

Honey Bee Monitoring for Aerial Mosquito Adulticide Application Summary Report – 2020

Kim Skyrn, Ph.D and Hotze Wijnja, Ph.D

Massachusetts Department of Agricultural Resources – Division of Crop and Pest Services



Aerial Application –The single aerial application for mosquito control occurred from 8pm on August 10, 2020 and lasted until 2am on August 11, 2020 in 25 towns located in Bristol and Plymouth counties during the peak honey bee activity season. Similar to 2019, the mosquito adulticide product used in the aerial application was Anvil 10+10® ULV¹ containing the active ingredients Sumithrin® (d-Phenothrin) and piperonyl butoxide (PBO), that acts as a synergist increasing potency and duration of effectiveness. d-Phenothrin is a synthetic pyrethroid insecticide² and has been registered by EPA since 1976 for use to control adult mosquitos and other nuisance insects indoors and outdoors in residential yards and public recreational areas. The product Anvil 10+10® ULV is labeled for use in residential and recreational areas. d-Phenothrin is classified as being highly toxic to honey bees³. Risk mitigation language on the product label for Anvil 10+10® ULV includes the following Environmental Hazard statement as it relates to honey bees:

This product is highly toxic to bees exposed to direct treatment on blooming crops or weeds. Do not apply this product or allow it to drift to blooming crops or weeds while bees are actively visiting the area, except when applications are made to prevent or control a threat to public and/or animal health determined by a state, tribal or local health or vector control agency on the basis of documented evidence of disease causing agents in vector mosquitoes, or the occurrence of mosquito-borne disease in animal or human populations, or if specifically approved by the state or tribe during a natural disaster recovery effort.

Relative to the risk to honey bees from the aerial application, it should be noted that the potential hazard to direct exposure from the application was minimized since sprays occurred at night when honey bees are not typically active outside the hive box. However, as observed in 2019⁴, environmental conditions may cause honey bees to congregate on the outside of hive boxes at night (i.e. bee bearding), therefore potentially increasing the likelihood of some limited exposure in the spray area.

Beekeepers – At the time of the aerial application, a total of 108 registered beekeepers were managing apiaries in the spray area, which likely represents only a fraction of the total apiaries given that apiary registration is voluntary in the Commonwealth.

Stakeholder Communication – During the months of June and July 2020, Apiary Inspectors contacted and inspected all beekeepers whose apiaries were monitored during the 2019 aerial application. The goal of these actions was to assess hive health and interest in participating in 2020 monitoring should aerial applications take place. A list of apiaries was then compiled and used to select the 2020 honey bee monitoring sites.

Beekeeper communication consisted of a mass alert sent to officers of the state and county level beekeeping associations via email, Facebook posts, and Mass.gov website notifications. Communication took place pre-application, during and post-application. The Mass.gov website was updated this year to include a beekeeper-

¹ Clarke. Anvil® 10+10 ULV Pesticide Label: <https://www.clarke.com/filebin/productpdf/anvil1010.pdf>

² U.S. EPA. Permethrin, Resmethrin, d-Phenothrin (Sumithrin®): Synthetic Pyrethroids for Mosquito Control:

<https://www.epa.gov/mosquitocontrol/permethrin-resmethrin-d-phenothrin-sumithrin-synthetic-pyrethroids-mosquito-control>

³ National Pesticide Information Center (NPIC). d-Phenothrin Technical Fact Sheet: <http://npic.orst.edu/factsheets/archive/dphentech.html#references>

⁴ MDAR Honey Bee Monitoring for Aerial Mosquito Adulticide Application Summary Report - 2019: <https://www.mass.gov/doc/honey-bee-monitoring-for-aerial-mosquito-adulticide-application-summary-report-2019/download>

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specific factsheet with pre-cautionary recommendations ([EEE Spray FAQ for Beekeepers](#)) and a comment form ([Massachusetts Aerial Mosquito Spray Comment Form](#)) linked on the [2020 Massachusetts Aerial Mosquito Spray Map](#) which allowed stakeholders to submit real time information directly to MDAR. The Apiary Program only received one comment form submission related to honey bees and promptly responded to the question. Beekeepers of monitored apiaries were directly communicated with throughout the monitoring process. Unmonitored beekeepers in the spray area were also contacted post-application to determine the status of colony health in their apiaries. The Apiary Program did not receive any reports of Bee Kills during the aerial application this year. In addition to this final report, beekeepers of monitored apiaries were also provided an individual report.

