Summer of 2012 through Fall of 2013 was expanded to capture some states not previously sampled and the sampling season was lengthened with the inclusion of more southern states. This allowed us to greatly increase viral and pest data for the winter months as shown by the prevalence graphs for the more common viruses. Varroa mite loads were seen to increase over the first three years of the survey and remained high for this fourth year but did not show further increase .

Results that are being monitored in the 2013-2014 survey include Varroa mite loads to determine if the increasing trend continues and what, if any treatments, are being applied to the sampled colonies. Additional data will be collected on BQCV as not much is known about this very common and established virus. Observing the dynamics and possible seasonality between *N. apis* and *N. ceranae*, in conjunction with treatment data we hope to collect will also be core to the 2013 survey. By gathering yearly, sequential samples from a growing number of states, we may be able to see trends and patterns that relate to colony health. The survey does provide strong evidence that *Tropilaelaps*, Slow Paralysis Virus and *Apis cerana* are not present in the U.S.



Appendix

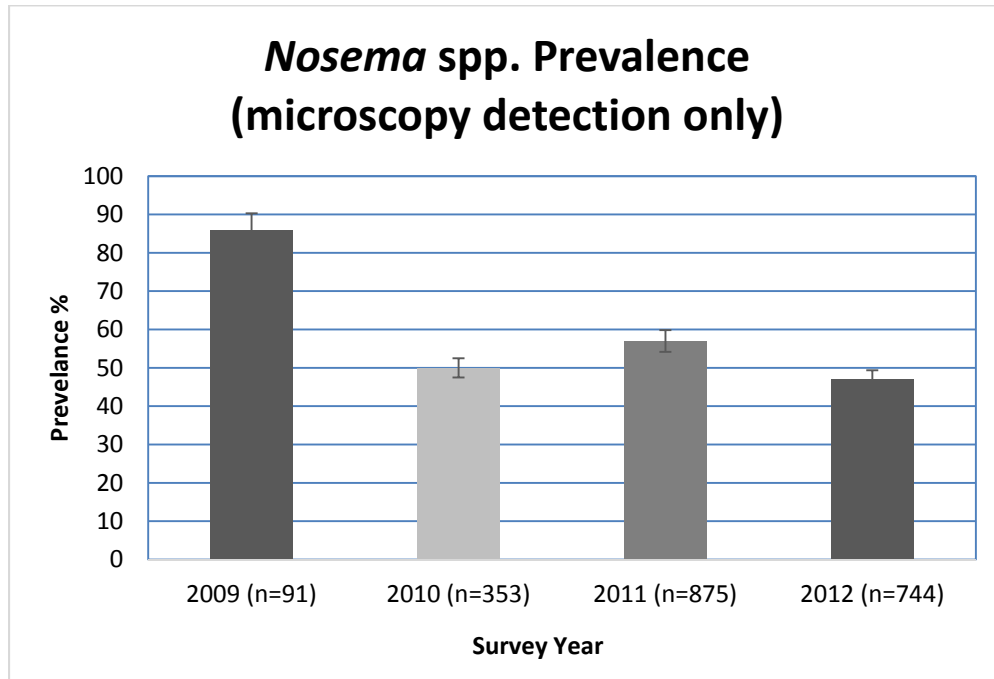


Figure 1: *Nosema* spp. prevalence over 4 years of survey (95% Confidence Intervals shown)

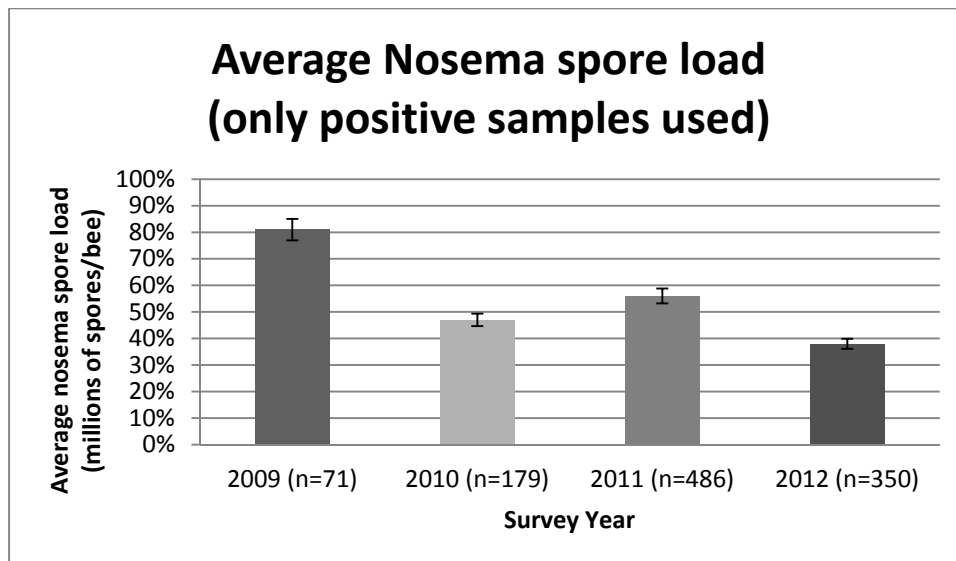


Figure 2: *Nosema* spp. spore load over 3 years of survey (95% Confidence Intervals shown)

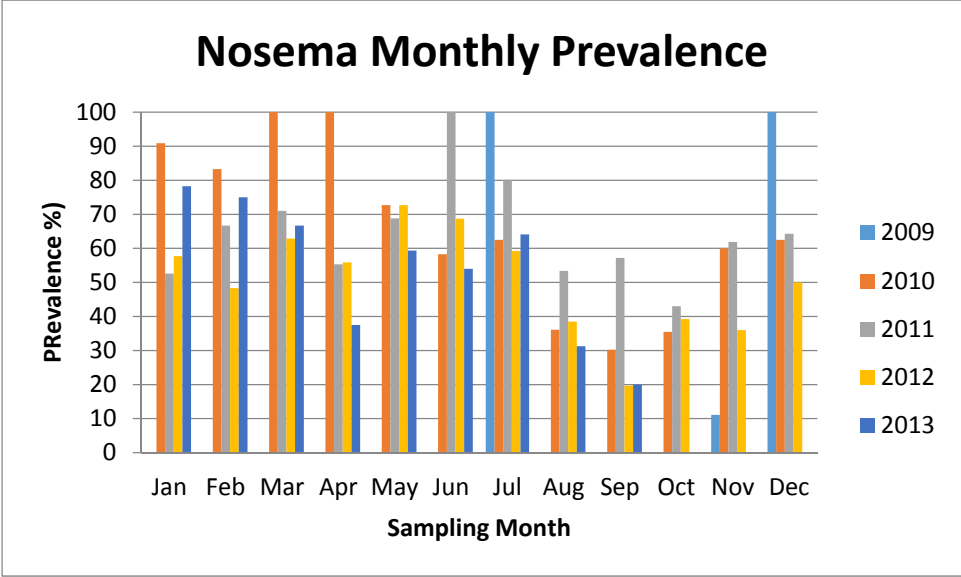


Figure 3: Monthly prevalence for *Nosma* spp. (95% Confidence Intervals shown)

