Honey Bee Monitoring for Aerial Mosquito Adulticide Application 2024 Summary Report

Kim Skyrm, Ph.D, Sarah Miller, Ph.D., and Hotze Wijnja, Ph.D
Massachusetts Department of Agricultural Resources – Division of Crop and Pest Services

Aerial Application – A single aerial application for mosquito control occurred in eight (8) towns (Carver, Halifax, Kingston, Middleborough, Plymouth, Plympton, Rochester and Wareham) located in Plymouth County on August 27, 2024. This time of the year coincides with peak honey bee activity (i.e. foraging for floral resources and colony development) in Massachusetts. Like 2019 and 2020, the mosquito adulticide product used in the aerial application was Anvil® 10+10 ULV[1] (Ultra-Low Volume) containing the active ingredients Sumithrin® (d-phenothrin) and piperonyl butoxide (PBO). Anvil® 10+10 ULV is labeled for use in residential and recreational areas. D-phenothrin[2] is a synthetic pyrethroid insecticide that has been registered by the United States (US) Environmental Protection Agency (EPA) since 1976 for use to control adult mosquitos and other nuisance insects located indoors and outdoors in residential yards and public recreational areas. Piperonyl butoxide[3] is a pesticide synergist that has been registered by the EPA since the 1950's and acts to enhance the potency, duration, and effectiveness of other insecticide ingredients, such as pyrethroids. Ultra-low volume[4] describes the method of sprayer application used to treat large areas characterized by a dispersal of very fine aerosol droplets that stay in the air to kill adult mosquitos on contact. D-phenothrin and PBO both break down rapidly and inactivation occurs due to exposure to sunlight, air, and soil and has a typical half-life of less than one (1) day in the environment.

D-phenothrin is classified as being highly toxic to honey bees.[5] 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 treatment 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.*

Potential hazards to honey bees from direct exposure from the aerial application were minimized since the application occurred at night when honey bees are not typically actively flying or foraging but instead situated inside the hive box. However, as observed during 2019 and 2020[6], environmental conditions during this time of year 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 the application area.

---

[1] US EPA. Clarke Anvil® 10+10 ULV Pesticide Label: https://ordspub.epa.gov/ords/pesticides/f?p=PPLS:8:805623268384::NO::P8_PUID,P8_RINUM:37136,8329-62
[2] National Pesticide Information Center (NPIC). d-Phenothrin General Fact Sheet: https://npic.orst.edu/factsheets/dphengen.html
[3] National Pesticide Information Center (NPIC). Piperonyl Butoxide (PBO) General Fact Sheet: https://npic.orst.edu/factsheets/pbogen.html
[4] U.S. EPA. Permethrin, Resmethrin, d-Phenothrin (Sumithrin®): Synthetic Pyrethroids for Mosquito Control: https://www.epa.gov/mosquitocontrol/permethrin-resmethrin-d-phenothrin-sumithrinr-synthetic-pyrethroids-mosquito-control
[5] National Pesticide Information Center (NPIC). d-Phenothrin Technical Fact Sheet: http://npic.orst.edu/factsheets/archive/dphentech.html#references
[6] Massachusetts Department of Agricultural Resources. Mosquito Spray FAQ for Honey Beekeepers: https://www.mass.gov/info-details/mosquito-spray-faq-for-honey-beekeepers

Kim Skyrm, Ph.D, Sarah Miller, Ph.D., and Hotze Wijnja, Ph.D
Massachusetts Department of Agricultural Resources – Division of Crop and Pest Services

Beekeeping Community – At the time of the aerial application, a total of 197 registered beekeepers were managing apiaries in the application area. This likely represents only a fraction of the total apiaries in this area, given that apiary registration is voluntary in Massachusetts.

Stakeholder Communication – Notifications via email pre-application were sent to officers of state and county level beekeeping associations and beekeepers with apiaries registered and located inside the targeted aerial application area. Disseminated information included details regarding the application (i.e. pesticide, type, date, and location) and were shared in this email as well as posted on the mass.gov website. Beekeepers of monitored apiaries inside and outside the application area were contacted directly throughout the monitoring process and afterward during a seasonal follow up on colony health. The Apiary Program did not receive any reports of Bee Kills (i.e. visible honey bee death suspected to be due to pesticide exposure) during or after the aerial application so no additional investigations were conducted this year. In addition to this report, beekeepers of monitored apiaries were also emailed a summary of their individual sample results.

Monitoring Methods – The *Honey Bee Monitoring Protocol for Aerial Mosquito Adulticide Application* from *The Mosquito Emergency Operations Response Plan for Mosquito-Borne Illness*[7] was utilized for monitoring with modification, as needed. Beekeepers were selected for monitoring based on their geographic location, colony health, and absence of miticides in colonies during the monitoring period (Figure 1). Selected apiaries were categorized geographically and designated as those inside (treatment) or outside (control) the application area. Hobby (i.e. those keeping honey bees as a hobby) and sideliner (i.e. those keeping honey bees to generate profit as a part time business) beekeepers comprised both the treatment and control groups. Colony health (i.e. queenright, no visible signs of a Bee Kill, no visible pathogens or developmental issues, and low Varroa mite levels) was determined through pre-inspections to ensure the absence of adverse health parameters that could introduce potential confounding variables when evaluating the impacts of the aerial application. Only colonies that were found to be visibly healthy during these inspections and free of the health issues noted above 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 as well as those employed during health emergencies and Bee Kill investigations where colony death is investigated due to suspected pesticide exposure. Exterior monitoring consisted of evaluating foraging activity at colony entrances and dead bee accumulation outside hive boxes. Dead bees were collected by positioning white 130 thread count muslin cotton/polyester cloths (104 inches x 66 inches) flat bed sheets (MassCor Industries) in front of hive boxes. Sheets were affixed to the ground using staples (5 inches x 1 inch) (TrafficMaster Artificial Grass Staples (100-pack), Home Depot) (Figure 2). Dead bees present on the cloths were counted and collected for analysis. Interior health assessments included evaluating food stores,

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

queen, brood and adult bee population, as well as behavior to identify signs of acute pesticide impacts or other health issues.