Monitoring Methods – The *Honey Bee Monitoring Protocol for Aerial Mosquito Adulticide Application* from [The Mosquito Emergency Operations Response Plan for Mosquito-Borne Illness](#)⁵ was utilized for monitoring with modification, as needed. Beekeepers were selected for monitoring based on their geographic location and colony health (Fig. 1). Selected apiaries were either categorized as those inside (treatment) or outside (control) the application area. Hobby beekeepers comprised the treatment group whereas, the MDAR State Apiaries and a commercial beekeeper were the control group. Colony health was determined by health inspections of colonies to ensure the absence of visible issues (i.e. queenright, no visible signs of pesticide-related Bee Kill, no visible pathogens, and low Varroa mite levels) which could confound potential negative impacts of the aerial application. Only colonies that were found to be visibly healthy during these inspections were included in monitoring efforts.

The monitoring protocol was defined by a series of visits to apiaries where inspectors performed health inspections on both the interior and exterior of honey bee colonies. These health inspections consisted of a combination of the standard health inspection procedures utilized by the MDAR Apiary Program Team for routine annual inspections, health emergencies and those involved in Bee Kill investigations where colony death is investigated due to suspected impacts of pesticide mis-use. Exterior monitoring consisted of evaluating foraging activity at colony entrances and dead bee accumulation outside hive boxes. Dead bee monitoring was evaluated through the use of white 130 muslin cotton/polyester cloths (66”W X 104”L flat bed sheets) situated and affixed on top of the ground using staples (1”W X 4”L) in front of hive boxes (Fig. 2). Interior health assessments included evaluating queen, brood, food, adult bee population quantity, quality and behavior to determine signs of acute or sublethal pesticide impacts or other health issue. Each apiary and honey bee colony was visited a total of three (3) times throughout the monitoring process during pre-set time intervals of pre-application (0-2 days pre-spray) and post-application (1-3 days and 7-10 days post-spray). Inspectors also relied on beekeepers to continuously monitor hive health and provide immediate reports of suspected negative impacts to MDAR outside of these monitoring visits.

During each apiary visit, the following data were collected: photo of apiary, counts of dead bees in front of hives and samples of bees. Though they were made in 2020, dead bee counts were shown to be inconsistent in the 2019 monitoring given the potential for weather, predators and worker bee hygienic behavior to remove

⁵ Massachusetts Emergency Operations Response Plan for Mosquito-Borne Illness: <https://www.mass.gov/massachusetts-emergency-operations-response-plan-for-mosquito-borne-illness>

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dead and dying individuals. Samples of adult bees were taken from live foragers entering/exiting hives and dead bees on cloths situated in front of hives, when available. All samples collected from individual colonies were pooled together for each monitoring visit to create a single apiary sample (i.e. live bee sample per apiary/per date and dead bee sample per apiary/per date). However, samples of dead bees were so few this year (ranging from only 1-20 individual bees per apiary visit with the majority less than 5 bees per apiary visit) that only samples of live bees were in large enough quantities to allow for lab analysis. After collection, all samples were stored in the freezer at -10°C until they were submitted for lab analysis on August 27, 2020. Samples were partitioned into subsets with half of each sent to the National Agricultural Genotyping Center (NAGC) for molecular analysis of viruses, bacteria and fungi and half sent to the Massachusetts Pesticide Analysis Laboratory (MPAL) for pesticide analysis targeting the mosquito adulticide active ingredients used in the aerial application.

Monitoring Results – A total of six (6) beekeepers managing seven (7) apiaries consisting of 66 colonies were monitored (Table 1). Of these, 12 colonies managed by four (4) beekeepers were located inside (treatment) and 54 colonies managed by two (2) beekeepers were located outside (control) the application area. The control apiaries were in three (3) counties and three (3) towns. The treatment apiaries were only located in Plymouth County but spread out between four (4) towns, representing the largest portion of the application area and thus most representative of the entire spray zone. A total of 21 samples (9 from control and 12 from treatment) were collected and submitted for lab analysis of viruses, bacteria, fungi, and pesticides.

Results from the pesticide analysis revealed that only four (4) samples (19.05%) contained PBO while the remaining 17 samples were Non-Detect (ND) or did not contain the target pesticides at the Limit of Detection (0.65-4.64 µg/kg (ppb)) (Table 2). The acute risk of measured pesticide residues to honey bees was assessed by comparing the measured residue levels in bees with the acute toxicity endpoints (50% Lethal Dose values or LD₅₀ values) for d-Phenothrin and PBO (Table 3). The LD₅₀ values were obtained from the Sanchez-Bayo and Goka (2014)⁶ and EPA risk assessment documents⁷. The contact and oral LD₅₀ values for these pesticides are listed in Table 3. To allow comparison of the measured pesticide levels in bees with toxicity endpoints, the standard LD₅₀ values were converted to LD₅₀ values in ppb relative to body weight⁸. These LD₅₀ values in ppb relative to body weight are also listed in Table 3.