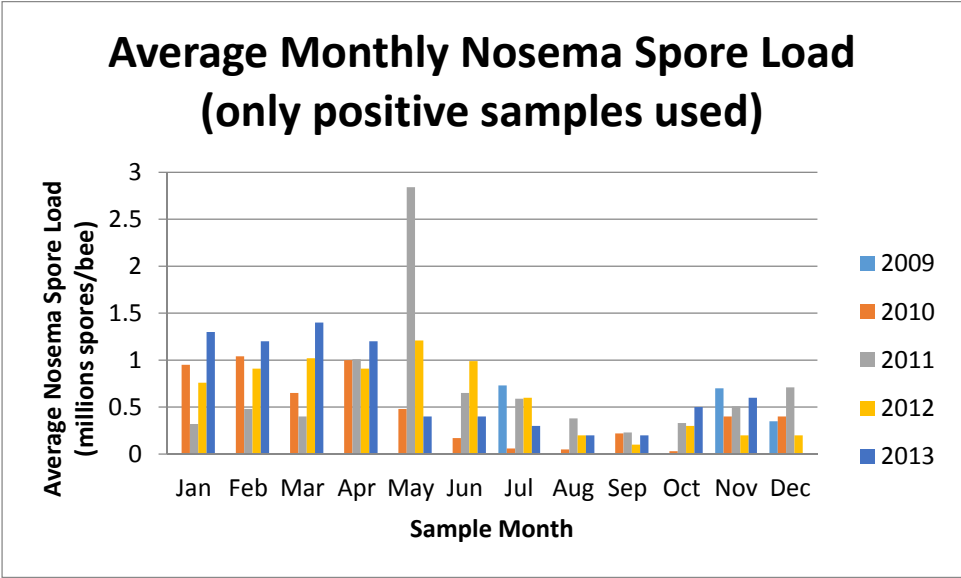


Figure 4: Average Monthly *Nosema* spore load (for samples testing positive by microscopic spore count) (95% Confidence Intervals shown)

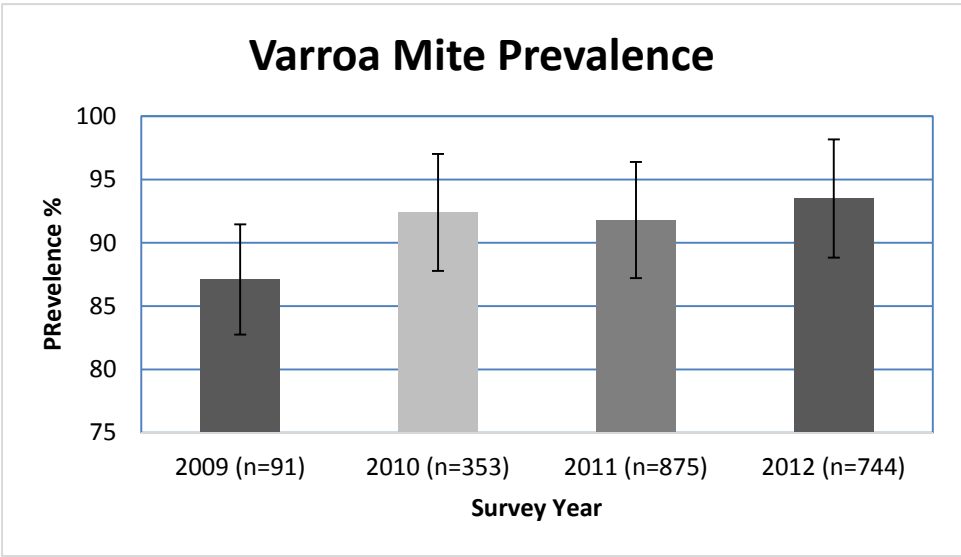


Figure 5: Varroa mite prevalence over 4 years of survey (95% Confidence Intervals shown)

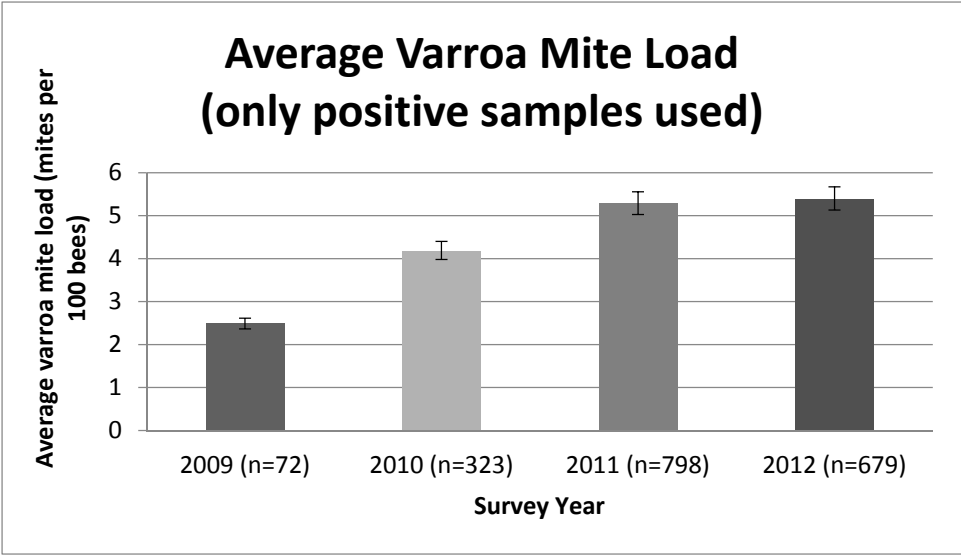


Figure 6: Average Varroa mite load over 4 years of survey (for samples testing positive) (95% Confidence Intervals shown)