Each apiary and honey bee colony were visited a total of three (3) times throughout the monitoring process during scheduled time intervals of pre-application (0-2 days pre-spray) 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 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 adult live and dead bees, when available. Samples of adult bees were taken from live foragers entering/exiting hives and dead bees on cloths situated in front of hives. Though they were conducted in 2024, dead bee counts have been shown in previous year monitoring efforts (2019-2020) to be an inconsistent indicator of colony risk. Weather (e.g., wind), predation, and worker bee hygienic behavior all have potential to remove dead and dying individuals from cloths away from the hive, resulting in undercounting of bee deaths.

All samples collected from individual colonies were pooled together from 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). Samples were collected and stored in sterile leak-proof dark amber centrifuge tubes (50ml) (VWR Ultra High Performance Light Sensitive Centrifuge Tubes) to preserve integrity. After collection, all samples were stored in a freezer at –10 °C until submitted for lab analysis. Samples were partitioned into separate tubes prior to lab submission, with half of each sample submitted for molecular analysis and the other half sent for pesticide analysis. Samples were sent via USPS Priority Mail on September 7, 2024, to the National Agricultural Genotyping Center (NAGC) for molecular analysis of viruses, bacteria, parasites, and fungi. On September 9, 2024, samples were hand-delivered to the Massachusetts Pesticide Analysis Laboratory (MPAL) for analysis of the mosquito adulticide active ingredients used in the aerial application.

Monitoring Results – The monitored apiaries were in four (4) counties and eight (8) towns (Table 1). A total of nine (9) beekeepers managing nine (9) apiaries consisting of 81 colonies were monitored. Of these, 26 colonies managed by four (4) beekeepers were located inside (treatment) and 55 colonies managed by five (5) beekeepers were located outside (control) the application area. Visual inspections did not indicate the presence of any health issues in any apiary, including bee mortality associated with a potential Bee Kill, at any time during the monitoring period. A total of 22 colonies were sampled as part of monitoring efforts, of which 8 were from treatment apiaries and 14 from control apiaries. A total of 83 adult bee samples were submitted for analysis, with 54 live adult bee samples (24 from treatment and 30 from control) and 29 dead adult bee samples (11 from treatment and 18 from control).

Counts of dead bees one (1) day after the aerial application (August 28, 2024) ranged from 12-26 adult bees per monitored treatment apiary (average of 18 adult bees) and 18-109 adult bees per control monitored apiary (average 47 adult bees). Numbers of dead bees in the treatment area were lower seven (7) days after the aerial application (September 3, 2024) with a range of 0-28 adult bees per monitored treatment apiary (average of 10 adult bees) and 0-143 dead adult bees (average 81 adult bees) per monitored control apiary. The daily forager mortality rate is estimated to be between 100 and 163 adult bees per colony per day for a honey bee colony at

this time of year, given an estimated colony size of 40-65 thousand adult individuals and assuming a 25% adult bee forager population.[8,9] Based on this estimate, the counts of dead bees observed were well below this range for an entire apiary in most cases, indicating little risk of the application to acute adult bee mortality. However, these counts should be taken with caution since observed dead bees are not always a reliable representation of bee health, as discussed above.

A. **Pesticide Analysis -** Samples submitted for pesticide analysis consisted of a total of 45 adult bee samples, with 20 from the treatment group (8 dead bees, 12 live bees) and 25 from the control group (10 dead bees, 15 live bees) (Table 2). Results from the pesticide analysis found 13 samples (29%) positive for pesticides while the remaining 32 samples (71%) were Non-Detect (ND) or not positive for pesticides at the Limit of Detection (LOD) (0.65-3.25 µg/kg bee (ppb)) (Figure 3). Samples collected from the control group were all ND for pesticides except for a single live bee sample that was positive for PBO. Samples collected from the treatment area group consisted of two (2) positives for d-phenothrin (10% of treatment group samples) and 12 positives for PBO (60% of treatment group samples). All positive samples were collected after the spray date, though only two (2) samples were positive for both d-phenothrin and PBO. Those samples were collected the day after the aerial application.

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 LD_{50} values) for d-phenothrin and PBO (Table 3). The LD_{50} values were obtained from Sanchez-Bayo and Goka (2014)[10] and EPA risk assessment documents.[11] To allow comparison of the measured pesticide levels in bees with toxicity endpoints, the standard LD_{50} values were converted to LD_{50} values in ppb relative to body weight by multiplying the standard LD_{50} values (ug/bee) using a factor of ~7800 (assuming an average bee weight of 0.128 g).[12] The contact and oral LD_{50} values for these pesticides along with the LD_{50} values in ppb relative to body weight are listed in Table 3. A comparison of the measured ppb residue levels in Table 2 with the LD_{50} 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 LD_{50} 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 LD_{50} value (ppb) and are included in

[8] Visscher, P.K. and Dukas, R. 1997. Survivorship and foraging of honey bees. Insectes Sociaux 44(1). https://link.springer.com/article/10.1007/s000400050017
[9] Seeley, T.D. 1995. The Wisdom of the Hive. Harvard University Press, Cambridge, MA, USA.
[10] 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
[11] 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
[12] U.S. EPA 2012. Ecological Effects Test Guidelines OPPTS 850.3020 Honey Bee Acute Contact Toxicity. https://nepis.epa.gov/Exe/ZyNET.exe/P100IRFL.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C11thru15%5CTxt%5C00000010%5CP100IRFL.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL

Table 3. The LOC is 0.4 for acute risk.[13] The calculated RQ values in Table 3 are well below the acute LOC. Therefore, again it is very unlikely that the measured residues of d-phenothrin and PBO caused lethal effects to the bees.

