

Honey Bee Monitoring for Aerial Mosquito Adulticide Application Summary Report – 2019

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

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



Aerial Application – The statewide aerial applications for mosquito control occurred during August 8-27, 2019 and September 10-18, 2019 in 7 Massachusetts counties (Bristol, Hampden, Hampshire, Middlesex, Norfolk, Plymouth and Worcester) during the peak honey bee activity season. At the time of the applications, these counties consisted of a total of 259 registered beekeepers managing apiaries in the application areas which represents only a fraction of the total apiaries in these areas given that apiary registration is voluntary in the Commonwealth. A total of 34 beekeepers registered during the time of the aerial applications representing a 12% increase in overall in current statewide registration.

The mosquito adulticide product used in the aerial applications was Anvil 10+10® ULV¹ containing the active ingredient Sumithrin® (d-Phenothrin) and synergist piperonyl butoxide (PBO), that increases its 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 applications, it should be noted that the potential hazard to direct application exposure from the aerial application was minimized since sprays occurred at night when honey bees are typically inside the hive box. However, the following 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 to honey bees in spray areas:

1. Large colony population inside hive box;
2. Outside temperature above 85°F; and
3. Beekeeper applied miticide treatment to the hive box interior.

Stakeholder Communication – Communication to beekeepers consisted of a variety of media outlets including phone calls, emails, Facebook posts, and Mass.gov website notifications that took place pre-application, during and post-application. Individual pre-application notification was sent via email to a total of 803 beekeepers located in the counties of the spray areas. These beekeepers consisted of those voluntarily registered, with past inspection records with the Apiary Program and to the officers of the state and county level beekeeping

¹ U.S. EPA. Multicide Mosquito Adulticiding Concentrate 2705:

https://iaspub.epa.gov/apex/pesticides/f?p=PPLS:102:::NO::PI02_REG_NUM:1021%2D1688

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

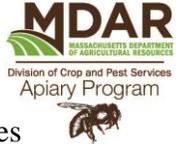
<https://www.epa.gov/mosquitocontrol/permethrin-resmethrin-d-phenothrin-sumithrin-synthetic-pyrethroids-mosquito-control>

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

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associations within the application areas. Each email consisted of links to the [EEE in Massachusetts](#) Mass.gov service pages as well as a Frequently Asked Questions (FAQ) list containing general recommendations tailored specifically for beekeepers. Additional communication included responding to the many stakeholder phone calls, phone messages, text messages, Facebook messages, and emails received during this time period. Beekeepers were also contacted post-application to determine status of colony health following spray events. All follow up communication and investigations of suspected Bee Kills were conducted in a timely manner. In addition to this final report, beekeepers were emailed a final report of their individual sample results taken from their apiaries.

Honey Bee Monitoring Methods – *The Honey Bee Monitoring Protocol for Aerial Mosquito Adulticide Application* from [The Mosquito Emergency Operations Response Plan for Mosquito-Borne Illness](#)⁴ 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 within (treatment group) or outside (control group) the application area based on their geographic location and health inspection prior to application. The MDAR State Apiaries in Amherst and Danvers were amongst those monitored outside the application area (control group). Monitored apiaries inside the application areas which received multiple applications were monitored for each spray application, when possible. Some apiaries had to be removed from these repeated monitoring attempts given the application of miticides on hives as part of seasonal management. 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 applications. Only colonies that were found to be visibly healthy during these inspections were included in monitoring efforts. Commercial, hobby and sideliner classified beekeepers comprised the monitored apiaries accurately representing the diversity of apiculture in Massachusetts.

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. Interior health assessments included evaluating queen, brood, food stores, and population levels to determine impacts of pesticides or presence of other health issues. Exterior monitoring consisting of evaluating foraging activity at colony entrances and dead bee accumulation outside of the hive boxes. Dead bee monitoring was conducted using clear plastic (drop cloth) and light colored canvas (drop cloth) or muslin cotton cloths (66”W x 104”L) situated on the ground in front of hive boxes (Fig. 2). To prevent contamination in apiaries monitored repeatedly during multiple spray events, cloths were replaced prior to additional application(s). Each apiary and honey bee colony were visited a total of 3 times throughout the monitoring process during pre-set time intervals of pre-application (0-2 days) and post-application (1-3 days and 7-10 days). Inspectors also relied on beekeepers to continuously monitor hive health and provide immediate reports of suspected negative impacts to MDAR during times outside of monitoring visits.

⁴ 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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During each apiary visit, the following data were collected, when possible: photo of apiary, counts of dead bees in front of hive and sample of bees. Dead bee counts were not consistently possible given the following un-anticipated issues that occurred at some locations:

- Weather conditions removing cloths from in front of hives;
- Predators consuming or inclement weather conditions removing dead bees away from hives or cloths;
- Colony hygiene behavior of worker bees removing dead bees away from hives or cloths;
- Cloths removed due to beekeeper concerns about damaging vegetation around hives or need for land management of area around apiary;
- Beekeeper hive management which increased dead bee populations given exposure to in-hive applied miticides; and
- Beekeeper installed hive covers were not made of, installed or removed properly therefore caused colony stress.