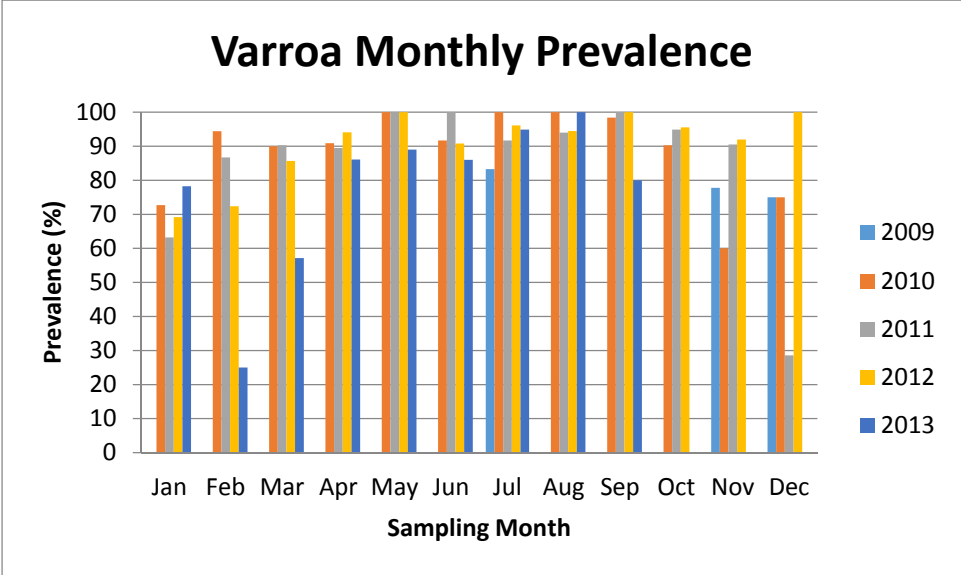


Figure 7: Monthly prevalence for Varroa mites

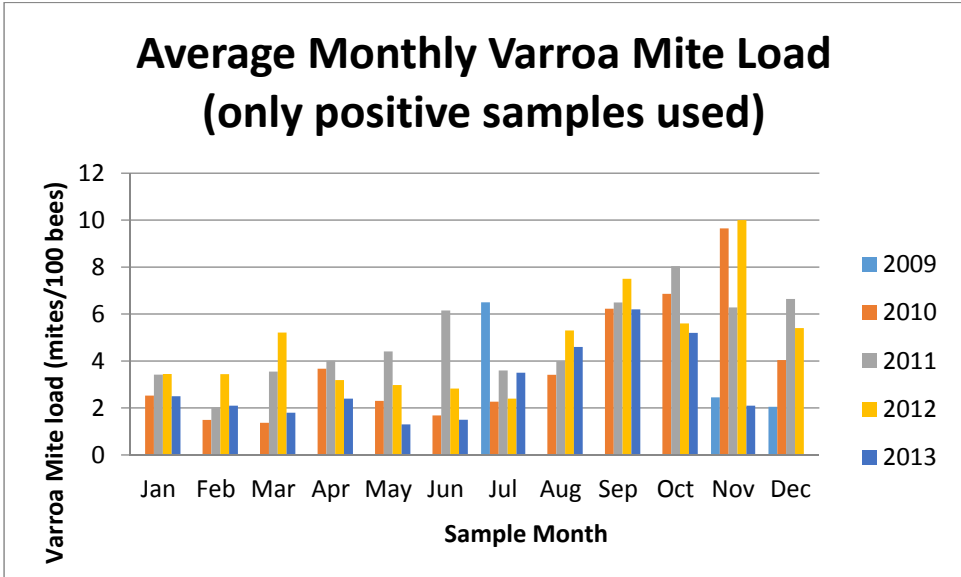


Figure 8: Average monthly Varroa mite load (for samples testing positive)

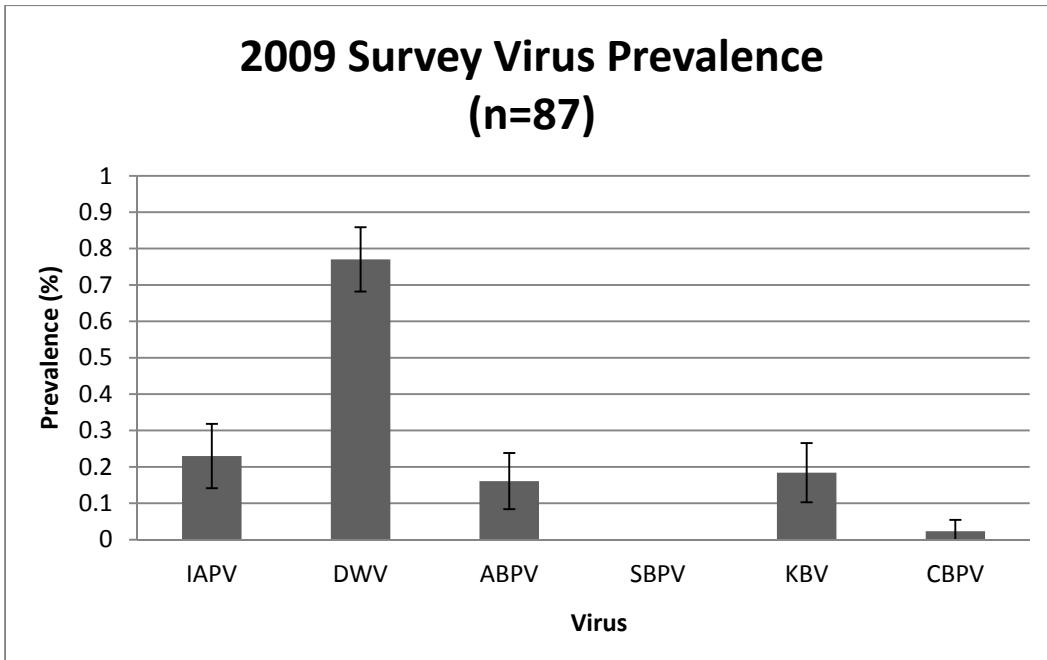


Figure 9: Virus prevalence for 2009 Pilot Survey (95% Confidence Intervals shown)