PBO is included in adulticide formulations to enhance the efficacy of insecticidal compounds. The exact magnitude of this synergistic effect and its potential impact on honey bees is not known here, but an estimate has been included in Table 3 from a study by Rinkevich et al. (2015), which suggests PBO may increase honey bee mortality by a factor of three (3) when exposed to d-phenothrin, depending on the bee subspecies. To account for this synergism, RQs were also calculated for the scenario of the application formulation decreasing the d-phenothrin LD_{50} by 3x, meaning honey bees being 3x more sensitive to the formulation than if it only contained d-phenothrin. RQ values for hypothetical oral and contact exposure fall into ranges below EPA-established levels of concern, even when considering potential synergistic effects.

B. **Molecular Analysis -** Samples submitted for molecular analysis consisted of 38 adult bee samples, of which 15 were from the treatment group (3 dead adult bees, 12 live adult bees) and 23 from the control group (8 dead adult bees, 15 live adult bees) (Table 4 and Table 5). Every sample contained co-infections of multiple viruses, indicating the widespread occurrence and diversity of viruses in honey bees in the Commonwealth. A total of eight (8) viruses were detected, listed here in order of prevalence in samples: Deformed Wing Virus B (DWV-B) (79%), Sacbrood Virus (SBV) (76%), Deformed Wing Virus-A (DWV-A) (53%), Black Queen Cell Virus (BQCV) (45%), Lake Sinai Virus-1 (LSV1) (26%), Israeli Acute Bee Paralysis Virus (IABPV) (13%), Chronic Bee Paralysis Virus (CBPV) (8%), and Lake Sinai Virus-2 (LSV2) (3%). All viruses were found in both the control and treatment apiaries except for CBPV, IABPV, and LSV2, which were only detected in samples taken from control apiaries (Figure 4). Of particular importance is the occurrence of CBPV, IABPV, LSV1 and LSV2, which have been found to cause acute colony death during investigations of Bee Kills in the Commonwealth, often mimicking symptoms of bee death due to pesticide exposure.[14]

Unfortunately, viruses are the least understood and most omnipresent health issue impacting honey bees. Viruses also often act synergistically with other biotic and abiotic stressors and have potential to be expressed asymptomatically, with little to no impact on individual bee health, or symptomatically, causing morphological, physiological, and behavioral issues leading to colony mortality.[15] Some viruses are also direct indicators of other health issues such as those associated with the ubiquitous ectoparasitic mite, *Varroa destructor*, a major vector or associate of many common honey bee viruses, including several of those detected in samples: BQCV,

[13] 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
[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/
[15] Beaurepaire A., Piot N., Doublet V., Antunez K., Campbell E., Chantawannakul P., Chejanovsky N., Gajda A., Heerman M., Panziera D., Smagghe G., Yañez O., de Miranda J.R., Dalmon A. 2020. Diversity and global distribution of viruses of the western honey bee, *Apis mellifera*. Insects.11(4): 239. https://pmc.ncbi.nlm.nih.gov/articles/PMC7240362/

DWV-A, DWV-B, IABPV, and SBV.[16][17] If left unmanaged, Varroa mites and associated viruses will cause colony mortality. Given this, beekeeper-driven Integrated Pest Management (IPM) strategies targeted for Varroa mites are critical because diligent management not only reduces mite loads but also the potential to directly impact the occurrence and severity of these viruses in the Commonwealth.

Molecular analysis further revealed that samples also contained the following pathogens and parasites in order of prevalence: *Nosema ceranae* (58%), *Lotmaria passim* (26%), Tracheal mites (26%), EFB (8%), and Chalkbrood (3%) (Figure 5). All pathogens were found in both the control and treatment apiaries except for Chalkbrood, which was only detected in control apiaries. The occurrence of *N. ceranae* in samples is not uncommon, but severe infections can cause colony mortality if left unmanaged and co-infections with *L. passim*, whose effects on honey bee health as a single pathogen are poorly understood.[18] Of the ten (10) samples positive for *L. passim* in this survey, eight (8) of those were co-infections with *N. ceranae*. Although *L. passim* is less virulent than *N. ceranae*, it has also been shown to cause decreased lifespan of infected bees and colony collapse.[19]

The most alarming parasite found in samples was the Tracheal mite, which was previously thought to be eradicated in US honey bee colonies since 2015, although detection data have been limited.[20] Also, it is interesting that detections occurred in samples taken this time of year, since this mite is considered to be uncommon during periods when adult bee populations in colonies are high and it is rarely found in foraging honey bees. Tracheal mites are a greater health threat to colonies in colder climates, given their ability to reduce honey yield, reduce brood production, and increase winter mortality.[21] They are also often associated with colonies that have high bacterial and viral loads, which seem to align with the apiaries monitored, given the diversity of pathogens detected in samples.

The occurrence of EFB in samples was concerning due to the potential for mortality and spread of infection, hence requiring beekeeper management through treatment.[22] Chalkbrood was only found in a single sample and while it rarely causes colony mortality, it does have the potential to weaken colonies by reducing population and subsequent honey production. The most detrimental and virulent pathogen, American Foulbrood (AFB), was not detected in any sample.

---

[16] 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/

[17] Traynor, K.S., Mondet, F., de Miranda, J.R., Techer, M., Kowallik, V., Oddie, M.A.Y., Chantawannakul, P., and McAfee, A. 2020. Varroa destructor: A complex parasite, crippling honey bees worldwide. Trends in Parasitology, 36(7). https://www.sciencedirect.com/science/article/pii/S147149222030101X

[18] 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/.