Given these challenges, a few protocol changes were made during the monitoring. The first was using landscape staples to affix cloths in front of hives therefore allowing them to remain stationary throughout monitoring. Next, the initial plastic and canvas drop cloths were replaced with muslin cotton cloths and this resulted in reduced damage to vegetation hives and less water retention. Some beekeepers of monitored apiaries elected to cover their hives as a pre-cautionary measure to provide protection during applications. This practice varied widely among beekeepers and could have imposed additional risk on honey bee health depending on the type of cover material, configuration, and duration of coverage time. We recommended in the FAQ sent to beekeepers that if used, covers should be made of cotton material, configured loosely over the hive box being careful to not restrict access of hive entrances and removed swiftly after application.

Despite the inability to record dead bee counts for each apiary during the monitoring period, inspectors were able to assess hives given foraging activity and interior health of hives. Pre-application samples of adult bees were taken of apiaries, when possible. Post-application samples of adult bees were only taken when deemed necessary (i.e. if hives presented visible symptoms indicating a possible Bee Kill resulting from pesticide use given the occurrence of large amounts of dead bees in front of hive or on cloths). After collection, samples were stored in the freezer at -10°C and evaluated at the end of the monitoring event to determine if collected quantities warranted lab analysis. Samples deemed necessary for lab analysis were those that contained higher than anticipated quantities of dead bees and were sent for both viral and pesticide analysis. Virus samples were analyzed by the National Agricultural Genotyping Center (NAGC) and pesticide samples were analyzed by the Massachusetts Pesticide Analysis Laboratory (MPAL).

The estimated populations of hives during the monitoring events ranged between 40-65,000 individuals of which the forager population comprises an estimated 25% (Seeley, 1995)⁵. The daily forager mortality rate in an active honey bee colony can range from 1-5% since the average lifespan of a foraging honey bee is only 7.7 days, but ranges between 2 to 17 days (Visscher and Dukas, 1997)⁶. This equates to a minimum estimated daily forager mortality rate of 100-163 individuals. Dead bees are removed from the hive box through the hygienic

⁵ Seeley, T.D. 1995. *The Wisdom of the Hive*. Harvard University Press, Cambridge, MA, USA.

⁶ Visscher, P.K. and Dukas, R. 1997. Survivorship and foraging of honey bees. *Insectes Society* 44, (1).
<https://link.springer.com/article/10.1007/s000400050017>

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behavior of undertaker bees (Seeley, 1985)⁷. If a colony is stressed or weakened from a health issue, it will also modify the hygienic behavior of undertaker bees to either not remove the dead or dying from the interior of the hive box or carry them to great distances outside the hive box. Instead dead or dying bees will be deposited at or right outside the hive entrance. This modification in behavior allows for ease in determining acute honey bee kills given the presence of a large accumulation of dead or dying bees in close proximity to the hive.

Inspections were also conducted of apiaries not part of the monitoring protocol for beekeepers who reported conditions consist with a potential Bee Kill suspected to be due to pesticide exposure. These complaints were followed up on with apiary visits and inspection by the MDAR Apiary Program team using the standard Bee Kill protocols. Samples from these investigations were evaluated in the same manner as those from the monitoring program in that only those samples that warranted pesticide analysis were submitted to MPAL. However, all these investigated apiaries were sampled for viruses and sent for analysis to NAGC.

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; LD₅₀ values) for d-Phenothrin and PBO. The LD₅₀ values were obtained from the Sanchez-Bayo and Goka (2014)⁸ and EPA risk assessment documents⁹. The risk of residues in pollen was assessed by using the BeeRex model¹⁰.

Honey Bee Monitoring Results – A grand total of 36 beekeepers managing 39 apiaries consisting of 535 colonies were monitored (Table 1). Of these, 436 colonies managed by 30 beekeepers were located inside (treatment) and 99 colonies managed by six (6) beekeepers were located outside (control) the application areas. Many of the monitored apiaries were in towns that received repeated aerial applications located in Plymouth, Bristol, and Worcester counties. Apiaries located inside the application area included 24 towns: Berlin, Brimfield, Dartmouth, Duxbury, East Taunton, Hopkinton, Lakeville, Marlborough, Milford, Millbury, Northborough, Northbridge/Whitinsville, North Dighton, North Grafton, Needham, Raynham, Shrewsbury, Southborough, Southbridge, Upton, Walpole, Westborough, West Bridgewater, West Brookfield. Apiaries located outside the application area included seven (7) towns: Amherst, Berlin, Danvers, Ware, Charlton, New Braintree, Sudbury.