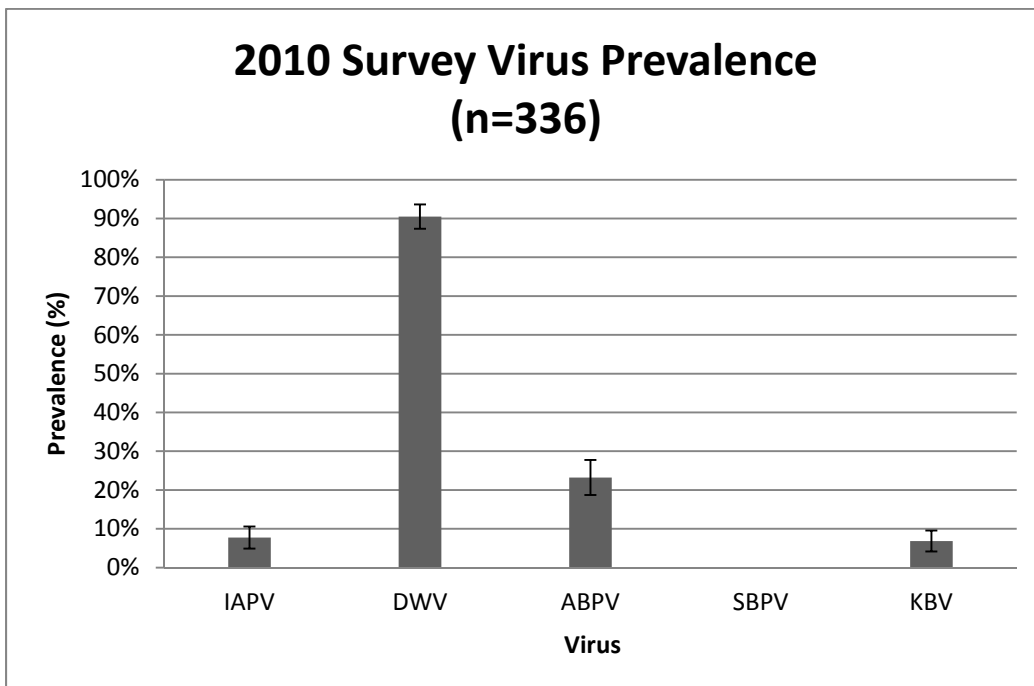


Figure 10: Virus prevalence for 2010 Limited National Survey (95% Confidence Intervals shown)

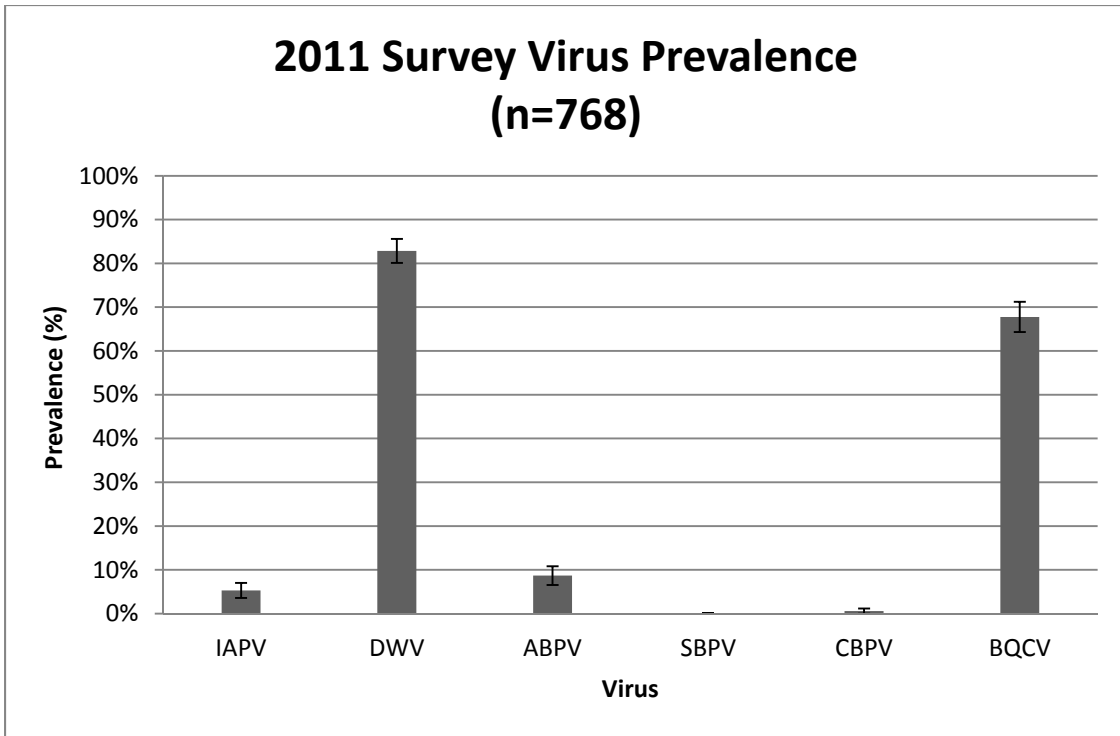


Figure 11: Virus prevalence for 2011 National Survey (95% Confidence Intervals shown)

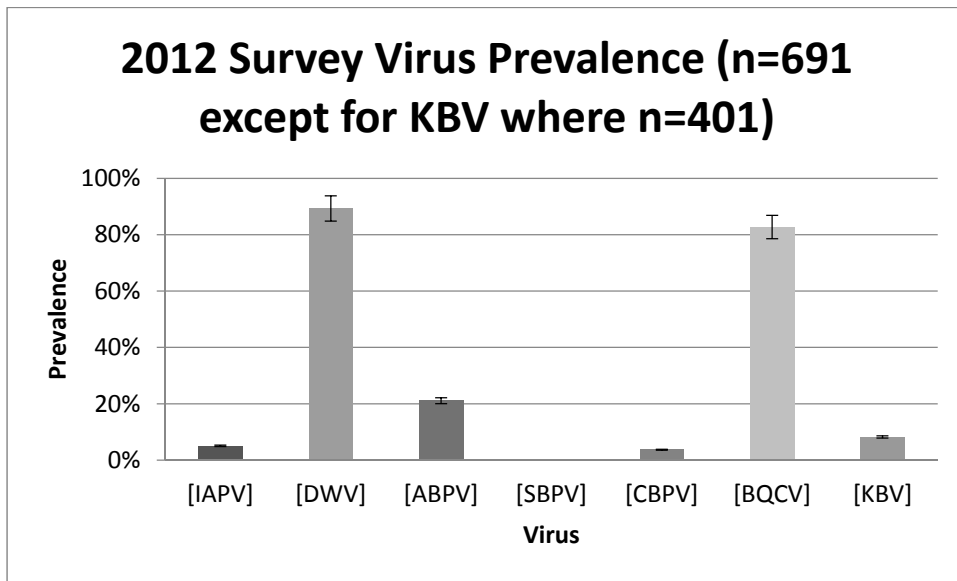


Figure 12: Virus prevalence for 2012 National Survey (95% Confidence Intervals shown)