A comparison of the measured ppb residue levels in Table 2 with the LD₅₀ values for honey bees (expressed in ppb relative to bee body weight) in Table 3 indicates that the measured levels are much lower than the LD₅₀ values and therefore not likely to cause acute effects. A formal risk assessment is based on Risk Quotient (RQ) values and comparison with EPA established Levels of Concern (LOC). Risk quotients were calculated by dividing the measured residue levels in bees with the LD₅₀ value (ppb) and are included in Table 3. The LOC is

⁶ Sanchez-Bayo, F. and Goka, K. 2014. Pesticide residues and bees – A risk assessment. PLoS One, 9(4).

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0094482#pone.0094482.s002>

⁷ U.S. EPA. 2017. Piperonyl Butoxide (PBO): Preliminary Ecological Risk Assessment for Registration Review.

<https://www.regulations.gov/document?D=EPA-HQ-OPP-2010-0498-0025>

⁸ Multiplying the standard LD₅₀ values (ug/bee) using a factor of 10,000 (assumes an average bee weight of 0.1g) (see Mullin et al. 2010:

<http://journals.plos.org/plosone/article/asset?id=10.1371%2Fjournal.pone.0009754.PDF>

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0.4 for acute risk.⁹ The calculated RQ values in Table 3 are well below the acute LOC. Therefore, again it is very unlikely that the measured residues of PBO caused lethal effects to the bees.

All samples contained multiple honey bee viruses (Table 4). The most common viruses were Sacbrood Virus (SBV), Black Queen Cell Virus (BQCV), Varroa Destructor Virus 1 (VDV1) and Deformed Wing Virus (DWV), which occurred in 100%, 100%, 90% and 81% of samples, respectively (Fig. 3). The only other viruses detected were Chronic Bee Paralysis Virus (CBPV), Lake Sinai Virus 1 (LSV1) and Lake Sinai Virus 2 (LSV2) which occurred in 14%, 10% and 10% of samples, respectively. Overall, these viruses, sometimes as co-infections, were found in a total of seven (7) samples (33%). The treatment group consisting of Plymouth County had the highest incidence of viruses while the control group's Hampden and Hampshire counties had the lowest (Fig. 4).

Samples also contained bacteria and fungi (Table 5). The most common detected, found in every sample (100%), was the fungus, *N. ceranae* (Fig. 5). The bacteria, European Foulbrood (EFB) and fungus, *Nosema apis* was detected in 10% and 5% of samples, respectively. The detrimental and virulent bacterial pathogen, American Foulbrood (AFB) was not detected in any sample. Control apiaries located in Hampden County were infected with the highest incidence of these pathogens with samples containing EFB, *N. apis* and *N. ceranae* (Fig. 6). Treatment apiaries located in Plymouth County were only infected with *N. ceranae*.

The high occurrence of DWV and VDV1 is similar to past honey bee samples taken from the Commonwealth and across the United States (Table 6). A comparison of the MDAR samples reveals that the occurrence of DWV greatly increased in prevalence from 2019 to 2020 while LSV2 and CBPV decreased. However, the percentages of samples with DWV, CBPV and *Nosema ceranae* is higher in Massachusetts samples compared with national surveys.

The honey bee monitoring activities associated with the aerial spray resulted in a total expense of \$11,529.60:

- \$1,083.00 inspector labor (57 hours);
- \$471.60 inspector travel (1,048 miles); and
- \$9,975.00 lab processing (42 samples).

Discussion – Viruses are considered to be the least understood of honey bee health issues with some being omnipresent in samples as asymptomatic infections and others known causes of acute or chronic colony mortality. Given the frequent occurrence of some viruses such as BQCV, national monitoring efforts now exclude this virus in analysis (USDA-APHIS, 2019)¹⁰. Other viruses are associated with honey bee parasites, such as the Varroa mite, *Varroa destructor*. This ubiquitous ectoparasitic mite is a major vector or associate of many common honey bee viruses including the DWV and VDV1 found in samples (Brutscher et al. 2016)¹¹. If left unmanaged, Varroa mites and associated viruses will cause colony mortality. Given this, the extent and

⁹ U.S. EPA. 2014. Guidance for Assessing Pesticide Risks to Bees. https://www.epa.gov/sites/production/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf

¹⁰ USDA-APHIS National Honey Bee Survey Reports. 2019. https://research.beeinformed.org/state_reports/

¹¹ Brutscher, L.M., McMenamin, A.J., and Flenniken, M.L. 2016. The buzz about honey bee viruses. PLoS Pathogens, 12(8). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4990335/>

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implementation of beekeeper driven Integrated Pest Management (IPM) strategies targeted for Varroa mite control impacts the occurrence and severity of these viruses in the Commonwealth.