A total of 37 samples (15 pesticide and 22 viral) were lab submitted for virus and pesticide analysis (Tables 2 and 3). Of these, a total of 16 samples were from monitored apiaries and 21 samples (3 pesticide samples and 18 virus samples) were taken from investigations of Bee Kill complaints from apiaries not monitored during the spray events. Samples for pesticides and viruses were submitted from the same five (5) counties (Bristol, Hampden, Norfolk, Plymouth and Worcester), whereas virus samples were submitted for only Middlesex county.

⁷ Seeley, T.D. 1985. Honeybee Ecology: A Study of Adaptation in Social Life. Princeton University Press, Princeton, NJ, USA.

⁸ 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>

⁹ 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>

¹⁰ U.S. EPA, BeeRex model and guidance: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment>

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Results from the pesticide analysis (Table 2) revealed that 10 samples were positive for one or both pesticides and five (5) samples were Non-Detect (ND) at the Limit of Detection (d-Phenothrin was 6.5-20.7 $\mu\text{g}/\text{kg}$ (ppb) and 1.3-4.1 $\mu\text{g}/\text{kg}$ (ppb) for PBO). A total of five (5) samples (33%) were positive for both d-Phenothrin and PBO, and a total of five (5) samples (33%) were only positive for PBO (Fig. 3). No samples were found to be positive only for d-Phenothrin. Plymouth county had the highest amount of positive samples for PBO with four (4), but the lowest amount of samples positive for d-Phenothrin with one (1) (Fig. 4). Norfolk and Worcester counties had the highest amount of positive samples for d-Phenothrin with two (2), but lower positive PBO samples (two (2) for Norfolk and three (3) for Worcester). Only a single pollen sample was taken from Worcester county and it was positive for both d-Phenothrin and PBO, but the dead bee samples analyzed from this same sampled colony only tested positive for PBO.

The contact and oral LD₅₀ values for these pesticides are listed in Tables 4 and 5. 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 weight¹¹. These LD₅₀ values in ppb relative to body weight are listed in Table 4.

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 4 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 4.

The LOC is 0.4 for acute risk.¹² The calculated RQ values in Table 4 are well below the acute LOC. Therefore, it is very unlikely that the measured residues of d-Phenothrin and PBO caused lethal effects to the bees. Regarding the pollen sample, the risk quotient of 0.15 for d-Phenothrin is below the level of concern for acute lethal effect to bees (Table 5). The very low risk quotient for PBO is consistent with its low toxicity to bees.

Viruses were prevalent in all samples, with a majority of samples positive for three (3) or more (Table 3). The most common viruses were Sacbrood Virus (SBV) and Varroa Destructor Virus 1 (VDV1), which occurred in 100% and 86% of samples, respectively (Fig. 5). Plymouth county had the highest incidence of viruses while Hampden county had the lowest (Fig. 6). The most detrimental parasitic mite, *Varroa destructor*, is a major vector of the following detected honey bee viruses: Deformed Wing Virus (DWV), Varroa Destructor Virus 1 (VDV1), and Israeli Acute Paralysis Virus (IAPV) (Brutscher et al. 2016)¹³. Of the viruses detected, Chronic Bee Paralysis Virus (CBPV), Israeli Acute Paralysis Virus (IAPV) and Lake Sinai Virus 1 (LSV1) which were found in 21 samples, sometimes as multiple-infections, can present symptoms similar to a pesticide related Bee Kill. The occurrence of CBPV is linked with crowding of honey bee colonies in concentrated geographic areas (Genersch & Aubert, 2010)¹⁴ and was detected in the most samples from Plymouth county.

¹¹ 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](http://journals.plos.org/plosone/article/asset?id=10.1371%2Fjournal.pone.0009754.PDF))

¹² 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

¹³ 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/>

¹⁴ 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/>

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The honey bee monitoring activities associated with the aerial spray resulted in a total expense of \$15,976.97 taken from the MDAR Apiary Program budget:

- \$6,829.75 inspector labor (588.5 labor hours including 228.5 for Chief Inspector and 360 for Inspectors consisting of 276.5 regular hours and 83.5 overtime hours);
- \$1,952.55 inspector travel (4,339 miles);
- \$999.67 supplies; and
- \$6,195.00 lab processing of samples (37 samples consisting of 22 virus lab and 15 for pesticide lab).

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 spray areas also reported no observable health issues resulting from the aerial application. Data analysis indicates that the pesticide residue levels in the bee and pollen 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 the bee mortality observed in these monitoring events and investigations. Many of the viruses found in samples are documented to cause bee mortality. Given this, the most likely cause of any higher than normal observed bee mortality from samples taken during these monitoring efforts were likely caused by a combination of the negative impacts of viruses detected in samples and that associated with standard daily bee mortality.