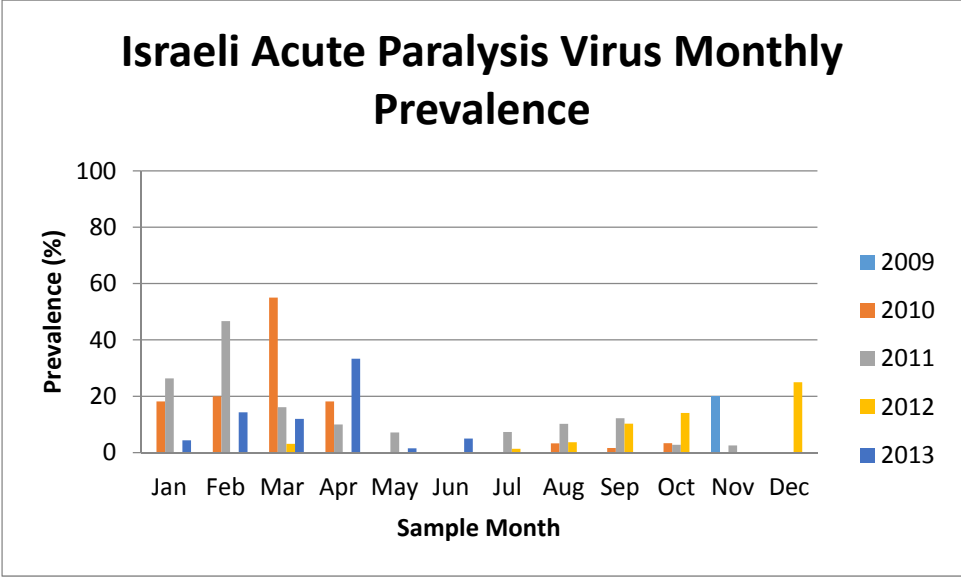


Figure 13: Israeli Acute Paralysis Virus prevalence over 4 years of survey

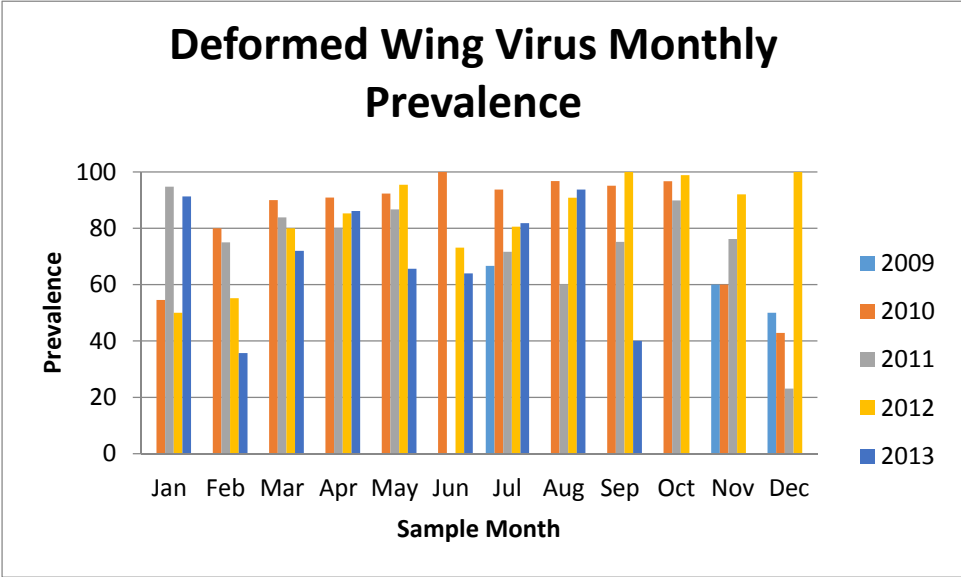


Figure 14: Deformed Wing Virus prevalence over 4 years of survey

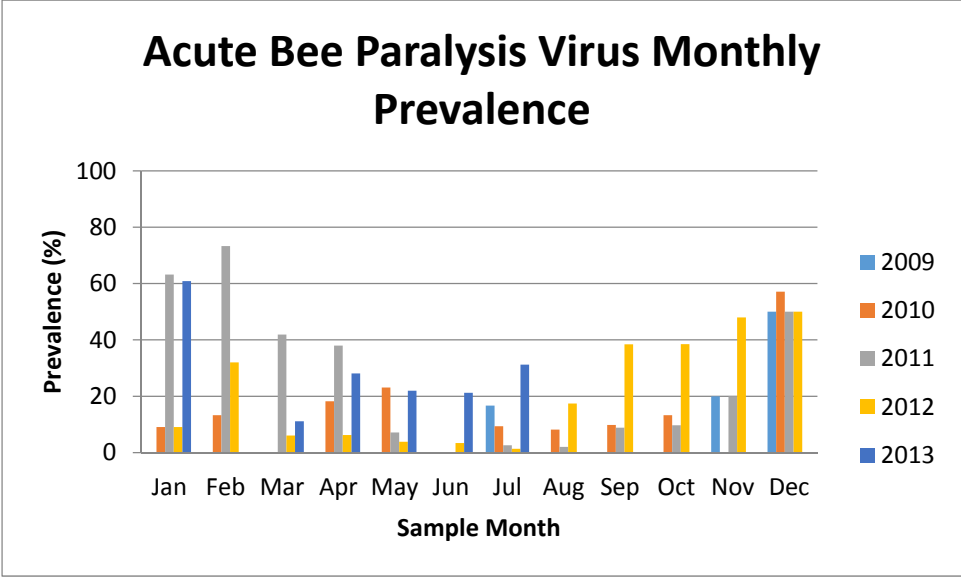


Figure 15: Acute Bee Paralysis Virus prevalence over 4years of survey

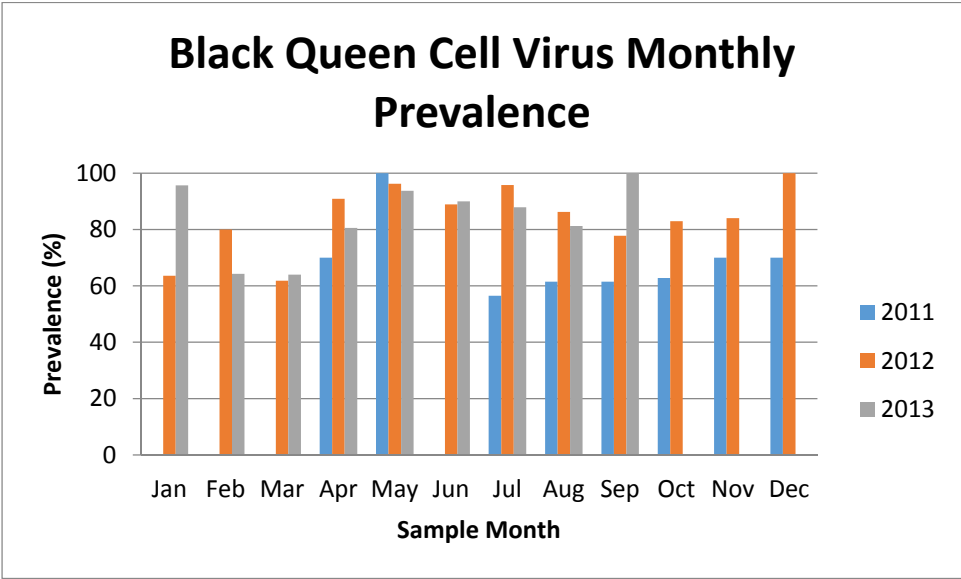


Figure 16: Black Queen Cell Virus prevalence, 2011/2012/2013 only



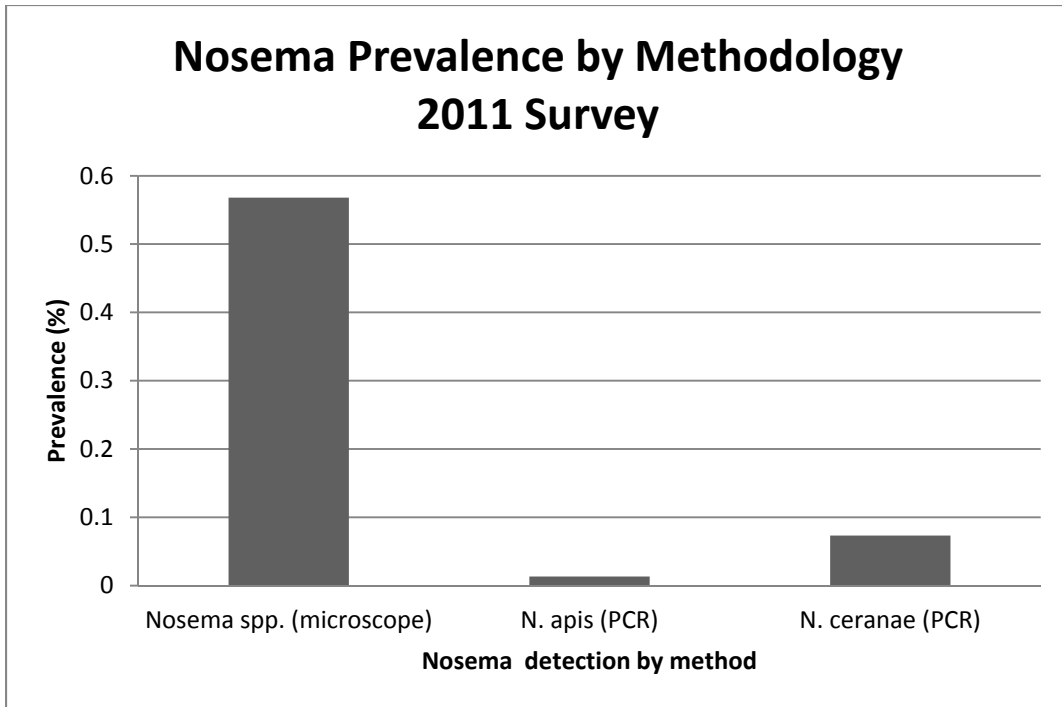


Figure 17: Nosema prevalence by methodology

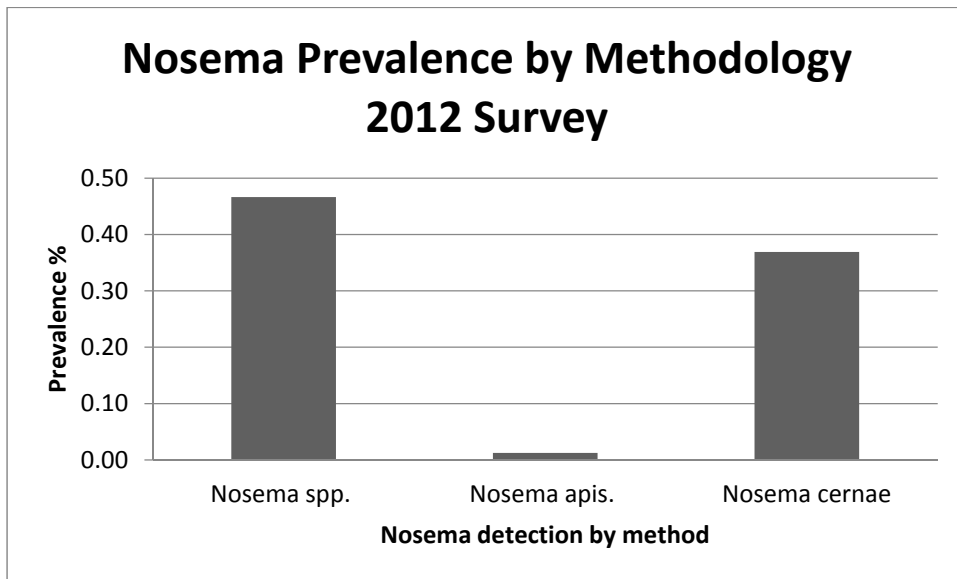


Figure 18: Nosema prevalence by methodology

Pesticide	LOD (ppb)	Prevalence %	Average Detection if positive for target (ppb)	Range if positive for target (ppb)
<b>1-Naphthol</b>	10	0.5	169.8	19.2 - 438
<b>2,4 Dimethylaniline</b>	50	n/a	n/a	n/a
<b>2,4 Dimethylphenyl formamide (DMPF)</b>	4	18.5	171.4	6.6 - 12700
<b>3-Hydroxycarbofuran</b>	4	n/a	n/a	n/a
<b>4,4 dibromobenzophenone</b>	4	n/a	n/a	n/a
<b>Acephate</b>	50	0.8	216.3	67.8 - 410