Other viruses such as CBPV are rare and have known associations with colony mortality, often causing acute mortality or visual symptoms of Bee Kills. The occurrence and spread of CBPV has been linked to high concentrations of honey bee colonies in a single geographic area given the spread of the virus through oral, fecal and contact routes (Genersch & Aubert, 2010)¹². Similarly, in the 2019 monitoring data, this virus was only detected in the treatment group samples which were taken from Plymouth County. The reoccurrence of CBPV in the region could be due to the high concentration of apiaries in the area combined with the sustained infection in colonies.

The occurrence of *N. ceranae* in samples is not uncommon, but severe infections can cause colony mortality if left unmanaged (Burnham, 2019)¹³. The occurrence of EFB in samples was concerning given the potential for mortality and spread of infection, hence required beekeeper management through antibiotic treatment (Vidal-Naquet, 2015)¹⁴.

Conclusion – The visual observations of the MDAR Apiary Program Team combined with that of the beekeepers whose apiaries were visited and consistently monitored for colony health, indicate that overall honey bee colonies were not acutely impacted by the aerial application. Beekeepers contacted in follow up communication, whose colonies were not monitored or investigated in this report but located in the spray zone, also reported no observable health issues resulting from the aerial application. Data analysis from sampling indicates that the pesticide residue levels in the live bee samples were well below the level that would cause lethal effects in adult honey bees. Given this, it can be concluded that the exposure to d-Phenothrin and PBO from the aerial application was not a major cause of any bee mortality observed in these monitoring events.

Future Recommendations – Future monitoring efforts should continue to be reduced to only a maximum of three (3) to five (5) monitored apiaries inside and outside the spray area for each application. If the same area is repeatedly sprayed, additional monitoring efforts of the same apiaries should be reduced or eliminated if previous monitoring efforts showed no negative impact. Sampling efforts during all monitoring, while costly, should be continued and include:

- live and dead honey bees (when available),
- pre-application and post-application,
- all colonies in monitored apiaries,
- pooled samples from all colonies representing the entire apiary; and
- submissions sent for molecular (viral, bacterial and fungal) and pesticide analysis.

¹² Genersch, E. and Aubert, M. 2010. Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). Veterinary Research, 41(6). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2883145/>

¹³ Burnham, A.J. 2019. Scientific advances in controlling *Nosema ceranae* (Microsporidia) infections in honey bees (*Apis mellifera*). Frontiers in Veterinary Science, 6(79). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6428737/>.

¹⁴ Vidal-Naquet, N. 2015. Honeybee Veterinary Medicine: *Apis Mellifera* L. 5M Publishing. Sheffield, United Kingdom.

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Given the difficulty and often inability of labs to process very small sample sizes, future samples of dead bees should only be submitted for lab analysis if they contain quantities of at least 30 individual honey bees per apiary. The [Honey Bee Monitoring Protocol for Aerial Mosquito Adulticide Application](#) from [The Mosquito Emergency Operations Response Plan for Mosquito-Borne Illness](#) should be updated to reflect the aforementioned.