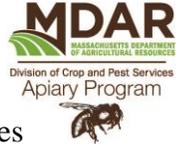
Future Recommendations – Based on the large amount of correspondence from beekeepers during these monitoring efforts, we suggest the use of a large group informational session for those in application areas to allow for common questions and concerns to be addressed prior to aerial applications. Additionally, a formal FAQ document targeted for beekeepers should be created and posted on the MDAR website which provides additional information that would serve as a reference to beekeepers. If this document, includes the pre-cautionary recommendation of covering hives, it should be re-visited given the unexpected confusion on the best recommended methods and procedures involved in using this practice and in some cases un-intended resulting negative impacts on colonies. Future monitoring efforts should continue to include the use of the cotton cloths in front of hives since they consistently allowed for highly visible detection of un-intended pesticide impacts given their ability to highlight large quantities of dead bees indicative of an acute bee kill.

Given the complexities and dynamic nature of honey bee colony health combined with regional variances of apiculture in Massachusetts, future monitoring efforts should re-evaluate the selection of control apiaries as part of monitoring efforts. The selection of these colonies should be done in advance to monitoring by ensuring that these colonies are comprised of similar health, development stage and geographic location in order to compare with those in application areas. If locating control colonies proves to be difficult, monitoring efforts could consider changing the protocol to just include pre-application (control) and post-application (treatment) monitoring of the same colonies in spray areas. In practice, this would entail using the same monitored colony pre-spray as the control treatment group and post-spray as the treatment group and eliminate the use of separate control colonies outside the treatment area. While not ideal, this is a possible alternative to the current monitoring protocol, if needed.

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If the same active ingredient and aerial application methodology are used in future sprays, the sample size of honey bee monitoring efforts should be reduced to a maximum of 3-5 apiaries within and outside the spray area. The reasoning behind this change is due to the observations taken during these monitoring efforts revealing an overall absence of negative impacts on health indicating minimal risk to exposed honey bee colonies. Similar results were obtained in previous years of state-level monitoring indicating a trend in the potential for low risk to honey bee colonies within treated areas. Reducing the sample size should allow for not only an accurate assessment of the potential impacts on honey bee health from the aerial application, but also increased time management for the inspection team to be more efficient in investigating complaints of Bee Kills that occur during this time period.

Finally, samples should be taken from all apiaries involved in monitoring efforts pre- and post-application for both pesticides and viruses to allow for more consistent sample results and greater resolution of the potential risks from aerial application. The [Honey Bee Monitoring Protocol for Aerial Mosquito Adulticide Application](#) from [The Mosquito Emergency Operations Response Plan for Mosquito-Borne Illness](#) protocols should be updated to reflect the aforementioned.

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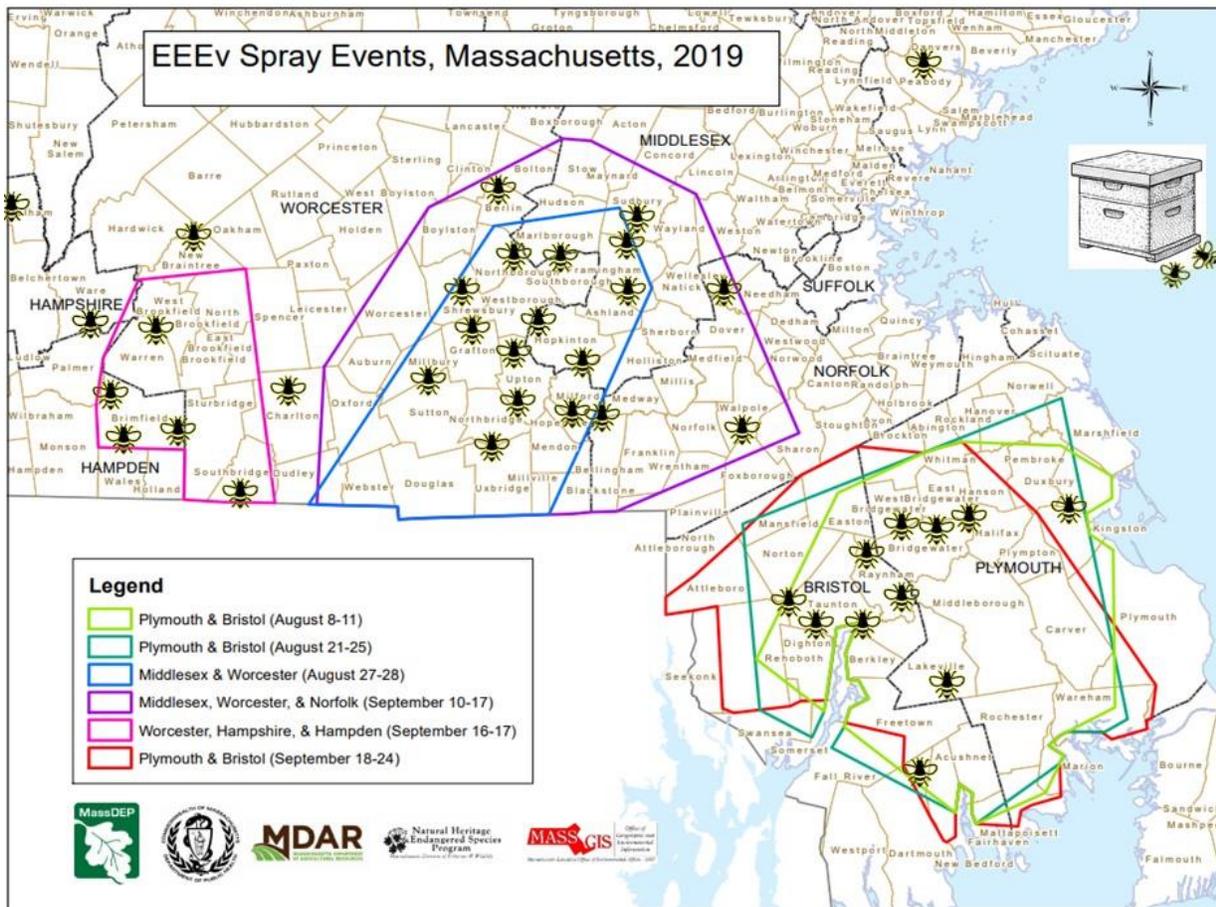