[19] MacInnis, C.I., Luong, L.T. and Pernal, S.F. 2023. A tale of two parasites: Responses of honey bees infected with *Nosema ceranae* and *Lotmaria passim*. Scientific Reports 13. https://www.nature.com/articles/s41598-023-49189-9

[20] Moore, P.A. Wilson, M.E. and Skinner, J.A. 2015. Honey bee tracheal mites: Gone? But not for good. https://bee-health.extension.org/honey-bee-tracheal-mites-gone-but-not-for-good/

[21] Downey, D.L. and Winston, M.L. 2001. Honey bee colony mortality and productivity with single and dual infestations of parasitic mite species. Apidologie 32(567-575). https://www.apidologie.org/articles/apido/abs/2001/06/downey/downey.html

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

Conclusion – The visual observations of the MDAR Apiary Program Team, combined with those 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 of Anvil® 10+10 ULV. Beekeepers contacted in follow up communication with colonies 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 indicates that detected pesticide residues in the live and dead bee samples were well below levels expected to cause concerning 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. It is likely that the cause of any adult bee mortality observed during the monitoring period was due to the presence and often co-infections of the viruses, pathogens, and parasites detected in samples.

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

- live and dead (when available) adult honey bees
- pre-application and post-application
- pooled samples from all monitored colonies representing the entire apiary
- molecular and pesticide analysis for samples

References

1. Beaurepaire A., Piot N., Doublet V., Antunez K., Campbell E., Chantawannakul P., Chejanovsky N., Gajda A., Heerman M., Panziera D., Smagghe G., Yañez O., de Miranda J.R., Dalmon A. 2020. Diversity and global distribution of viruses of the western honey bee, *Apis mellifera*. Insects.11(4): 239. https://pmc.ncbi.nlm.nih.gov/articles/PMC7240362/
2. 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/
3. 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/
4. Downey, D.L. and Winston, M.L. 2001. Honey bee colony mortality and productivity with single and dual infestations of parasitic mite species. Apidologie 32(567-575). https://www.apidologie.org/articles/apido/abs/2001/06/downey/downey.html
5. 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/

6. MacInnis, C.I., Luong, L.T. and Pernal, S.F. 2023. A tale of two parasites: Responses of honey bees infected with *Nosema ceranae* and *Lotmaria passim*. Scientific Reports 13. https://www.nature.com/articles/s41598-023-49189-9
7. Massachusetts Department of Agricultural Resources. Mosquito Spray FAQ for Honey Beekeepers: https://www.mass.gov/info-details/mosquito-spray-faq-for-honey-beekeepers
8. Massachusetts Emergency Operations Response Plan for Mosquito-Borne Illness: https://www.mass.gov/massachusetts-emergency-operations-response-plan-for-mosquito-borne-illness
9. Moore, P.A. Wilson, M.E. and Skinner, J.A. 2015. Honey bee tracheal mites: Gone? But not for good. https://bee-health.extension.org/honey-bee-tracheal-mites-gone-but-not-for-good/
10. National Pesticide Information Center (NPIC). d-Phenothrin General Fact Sheet: https://npic.orst.edu/factsheets/dphengen.html
11. National Pesticide Information Center (NPIC). d-Phenothrin Technical Fact Sheet: http://npic.orst.edu/factsheets/archive/dphentech.html#references
12. National Pesticide Information Center (NPIC). Piperonyl Butoxide (PBO) General Fact Sheet: https://npic.orst.edu/factsheets/pbogen.html
13. Rinkevich, F.D., Margotta, J.W., Pittman, J.M., Danka, R.G., Tarver, M.R., Ottea, J.A., and Healy, K.B. 2015. Genetics, synergists, and age affect insecticide sensitivity of the honey bee, *Apis mellifera*. PLOS ONE 10(10). https://doi.org/10.1371/journal.pone.0139841
14. 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
15. Seeley, T.D. 1995. The Wisdom of the Hive. Harvard University Press, Cambridge, MA, USA.
16. Traynor, K.S., Mondet, F., de Miranda, J.R., Techer, M., Kowallik, V., Oddie, M.A.Y., Chantawannakul, P., and McAfee, A. 2020. Varroa destructor: A complex parasite, crippling honey bees worldwide. Trends in Parasitology, 36(7). https://www.sciencedirect.com/science/article/pii/S147149222030101X
17. U.S. EPA 2012. Ecological Effects Test Guidelines OPPTS 850.3020 Honey Bee Acute Contact Toxicity. https://nepis.epa.gov/Exe/ZyNET.exe/P100IRFL.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C11thru15%5CTxt%5C00000010%5CP100IRFL.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL
18. 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
19. 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

20. U.S. EPA. Permethrin, Resmethrin, d-Phenothrin (Sumithrin®): Synthetic pyrethroids for mosquito control: https://www.epa.gov/mosquitocontrol/permethrin-resmethrin-d-phenothrin-sumithrinr-synthetic-pyrethroids-mosquito-control
21. U.S. EPA. Clarke Anvil® 10+10 ULV Pesticide Label: https://ordspub.epa.gov/ords/pesticides/f?p=PPLS:8:805623268384::NO::P8_PUID,P8_RINUM:37136,8329-62
22. Vidal-Naquet, N. 2015. Honeybee Veterinary Medicine: *Apis Mellifera* L. 5M Publishing. Sheffield, United Kingdom.
23. Visscher, P.K. and Dukas, R. 1997. Survivorship and foraging of honey bees. Insectes Sociaux 44(1). https://link.springer.com/article/10.1007/s000400050017