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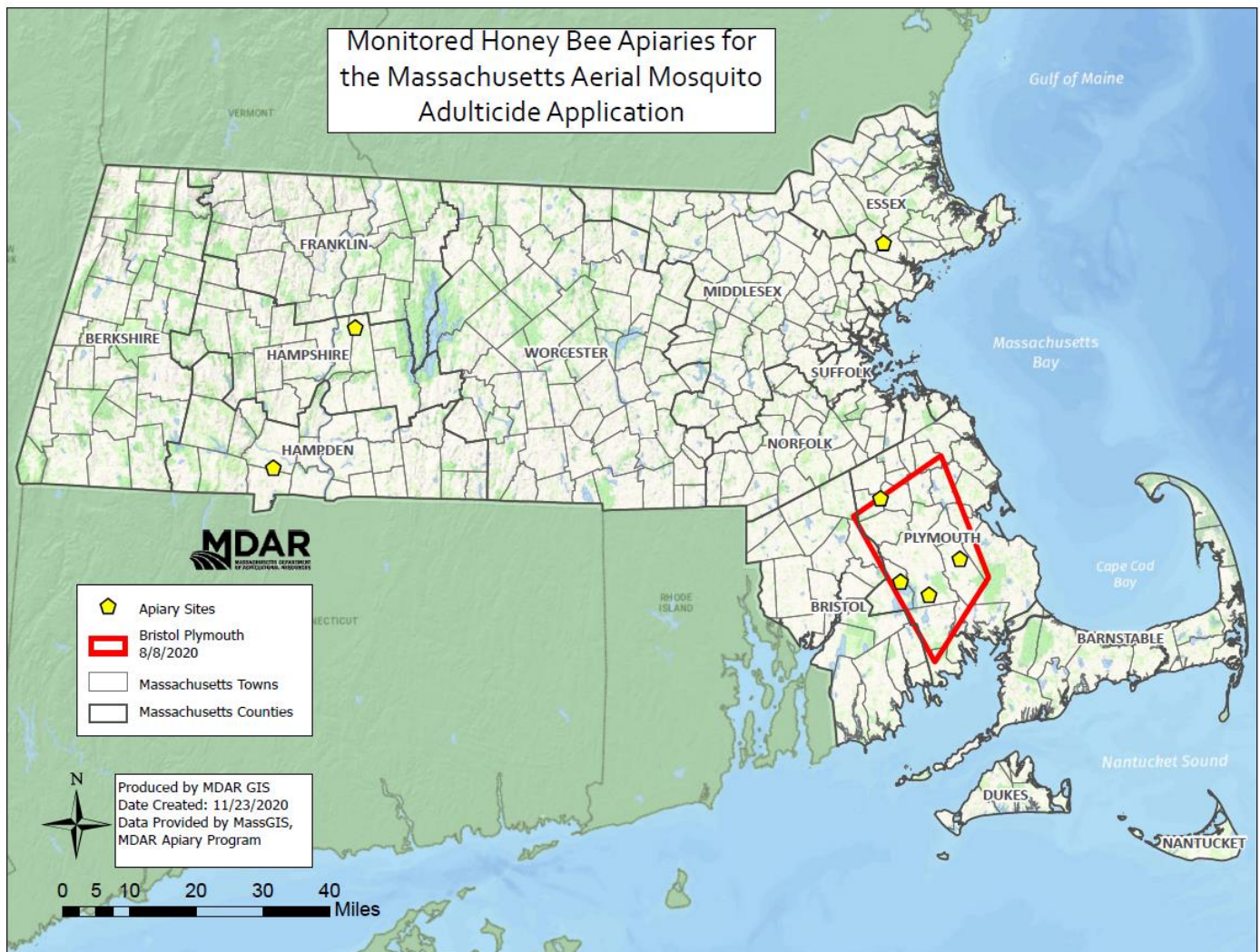


Figure 1. Map showing the aerial application spray area (red) and monitored apiary locations (yellow).

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Figure 2. Monitored apiary in treatment group with cloths installed.

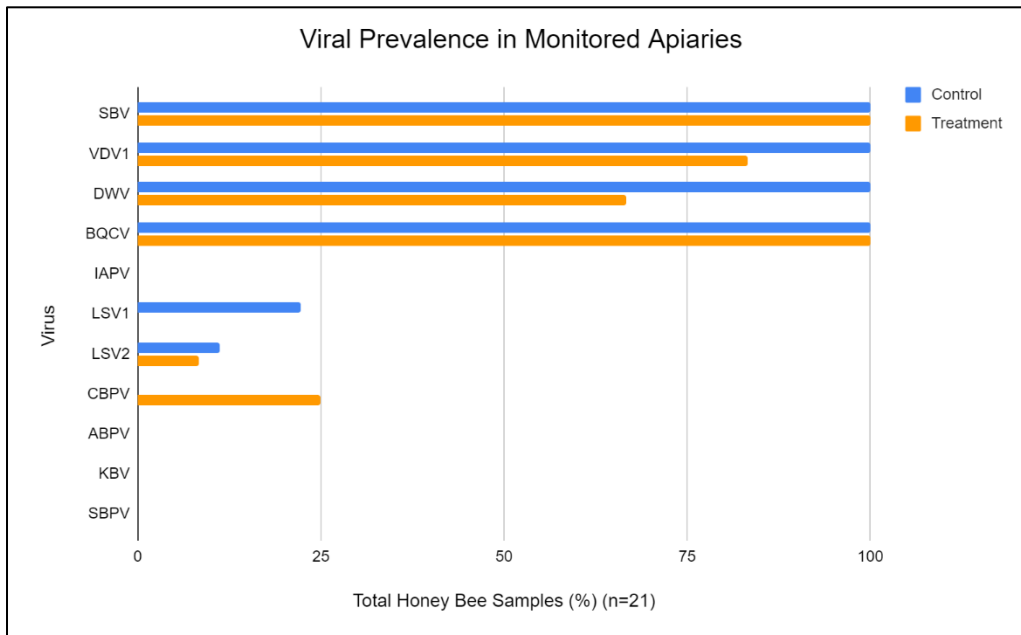


Figure 3. Pathogen (virus) prevalence in honey bee samples by group.