Figure 1. Map showing aerial applications with majority of monitored apiary locations indicated by the bee symbols. Note that given scale, apiaries are mapped based on town and general location.

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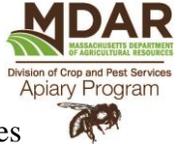


Figure 2. Hobby and commercial beekeeper monitored apiaries with cloths installed.

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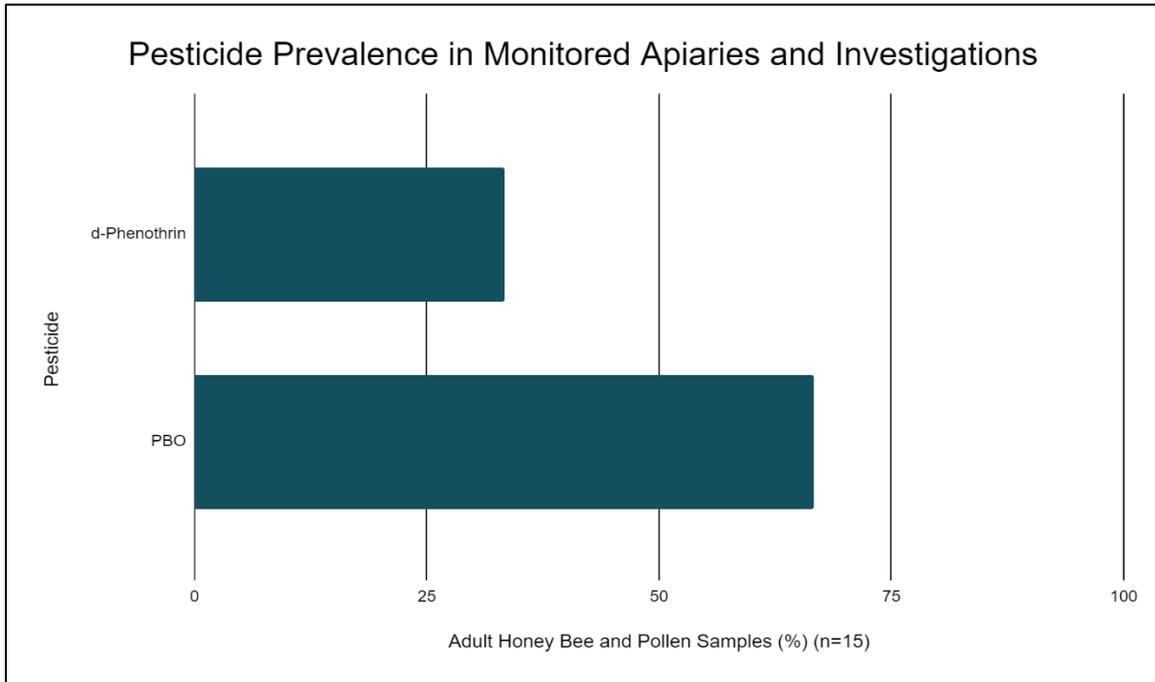
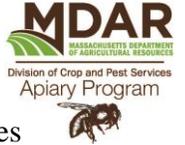


Figure 3. Pesticide prevalence in dead adult honey bees and pollen (n=15).