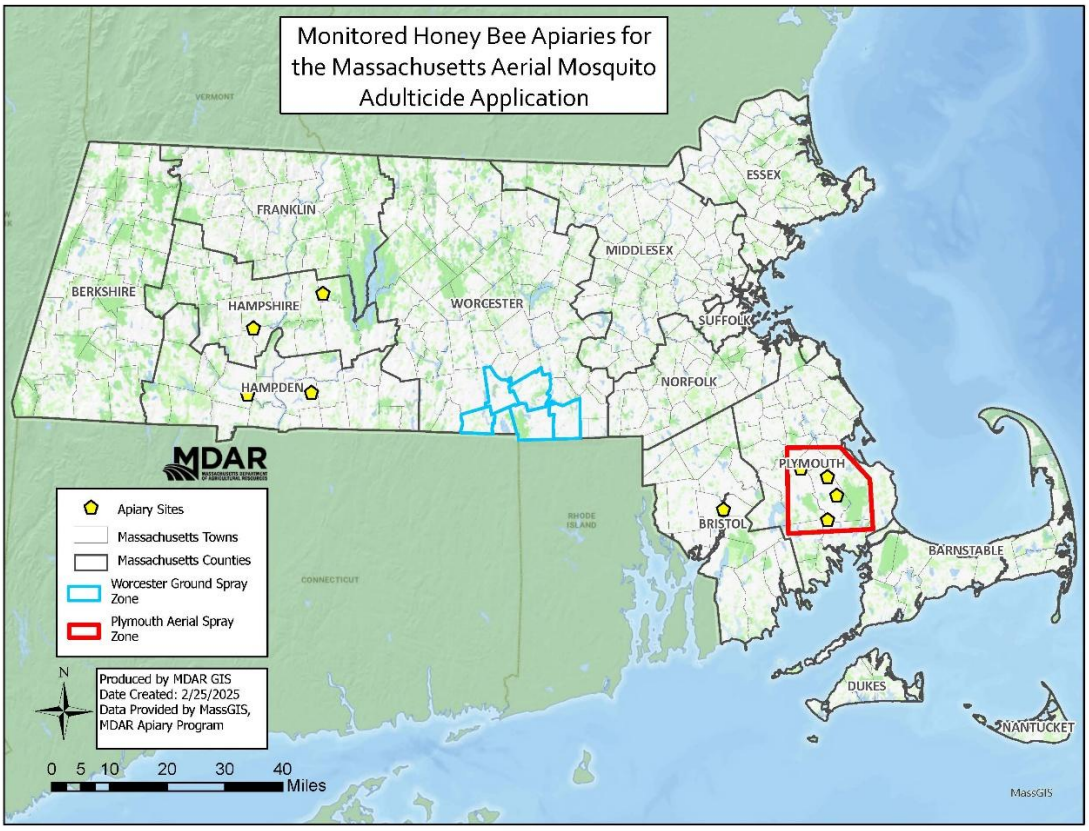

Figure 1. Map showing aerial application spray area (red) and monitored apiary locations (yellow).

Figure 2. Monitored apiaries in control and treatment groups, respectively, with cloths installed.

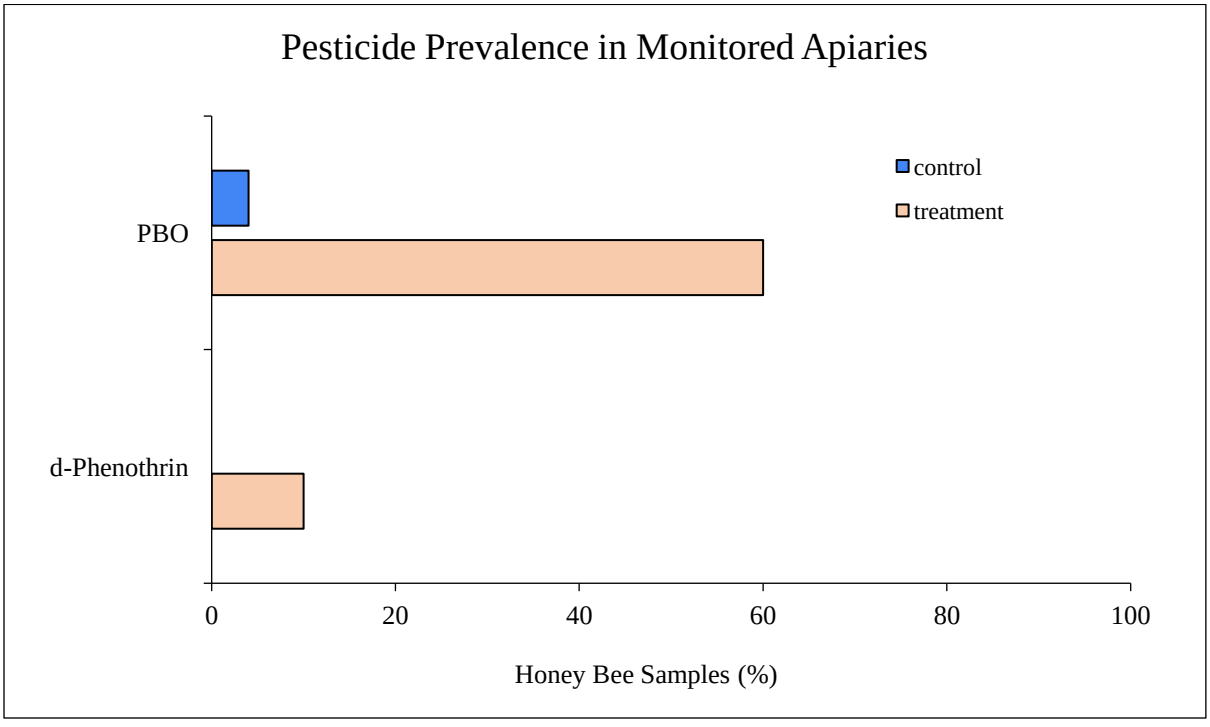

Figure 3. Pesticide prevalence in live and dead adult honey bee samples collected from monitored apiaries (n=45).