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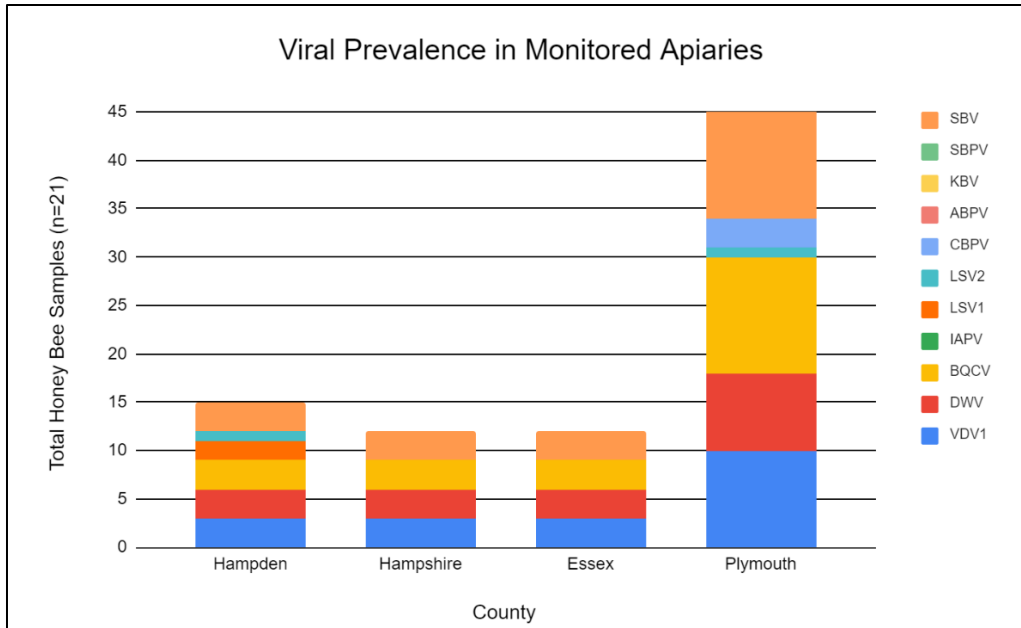


Figure 4. Pathogen (virus) prevalence in honey bee samples by county.

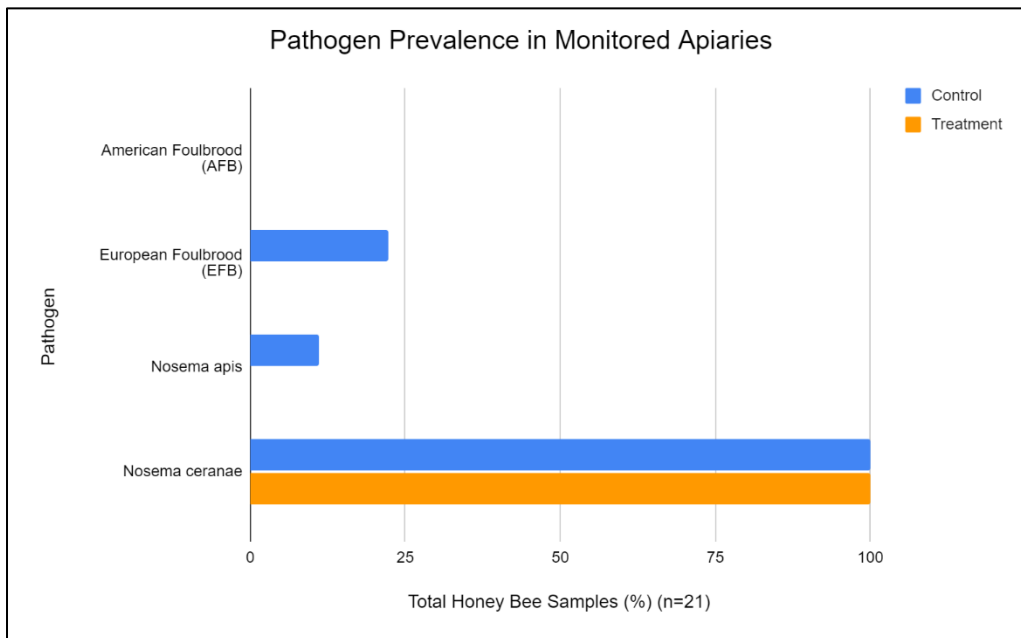


Figure 5. Pathogen (bacteria and fungi) prevalence in honey bee samples by group.