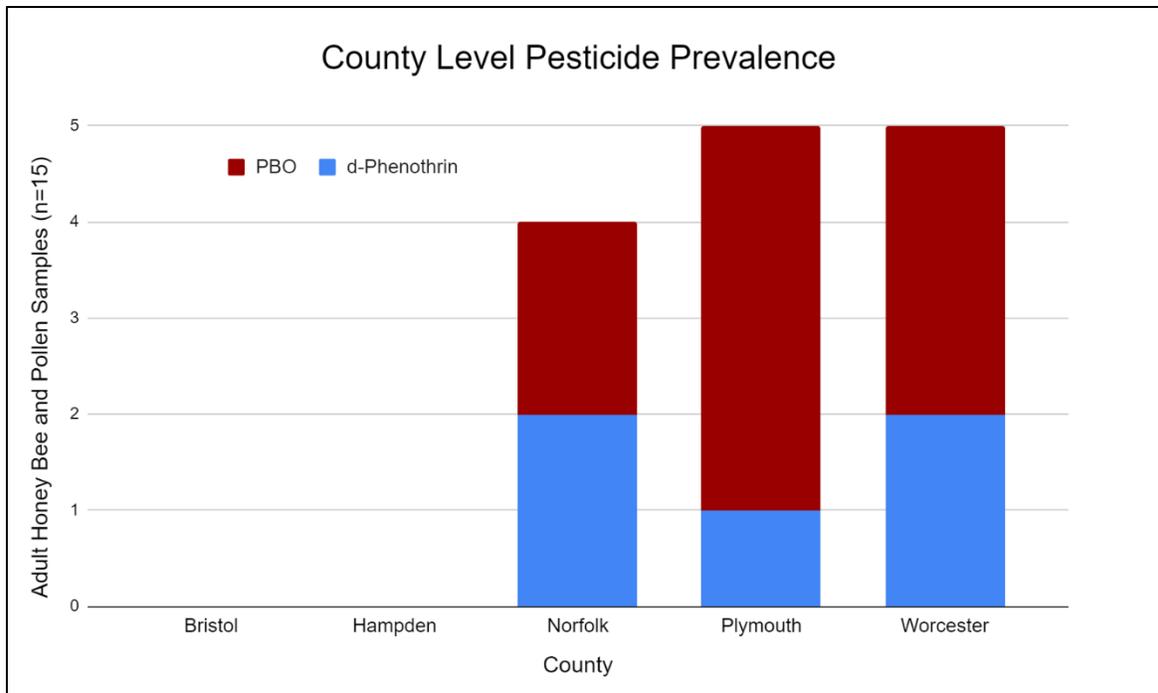


Figure 4. Pesticide prevalence in dead adult honey bees and pollen by county (n=15).

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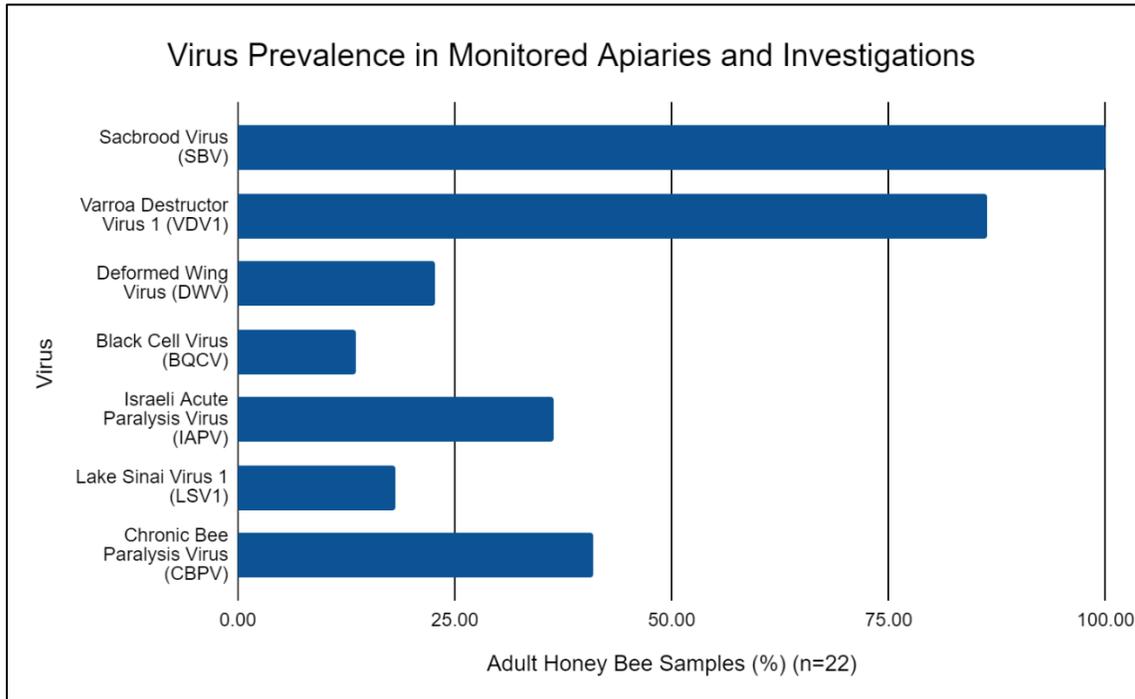
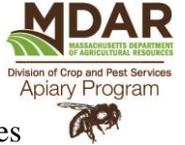


Figure 5. Virus prevalence in dead adult honey bees (n=22).