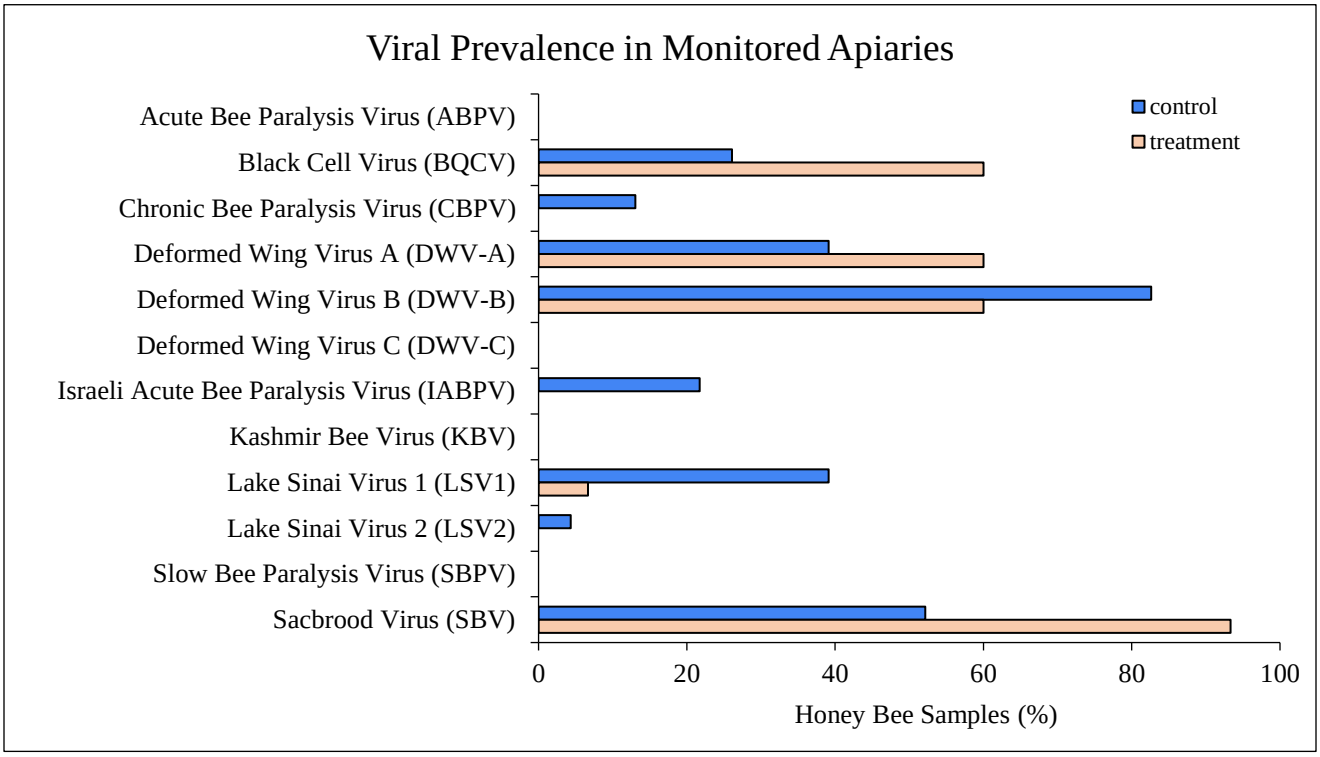

Figure 4. Viral prevalence in live and dead adult honey bee samples collected from monitored apiaries (n=38).

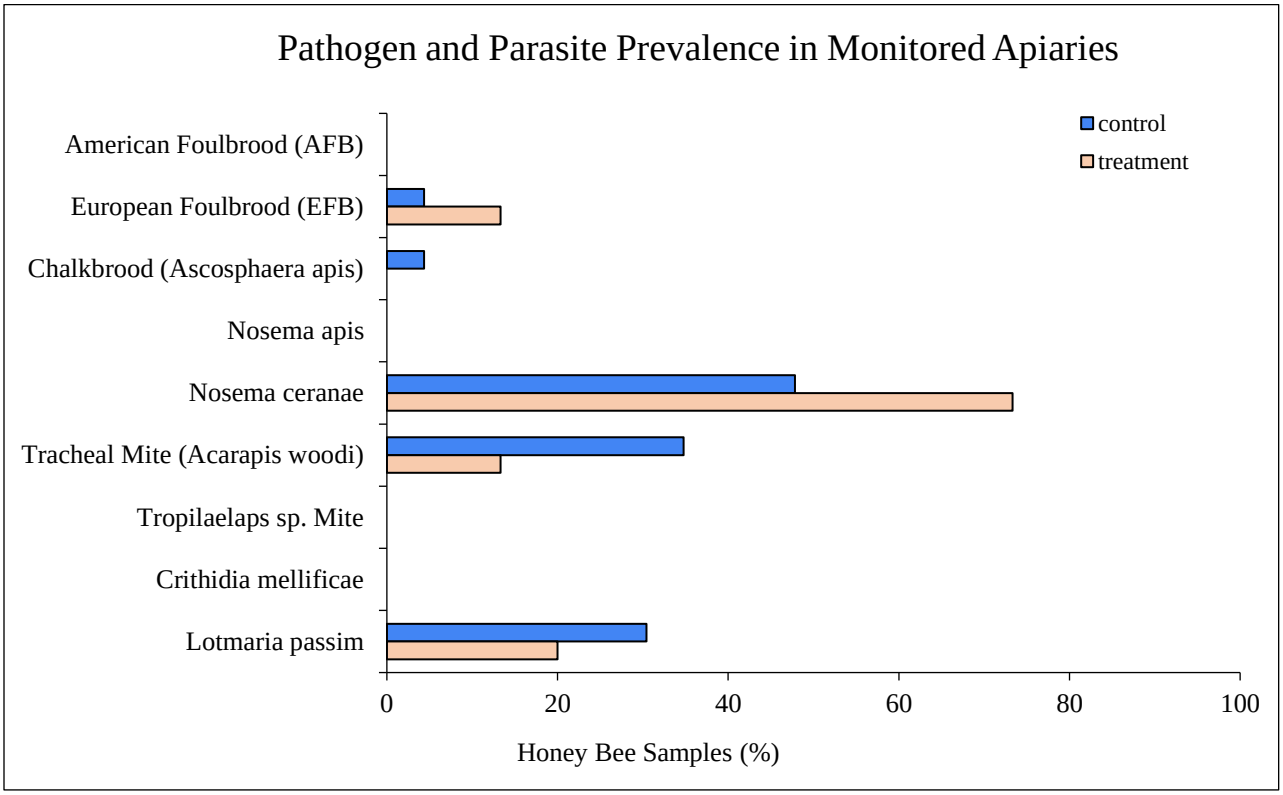

Figure 5. Pathogen (bacteria and fungi) and parasite (protozoa and mites) prevalence in live and dead adult honey bee samples collected from monitored apiaries (n=38).