<b>Acetamiprid</b>	8	0.3	32.8	9.4 - 56.1
<b>Acetachlor</b>	10	0.2	52.7	52.7*
<b>Alachlor</b>	10	0.5	52.0	10.8 - 93.1
<b>Aldicarb</b>	4	n/a	n/a	n/a
<b>Aldicarb sulfone</b>	3	0.2	14.0	14*
<b>Aldicarb sulfoxide</b>	20	0.2	35.9	35.9*
<b>Aldrin</b>	10	n/a	n/a	n/a
<b>Allethrin</b>	10	n/a	n/a	n/a
<b>Amicarbazone</b>	30	n/a	n/a	n/a
<b>Amitraz</b>	4	n/a	n/a	n/a
<b>Atrazine</b>	6	6.5	65.4	7.4 - 996
<b>Azinphos methyl</b>	6	n/a	n/a	n/a
<b>Azoxystrobin</b>	2	9.7	57.3	4.6 - 1870
<b>Bendiocarb</b>	2	n/a	n/a	n/a
<b>Benoxacor</b>	4	0.2	Trace	Trace
<b>BHC alpha</b>	4	n/a	n/a	n/a
<b>Bifenazate</b>	20	n/a	n/a	n/a
<b>Bifenthrin</b>	1	6.2	24.7	1.2 - 149
<b>Boscalid</b>	4	5.4	623.9	15.9 - 3510
<b>Bromuconazole</b>	20	n/a	n/a	n/a
<b>Buprofezin</b>	20	n/a	n/a	n/a
<b>Captan</b>	10	2.5	411.0	18.3 - 1580
<b>Carbaryl</b>	30	0.9	167.3	66.5 - 442
<b>Carbendazim (MBC)</b>	5	3.9	58.7	7.3 - 275
<b>Carbofuran</b>	5	n/a	n/a	n/a
<b>Carboxin</b>	4	n/a	n/a	n/a
<b>Carfentrazone ethyl</b>	1	n/a	n/a	n/a
<b>Chlorfenopyr</b>	1	n/a	n/a	n/a
<b>Chlorfenvinphos</b>	6	0.9	53.0	40.3 - 75.1