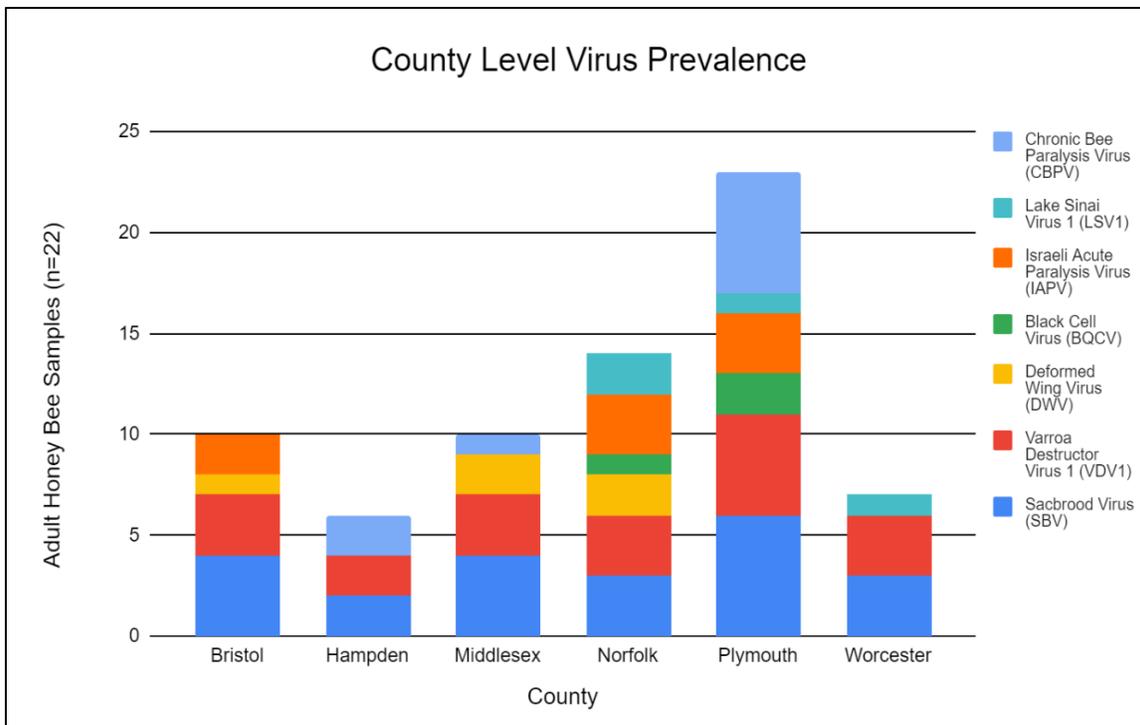


Figure 6. Virus prevalence in dead adult honey bees by county (n=22).

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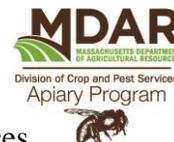


Table 1. Honey bee monitoring sites inside (treatment) and outside (control) the aerial application area.

Metric	Bristol/Plymouth			Middlesex/ Worcester	Middlesex/ /Norfolk/ Worcester	Hampden/ Hampshire/ Worcester	Total	
	8/8- 8/11/19	8/21- 8/25/19	9/18- 9/24/19	8/26- 8/27/19	9/10- 9/18/19	9/16- 9/17/19		
inside application area (treatment)	beekeepers	9	8	3	12	14	6	30
	apiaries	11	10	5	12	14	6	32
	colonies	125	122	69	45	55	20	436
	towns	7	7	3	11	13	3	24
	counties	2	2	2	2	3	2	7
outside application area (control)	beekeepers	1	1	1	3	1	3	6
	apiaries	2	2	2	4	1	3	7
	colonies	20	19	17	31	5	7	99
	towns	2	2	2	3	1	3	7
	counties	2	2	2	2	1	2	5

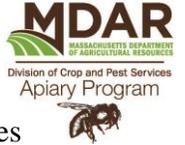
Table 2. Measured pesticide residues in samples of dead honey bees and pollen.

Sample ID	Sample County	MDAR Monitored Apiary	Apiary Location (i.e. inside or outside spray area)	Aerial Application Date (2019)	Sample Type	Sample Date (2019)	d- Phenothrin (µg/kg or ppb)	PBO (µg/kg or ppb)
WA	Bristol	no	inside	8/10; 8/24	bees	8/28	ND	ND
BM	Hampden	no	outside	N/A	bees	9/25	ND	ND
					bees	9/25	ND	ND
DS	Norfolk	yes	inside	9/15	bees	9/18	27	97.9
					bees	9/25	ND	ND
SS	Norfolk	yes	inside	9/15	bees	9/18	10.5	48.2
					bees	9/25	ND	ND
AR	Plymouth	yes	inside	8/9; 8/22; 9/22	bees	9/25	ND	14.6