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

Monitored Apiary	Metric Totals (n)							
	Counties	Towns	Apiaries	Beekeepers	Monitored Colonies	Sampled Colonies	Live Adult Bee Samples	Dead Adult Bee Samples
control	3	5	5	5	55	14	30	18
treatment	1	3	4	4	26	8	24	11
Total	4	8	9	9	81	22	54	29

Table 2. Pesticide prevalence in samples of live and dead adult honey bees taken from monitored apiaries.

Monitored Apiary	Sample ID	Sample Type	Sample County	Sample Date (2024)	d-Phenothrin (µg/kg or ppb)*	Piperonyl Butoxide (PBO) (µg/kg or ppb)*
control	SD082724L	live	Bristol	8/27	ND	ND
	SD082824L	live		8/28	ND	ND
	SD090324L	live		9/3	ND	5.00
	SD082824D	dead		8/28	ND	ND
	SD090324D	dead		9/3	ND	ND
	NS082724L	live	Hampden	8/27	ND	ND
	NS082824L	live		8/28	ND	ND
	NS090324L	live		9/3	ND	ND
	NS082824D	dead		8/28	ND	ND
	NS090324D	dead		9/3	ND	ND
	ML082724L	live		8/27	ND	ND
	ML082824L	live		8/28	ND	ND
	ML090324L	live		9/3	ND	ND
	ML082824D	dead		8/28	ND	ND
	ML090324D	dead		9/3	ND	ND
	DP082724L	live	Hampshire	8/27	ND	ND
	DP082824L	live		8/28	ND	ND
	DP090324L	live		9/3	ND	ND
	DP082824D	dead		8/28	ND	ND
	DP090324D	dead		9/3	ND	ND
	DC082724L	live		8/27	ND	ND
	DC082824L	live		8/28	ND	ND
	DC090324L	live		9/3	ND	ND
	DC082824D	dead		8/28	ND	ND
	DC090324D	dead		9/3	ND	ND
treatment	GH082724L	live	Plymouth	8/27	ND	ND
	GH082824L	live		8/28	ND	50.00
	GH090324L	live		9/3	ND	ND
	GH082824D	dead		8/28	ND	119.00
	GH090324D	dead		9/3	ND	ND
	JP082724L	live		8/27	ND	ND
	JP082824L	live		8/28	ND	117.00
	JP090324L	live		9/3	ND	8.00
	JP082824D	dead		8/28	ND	780.00
	JP090324D	dead		9/3	ND	26.00
	LR082724L	live		8/27	ND	ND
	LR082824L	live		8/28	ND	100.00
	LR090324L	live		9/3	ND	13.00
	LR082824D	dead		8/28	ND	278.00
	LR090324D	dead		9/3	ND	ND
	CS082724L	live		8/27	ND	ND
	CS082824L	live		8/28	5.30	104.00
	CS090324L	live		9/3	ND	25.00

CS082824D	dead		8/28	67.00	693.00
CS090324D	dead		9/3	ND	ND
		Total Samples	45	2	13
		Pesticide Prevalence (%)		4.44	28.89

*ND means that pesticide was not detected in sample at the Limit of Detection (LOD) (0.65-3.25 µg/kg bee ppb)

Table 3. Pesticide toxicity endpoints and calculated risk quotients in live adult honey bees

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)	Risk Quotient range, contact	Risk Quotient range, oral
d-phenothrin	0.13	0.16	1,016	1,250	5.3-67	0.005-0.066	0.004-0.054
piperonyl butoxide (PBO)	>25	-	195,313	-	5-780	<0.004	-
d-phenothrin, PBO-synergized (assume 3X sensitivity)[23]	0.04	0.05	339	417	5.3-67	0.02-0.2	0.01-0.16

Detection limit range for d-phenothrin and PBO was 0.65-3.25 µg/kg bee (ppb).
*Assuming 0.128g honey bee body weight (EPA 2012).

[23] Rinkevich, F.D., Margotta, J.W., Pittman, J.M., Danka, R.G., Tarver, M.R., Ottea, J.A., and Healy, K.B. 2015. Genetics, synergists, and age affect insecticide sensitivity of the honey bee, *Apis mellifera*. PLOS ONE 10(10). https://doi.org/10.1371/journal.pone.0139841

Table 4. Viral prevalence in samples of live and dead adult honey bees taken from monitored apiaries.