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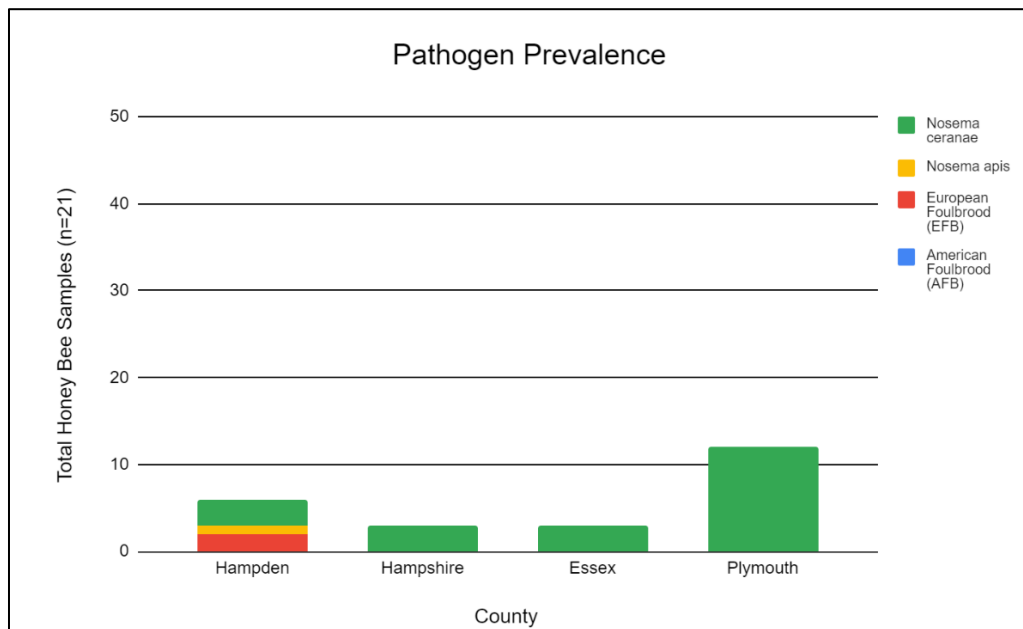


Figure 6. Pathogen (bacteria and fungi) prevalence in honey bee samples by county.

Table 1. Summary of honey bee monitoring for apiaries located inside (treatment) and outside (control) the aerial mosquito adulticide application area.

Monitored Apiary	Metric Totals						
	Beekeepers	Apiaries	Monitored Colonies	Sampled Colonies	Bee Samples	Towns	Counties
control	2	3	54	15	9	3	3
treatment	4	4	12	12	12	4	1
Total	6	7	66	27	21	7	4

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Table 2. Pesticide prevalence in honey bee samples.

Monitored Apiary	Sample ID	Sample County	Sample Date (2020)	d-Phenothrin (µg/kg or ppb)	Piperonyl Butoxide (PBO) (µg/kg or ppb)
control	BC080720L	Hampden	8/7	-	-
	BC081320L		8/13	-	-
	BC081920L		8/19	-	-
	SA080720L	Hampshire	8/7	-	-
	SA081220L		8/12	-	-
	SA081720L		8/17	-	-
	SD080720L	Essex	8/7	-	-
	SD081120L		8/11	-	-
	SD081720L		8/17	-	-
treatment	GB081020L	Plymouth	8/10	-	-
	GB081120L		8/11	-	1.54
	GB081720L		8/17	-	-
	CY081020L		8/10	-	-
	CY081120L		8/11	-	5.41
	CY081720L		8/17	-	1.29
	JC081020L		8/10	-	-
	JC081120L		8/11	-	-
	JC081720L		8/17	-	2.46
	NR081020L		8/10	-	-
	NR081120L		8/11	-	-
	NR081720L		8/17	-	-
	Total Samples			21	-
Pesticide Prevalence (%)				-	19.05

- pesticide non-detect (ND) or not detected in sample at the Limit of Detection (LOD) (0.65-4.64 µg/kg)

Table 3. Pesticide toxicity endpoints and calculated risk quotients in honey bee samples.

Pesticide	LD ₅₀ contact (µg/bee)	LD ₅₀ oral (µg/bee)	LD ₅₀ contact (ppb body weight)	LD ₅₀ oral (ppb body weight)	Range of Levels Detected (lowest-highest detected) (ppb)	Range of Risk Quotient contact	Range of Risk Quotient oral
d-Phenothrin	0.13	0.16	1,015	1,250	-	-	-
Piperonyl Butoxide (PBO)	>25	-	195,312	-	1.29-5.41	<0.0005	-

- pesticide non-detect (ND) or not detected in sample at the Limit of Detection (LOD) (0.65-4.64 µg/kg)

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Table 4. Pathogen (virus) prevalence in honey bee samples.