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					bees	8/12	15	49
HS	Plymouth	yes	inside	8/11; 8/21	bees	8/19	ND	1.5
					bees	8/24	ND	18.3
					bees	9/16	ND	2.7
DP	Worcester	yes	inside	9/15	bees	9/18	ND	4.1
					pollen	9/18	45.2	127.4
SJL	Worcester	yes	inside	9/15	bees	9/18	14.4	47.6
Total Samples					bees	14	4	9
					pollen	1	1	1
Pesticide Prevalence of Samples (%)							33.33	66.66

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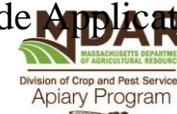


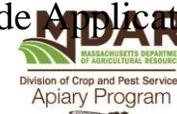
Table 3. Virus prevalence in samples of dead adult honey bees.

Sample ID	Sample County	MDAR Monitored Apiary	Apiary Location (i.e. inside or outside spray area)	Aerial Application Date (2019)	Sample Date (2019)	Sacbrood Virus (SBV)	Varroa Destructor Virus 1 (VDV1)	Deformed Wing Virus (DWV)	Black Cell Virus (BQCV)	Israeli Acute Paralysis Virus (IAPV)	Lake Sinai Virus (LSV)	Chronic Bee Paralysis Virus (CBPV)
LL	Bristol	no	inside	8/9; 8/23; 9/24	10/16	+	-	-	-	+	-	-
KC	Bristol	no	outside	N/A	10/25	+	+	-	-	+	-	-
NG	Bristol	no	inside	8/24; 9/20	10/7	+	+	-	-	-	-	-
WA	Bristol	no	inside	8/10; 8/24	8/28	+	+	+	-	-	-	-
BM	Hampden	no	outside	N/A	9/25	+	+	-	-	-	-	+
CP	Middlesex	no	outside	N/A	8/7	+	+	+	-	-	-	-
MR	Middlesex	no	inside	9/10	9/27	+	+	-	-	-	-	+
NM	Middlesex	no	inside	8/26; 9/15	9/24	+	-	-	-	-	-	-
AF	Norfolk	no	inside	9/14	7/15	+	+	-	-	+	+	-

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MB	Norfolk	no	outside	N/A	9/23	+	+	+	+	+	-	-
						+	+	+	-	+	+	-
AR	Plymouth	yes	inside	8/9; 8/22; 9/22	8/19 8/30	+	+	-	+	+	-	+
						+	+	-	-	+	-	+
AT	Plymouth	no	inside	8/9; 8/22; 9/21	9/27	+	+	-	-	-	-	+
						+	-	-	+	-	-	+
HS	Plymouth	yes	inside	8/11; 8/21	8/30	+	-	-	+	-	-	+
						+	+	-	-	-	-	+
JW	Plymouth	no	inside	8/21	10/23	+	+	-	-	-	-	+
						+	+	-	-	+	+	+
SF	Plymouth	no	inside	8/9	8/15	+	+	-	-	+	+	+
						+	+	-	-	-	-	-
DH	Worcester	no	inside	9/15	9/7	+	+	-	-	-	-	-
						+	+	-	-	-	-	-
DP	Worcester	yes	inside	9/15	9/16	+	+	-	-	-	-	-
						+	+	-	-	-	-	-
PM	Worcester	yes	inside	8/26; 9/11	9/24	+	+	-	-	-	+	-
						+	+	-	-	-	+	-
Total Samples					22	22	19	5	3	8	4	9
Virus Prevalence of Samples (%)					100.00	86.36	22.73	13.64	36.36	18.18	40.91	

+ virus detected in sample

- virus not detected in sample

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Table 4. Toxicity endpoints and calculated risk quotients for d-Phenothrin and piperonyl butoxide (PBO) in the dead honey bees.

Pesticide	LD ₅₀ (µg/bee) (contact)	LD ₅₀ (µg/bee) (oral)	LD ₅₀ (ppb body weight) (contact)	LD ₅₀ (ppb body weight) (oral)	Range of Levels Detected in Bees (lowest-highest detected) (ppb)	Range of Risk Quotient (contact)	Range of Risk Quotient (oral)
d-Phenothrin	0.13	0.16	1015	1250	10.5-27	0.01-0.03	0.004-0.02
piperonyl butoxide (PBO)	>25	-	195,312	-	1.5-97.9	<0.0005	-

Table 5. Toxicity endpoints and calculated risk quotients for d-Phenothrin and piperonyl butoxide (PBO) in the pollen sample.

Pesticide	LD ₅₀ (µg/bee) (contact)	LD ₅₀ (µg/bee) (oral)	Measured level in pollen (ppb)	Acute Risk Quotient (adult)
d-Phenothrin	0.013	0.016	45.2	0.15
piperonyl butoxide (PBO)	>25	-	127.4	<0.00005