Chlorferone	50	0.2	192.0	192*
Chlorothalonil	1	5.6	839.9	72.6 - 4900
Chlorpropham (CIPC)	40	n/a	n/a	n/a
Chlorpyrifos	1	18.5	20.5	1.1 - 303
Chlorpyrifos methyl	1	n/a	n/a	n/a
Clofentezine	100	n/a	n/a	n/a
Chlothianidin	1	1.5	27.7	5.5 - 62.8
Coumaphos	1	33.6	65.3	1.1 - 6260
Coumaphos oxon	1	4.3	28.1	5.5 - 180
Cyfluthrin	4	0.8	30.9	3.9 - 58.8
Cyhalothrin total	1	7.4	10.3	1.9 - 54.2
Cypermethrin	4	1.2	30.8	7.2 - 100
Cyphenothrin	20	n/a	n/a	n/a
Cyprodinil	4	5.4	147.5	4.3 - 2800
DDD p,p'	4	n/a	n/a	n/a
DDE p,p'	2	n/a	n/a	n/a
DDT p,p'	4	n/a	n/a	n/a
Deltamethrin	20	n/a	n/a	n/a
Diazinon	1	0.5	15.2	6.6 - 21.1
Dichlorvos (DDVP)	10	0.2	205.0	205*
Dicloran	1	0.2	25.0	25.0*
Dicofol	1	0.5	13.4	3.2 - 21
Dieldrin	10	0.2	12.4	12.4*
Difenoconazole	10	n/a	n/a	n/a
Diflubenzuron	20	0.6	159.0	84.3 - 252
Dimethenamid	10	n/a	n/a	n/a
Dimethoate	20	n/a	n/a	n/a
Dimethomorph	20	n/a	n/a	n/a
Dinotefuran	2	n/a	n/a	n/a
Diphenamid	1	n/a	n/a	n/a
Endosulfan I	2	1.9	34.9	2.2 - 124
Endosulfan II	2	1.7	21.0	2.1 - 54.9
Endosulfan sulfate	2	2.0	10.7	1.6 - 50.4
Endrin	10	n/a	n/a	n/a
Epoconazole	1	n/a	n/a	n/a
Esfenvalerate	2	3.2	14.4	3.7 - 77.4
Ethion	10	0.3	327.0	Trace-327
Ethofumesate	5	0.2	14.2	14.2*
Etoazole	1	n/a	n/a	n/a
Etridiazole	10	n/a	n/a	n/a

Famoxadone	20	n/a	n/a	n/a
Fenamidone	10	n/a	n/a	n/a
Fenbuconazole	2	1.9	462.4	9.2 - 3470
Fenhexamid	6	0.5	330.9	7.7 - 814
Fenpropathrin	1	0.9	43.2	20.7 - 93.6
Fenpropathrin N.D. 1.0	1	n/a	n/a	n/a
Fenpyroximate	5	6.3	31.0	2.1 - 266
Fenthion N.D. 10	10	n/a	n/a	n/a
Fipronil N.D. 10	10	n/a	n/a	n/a
Flonicamid	8	0.3	42.2	11.3 - 73.1
Flubendiamide N.D. 25.0	25	n/a	n/a	n/a
Fludioxonil	20	0.3	51.9	30.5 - 73.3
Fluoxastrobin N.D. 4.0	4	n/a	n/a	n/a
Fluridone	10	0.3	1279.0	108 - 4220
Flutolanil N.D. 4.0	4	n/a	n/a	n/a
Fluvalinate	1	49.4	70.6	2.2 - 1930
Heptachlor N.D. 4.0	4	n/a	n/a	n/a
Heptachlor epoxide N.D. 10	10	n/a	n/a	n/a
Hexachlorobenzene (HCB) N.D. 1.0	1	n/a	n/a	n/a
Hexythiazox N.D. 30	30	n/a	n/a	n/a
Hydoprene N.D. 10	10	n/a	n/a	n/a
Hydroxychlorothalonil	50	0.2	59.4	59.4*
Imazalil N.D. 5.0	5	n/a	n/a	n/a
Imidacloprid	1	2.2	22.2	2.8 - 216
Imidacloprid 5-hydroxy N.D. 25	25	n/a	n/a	n/a
Imidacloprid olefin N.D. 10	10	n/a	n/a	n/a
Indoxacarb	3	0.2	Trace	Trace
Iprodione N.D. 20	20	n/a	n/a	n/a
Lindane N.D. 4.0	4	n/a	n/a	n/a
Linuron N.D. 20	20	n/a	n/a	n/a
Malathion	4	0.2	63.9	63.9*
Metalaxyl	2	0.6	20.5	10.2 - 37.9
Methamidophos	4	1.1	15.8	5 - 36.5
Methidathion N.D. 10	10	n/a	n/a	n/a
Methomyl	10	0.3	19.2	14.7 - 23.6
Methoxyfenozide	2	2.2	200.1	5.7 - 1300
Metolachlor	6	0.8	921.4	14.7 - 2550
Metribuzin	1	0.2	3.5	3.5*
MGK-326	10	0.3	142.9	95.7 - 190
MGK-326 N.A. 10	10	n/a	n/a	n/a
Myclobutanil	15	1.2	448.0	30.1 - 1330
Norflurazon N.D. 6.0	6	n/a	n/a	n/a