Monitored Apiary	Sample ID	Sample County	Sample Date (2020)	Virus									
				Sacbrood Virus (SBV)	Varroa Destructor Virus 1 (VDV1)	Deformed Wing Virus (DWV)	Black Cell Virus (BQCV)	Israeli Acute Paralysis Virus (IAPV)	Lake Sinai Virus 1 (LSV1)	Lake Sinai Virus 2 (LSV2)	Chronic Bee Paralysis Virus (CBPV)	Acute Bee Paralysis Virus (ABPV)	Kashmir Bee Virus (KBV)
control	BC080720L	Hampden	8/7	+	+	+	+	-	+	-	-	-	-
	BC081320L		8/13	+	+	+	+	-	+	+	-	-	-
	BC081920L		8/19	+	+	+	+	-	-	-	-	-	-
	SA080720L	Hampshire	8/7	+	+	+	+	-	-	-	-	-	-
	SA081220L		8/12	+	+	+	+	-	-	-	-	-	-
	SA081720L		8/17	+	+	+	+	-	-	-	-	-	-
	SD080720L	Essex	8/7	+	+	+	+	-	-	-	-	-	-
	SD081120L		8/11	+	+	+	+	-	-	-	-	-	-
	SD081720L		8/17	+	+	+	+	-	-	-	-	-	-
treatment	GB081020L	Plymouth	8/10	+	+	+	+	-	-	-	+	-	-
	GB081120L		8/11	+	+	+	+	-	-	-	+	-	-
	GB081720L		8/17	+	+	+	+	-	-	-	-	-	-
	CY081020L		8/10	+	-	-	+	-	-	-	-	-	-
	CY081120L		8/11	+	-	-	+	-	-	-	-	-	-
	CY081720L		8/17	+	+	-	+	-	-	-	-	-	-
	JC081020L		8/10	+	+	+	+	-	-	-	-	-	-
	JC081120L		8/11	+	+	-	+	-	-	-	-	-	-
	JC081720L		8/17	+	+	+	+	-	-	-	+	-	-
	NR081020L		8/10	+	+	+	+	-	-	-	-	-	-
	NR081120L		8/11	+	+	+	+	-	-	+	-	-	-
NR081720L	8/17	+	+	+	+	-	-	-	-	-	-		
Total Samples			21	21	19	17	21	-	2	2	3	-	-
Viral Prevalence (%)				100.00	90.48	80.95	100.00	-	9.52	9.52	14.29	-	-

+ virus detected in sample
- virus not detected in sample

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Kim Skyrn, Ph.D and Hotze Wijnja, Ph.D

Massachusetts Department of Agricultural Resources – Division of Crop and Pest Services



Table 5. Pathogen (bacteria and fungi) prevalence in honey bee samples.

Monitored Apiary	Sample ID	Sample County	Sample Date (2020)	Bacteria		Fungi	
				American Foulbrood (AFB)	European Foulbrood (EFB)	<i>Nosema apis</i>	<i>Nosema ceranae</i>
control	BC080720L	Hampden	8/7	-	-	-	+
	BC081320L		8/13	-	+	+	+
	BC081920L		8/19	-	+	-	+
	SA080720L	Hampshire	8/7	-	-	-	+
	SA081220L		8/12	-	-	-	+
	SA081720L		8/17	-	-	-	+
	SD080720L	Essex	8/7	-	-	-	+
	SD081120L		8/11	-	-	-	+
	SD081720L		8/17	-	-	-	+
treatment	GB081020L	Plymouth	8/10	-	-	-	+
	GB081120L		8/11	-	-	-	+
	GB081720L		8/17	-	-	-	+
	CY081020L		8/10	-	-	-	+
	CY081120L		8/11	-	-	-	+
	CY081720L		8/17	-	-	-	+
	JC081020L		8/10	-	-	-	+
	JC081120L		8/11	-	-	-	+
	JC081720L		8/17	-	-	-	+
	NR081020L		8/10	-	-	-	+
	NR081120L		8/11	-	-	-	+
NR081720L	8/17	-	-	-	+		
Total Samples			21	-	2	1	21
Pathogen Prevalence (%)				-	9.52	4.76	100.00

+ pathogen detected in sample

- pathogen not detected in sample

Table 6. Comparison of state and national pathogen prevalence in honey bee samples.

Pathogen	MDAR (%)		USDA-APHIS Survey (%)*			
	2019 (n=22)	2020 (n=21)	2019 Massachusetts (n=24)	2019 United States (n=750)	2013-2019 United States (n=5,453)	
virus	Deformed Wing Virus (DWV) (or DWV-A*)	23	81	79	64	80
	Varroa Destructor Virus 1 (VDV1) (or DWV-B*)	86	90	96	90	80
	Lake Sinai Virus 2 (LSV2)	18	10	17	30	34
	Chronic Bee Paralysis (CBPV)	41	14	12	8	11
fungi	<i>N. ceranae</i>	N/A	100	75	68	67

*USDA-APHIS, 2019