<b>Oxamyl N.D. 5.0</b>	5	n/a	n/a	12.4*
<b>Oxyfluorfen</b>	1	2.9	7.7	1.7 - 13.7
<b>Paradichlorobenzene</b>	10	4.9	420.3	80.9 - 1820
<b>Parathion methyl</b>	2	0.2	6.6	6.6*
<b>Pendimethalin</b>	6	9.7	38.4	5.1 - 188
<b>Permethrin total</b>	10	0.6	175.6	20 - 421
<b>Phenothrin N.D. 10</b>	10	n/a	n/a	n/a
<b>Phorate N.D. 10</b>	10	n/a	n/a	n/a
<b>Phosalone N.D. 10</b>	10	n/a	n/a	n/a
<b>Phosmet</b>	10	1.1	149.9	7.3 - 785
<b>Piperonyl butoxide N.D. 6.0</b>	6	n/a	n/a	n/a
<b>Pirimiphos methyl N.D. 4.0</b>	4	n/a	n/a	n/a
<b>Prallethrin</b>	4	2.0	195.7	10.7 - 800
<b>Profenofos N.D. 10</b>	10	n/a	n/a	n/a
<b>Pronamide N.D. 1.0</b>	1	n/a	n/a	n/a
<b>Propachlor</b>	10	0.2	Trace	Trace
<b>Propanil N.D. 10</b>	10	n/a	n/a	n/a
<b>Propargite N.D. 10</b>	10	n/a	n/a	n/a
<b>Propazine</b>	4	0.2	34.3	34.3*
<b>Propetamphos N.D. 4.0</b>	4	n/a	n/a	n/a
<b>Propham</b>	20	0.2	Trace	Trace
<b>Propiconazole N.D. 10</b>	10	n/a	n/a	n/a
<b>Pymetrozine N.D. 20</b>	20	n/a	n/a	n/a
<b>Pyraclostrobin</b>	15	5.1	217.7	2.6 - 1400
<b>Pyrethrins N.D. 50</b>	50	n/a	n/a	n/a
<b>Pyridaben</b>	1	0.5	1.5	1.2 - 1.8
<b>Pyrimethanil</b>	3	1.9	12.7	3.2 - 60.8
<b>Pyriproxyfen</b>	2	0.9	7.7	1.5 - 13.6
<b>Quinoxyfen N.D. 10</b>	10	n/a	n/a	n/a
<b>Quintozene (PCNB) N.D. 1.0</b>	1	n/a	n/a	n/a
<b>Resmethrin total N.D. 10</b>	10	n/a	n/a	n/a
<b>Sethoxydim N.D. 2.0</b>	2	n/a	n/a	n/a
<b>Simazine N.D. 10</b>	10	n/a	n/a	n/a
<b>Spinosad N.D. 28</b>	28	n/a	n/a	n/a
<b>Spirodiclofen N.D. 1.0</b>	1	n/a	n/a	n/a
<b>Spiromesifen N.D. 10</b>	10	n/a	n/a	n/a
<b>Tebuconazole</b>	8	2.2	70.0	9.9 - 277
<b>Tebufenozide</b>	5	0.2	22.7	22.7*
<b>Tebuthiuron</b>	2	0.6	4.8	2.2 - 12.1
<b>Tefluthrin</b>	1	0.2	Trace	Trace
<b>Tetrachlorvinphos N.D. 4.0</b>	4	n/a	n/a	n/a
<b>Tetraconazole N.D. 6.0</b>	6	n/a	n/a	n/a

<b>Tetradifon N.D. 1.0</b>	1	n/a	n/a	n/a
<b>Tetramethrin N.D. 10</b>	10	n/a	n/a	n/a
<b>Thiabendazole</b>	1	1.2	2.6	1.1 - 4.7
<b>Thiacloprid</b>	1	0.5	151.2	49.1 - 326
<b>Thiamethoxam</b>	1	1.7	13.5	1.2 - 39.6
<b>THPI</b>	50	1.5	2147.8	37.6 - 7060
<b>Thymol</b>	50	20.7	2581.5	26.3 - 55800
<b>Triadimefon N.D. 2.0</b>	2	n/a	n/a	n/a
<b>Triadimenol N.D. 45</b>	45	n/a	n/a	n/a
<b>Tribufos (DEF) N.D. 2.0</b>	2	n/a	n/a	n/a
<b>Trifloxystrobin</b>	1	1.1	173.2	34.1 - 638
<b>Triflumizole N.D. 10</b>	10	n/a	n/a	n/a
<b>Trifluralin</b>	1	5.2	87.6	Trace-3.3
<b>Triticonazole</b>	10	0.6	310.8	19.2 - 438
<b>Vinclozolin</b>	1	0.5	3.3	6.6 - 12700

\*Only a single detection made for this pesticide so there is no range for level of detection, just the value. Pesticides highlighted in yellow have been detected in pollen samples to date.

Figure 19: Pesticide analysis up through 2013 survey (451 samples)  
(\*denotes single detection only)

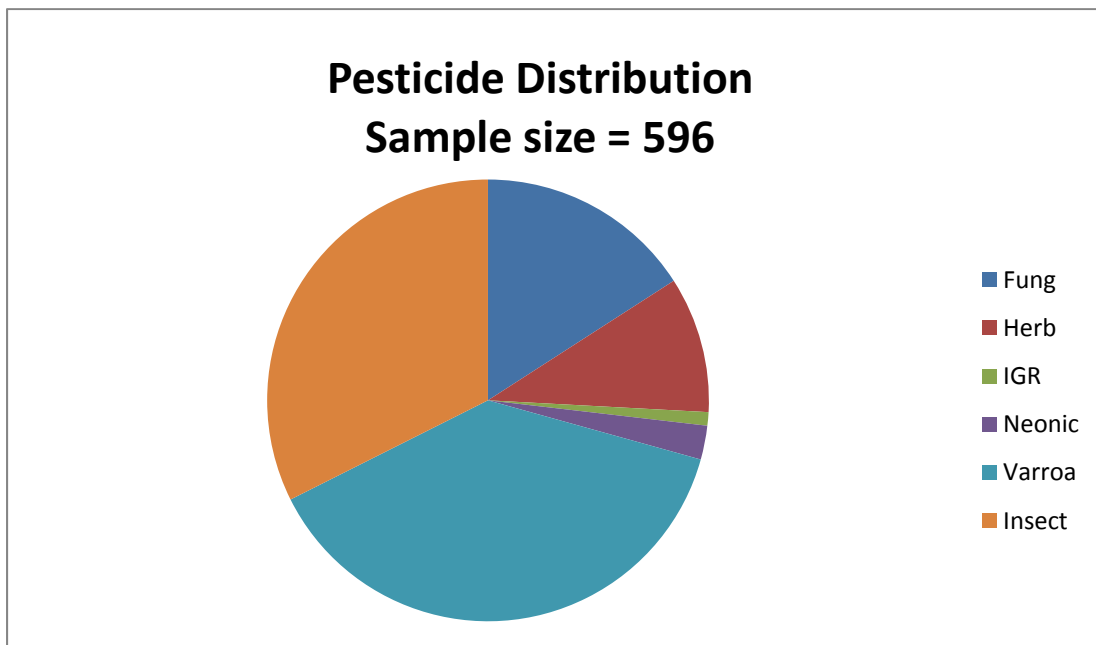


Figure 20: Classification of types of pesticide detected in pollen samples through 2014 .

## References

Evans J.D. (2006). Beepath: An ordered quantitative-PCR array for exploring honey bee immunity and disease, *Journal of Invertebrate Pathology*, 93 (2), pp. 135-139.

Pettis, J. S., R. Rose, E. M. Lichtenberg, P. Chantawannakul, N. Buawangpong, W. Somana, P. Sukumalanand, and D. vanEngelsdorp. 2013. A Rapid Survey Technique for *Tropilaelaps* Mite (Mesostigmata: Laelapidae) Detection. *Journal of Economic Entomology* 106: 1535-1544.