					Virus												
Monitored Apiary	Sample ID	Sample Type	Sample County	Sample Date (2024)	Acute Bee Paralysis Virus (ABPV)	Black Cell Virus (BQCV)	Chronic Bee Paralysis Virus (CBPV)	Deformed Wing Virus A (DWV-A)	Deformed Wing Virus B (DWV-B)	Deformed Wing Virus C (DWV-C)	Israeli Acute Bee Paralysis Virus (IABPV)	Kashmir Bee Virus (KBV)	Lake Sinai Virus 1 (LSV1)	Lake Sinai Virus 2 (LSV2)	Slow Bee Paralysis Virus (SBPV)	Sacbrood Virus (SBV)
control	SD082724L	live	Bristol	8/27	-	-	-	+	-	-	-	-	-	-	-	+
	SD082824L	live		8/28	-	+	-	-	+	-	-	-	-	-	-	+
	SD090324L	live		9/3	-	+	-	+	+	-	-	-	-	-	-	+
	NS082724L	live	Hampden	8/27	-	-	-	-	+	-	-	-	+	-	-	+
	NS082824L	live		8/28	-	+	-	-	-	-	-	-	-	-	-	+
	NS090324L	live		9/3	-	-	+	-	+	-	-	-	+	-	-	+
	NS082824D	dead		8/28	-	-	-	-	+	-	-	-	-	+	-	+
	NS090324D	dead		9/3	-	+	-	+	+	-	+	-	-	-	-	+
	ML082724L	live		8/27	-	-	-	-	+	-	-	-	+	-	-	-
	ML082824L	live		8/28	-	-	-	-	+	-	-	-	+	-	-	-
	ML090324L	live		9/3	-	-	-	-	+	-	-	-	+	-	-	-
	ML082824D	dead		8/28	-	+	-	-	+	-	-	-	+	-	-	-
	ML090324D	dead		9/3	-	-	-	-	+	-	-	-	+	-	-	-
	DP082724L	live	Hampshire	8/27	-	-	-	-	+	-	-	-	+	-	-	+
	DP082824L	live		8/28	-	+	-	-	+	-	-	-	+	-	-	+
	DP090324L	live		9/3	-	-	-	+	+	-	+	-	-	-	-	+
	DP082824D	dead		8/28	-	-	-	+	+	-	+	-	-	-	-	+
	DP090324D	dead		9/3	-	-	-	+	+	-	+	-	-	-	-	-
	DC082724L	live		8/27	-	-	-	+	+	-	-	-	-	-	-	-
	DC082824L	live		8/28	-	-	-	+	+	-	-	-	-	-	-	-
	DC090324L	live		9/3	-	+	+	+	+	-	-	-	-	-	-	+
	DC082824D	dead		8/28	-	-	-	+	+	-	-	-	-	-	-	+
	DC090324D	dead		9/3	-	+	+	+	+	-	+	-	-	-	-	+
treatment	GH082724L	live	Plymouth	8/27	-	-	-	-	-	-	-	-	-	-	-	+
	GH082824L	live		8/28	-	+	-	+	+	-	-	-	-	-	-	+
	GH090324L	live		9/3	-	+	-	+	+	-	-	-	-	-	-	+
	GH090324D	dead		9/3	-	+	-	-	-	-	-	-	-	-	-	+
	JP082724L	live		8/27	-	+	-	+	+	-	-	-	-	-	-	+
	JP082824L	live		8/28	-	+	-	+	+	-	-	-	-	-	-	+
	JP090324L	live		9/3	-	-	-	+	+	-	-	-	-	-	-	+
	LR082724L	live		8/27	-	+	-	-	-	-	-	-	-	-	-	+
	LR082824L	live		8/28	-	+	-	-	-	-	-	-	+	-	-	+
	LR090324L	live		9/3	-	+	-	-	-	-	-	-	-	-	-	+
	LR082824D	dead		8/28	-	-	-	-	-	-	-	-	-	-	-	+
	CS082724L	live		8/27	-	-	-	+	+	-	-	-	-	-	-	+

CS082824L	live	8/28	-	-	-	+	+	-	-	-	-	-	-	+
CS090324L	live	9/3	-	+	-	+	+	-	-	-	-	-	-	+
CS082824D	dead	8/28	-	-	-	+	+	-	-	-	-	-	-	-
Total Samples		38	0	17	3	20	30	0	5	0	10	1	0	29
Prevalence (%)			0	44.74	7.89	52.63	78.95	0	13.16	0	26.32	2.63	0	76.32

(+) Indicates that the virus was detected in sample. (-) Indicates that the virus was not detected in the sample.

Table 5. Pathogen (bacteria and fungi) and parasite (protozoa and mites) prevalence in samples of live adult honey bees taken from monitored apiaries.

					Bacteria		Fungi			Protozoa		Mites	
Monitored Apiary	Sample ID	Sample Type	Sample County	Sample Date (2024)	American Foulbrood (AFB)	European Foulbrood (EFB)	Chalkbrood *(Ascosphaera apis)*	*Nosema apis*	*Nosema ceranae*	*Crithidia mellificae*	*Lotmaria passim*	Tracheal Mite *(Acarapis woodi)*	*Tropilaelaps spp.* Mite
control	SD082724L	live	Bristol	8/27	-	-	+	-	-	-	-	-	-
	SD082824L	live		8/28	-	-	-	-	-	-	-	-	-
	SD090324L	live		9/3	-	-	-	-	+	-	-	+	-
	NS082724L	live	Hampden	8/27	-	-	-	-	+	-	-	-	-
	NS082824L	live		8/28	-	-	-	-	+	-	-	-	-
	NS090324L	live		9/3	-	-	-	-	+	-	-	-	-
	NS082824D	dead		8/28	-	-	-	-	+	-	+	-	-
	NS090324D	dead		9/3	-	-	-	-	+	-	+	-	-
	ML082724L	live		8/27	-	-	-	-	+	-	+	-	-
	ML082824L	live		8/28	-	-	-	-	+	-	+	+	-
	ML090324L	live		9/3	-	-	-	-	+	-	+	-	-
	ML082824D	dead		8/28	-	-	-	-	-	-	+	+	-
	ML090324D	dead		9/3	-	-	-	-	-	-	+	+	-
	DP082724L	live	Hampshire	8/27	-	-	-	-	-	-	-	+	-
	DP082824L	live		8/28	-	-	-	-	-	-	-	-	-
	DP090324L	live		9/3	-	-	-	-	+	-	-	-	-
	DP082824D	dead		8/28	-	-	-	-	+	-	-	+	-
	DP090324D	dead		9/3	-	+	-	-	-	-	-	+	-
	DC082724L	live		8/27	-	-	-	-	-	-	-	-	-
	DC082824L	live		8/28	-	-	-	-	-	-	-	-	-
	DC090324L	live		9/3	-	-	-	-	-	-	-	-	-
	DC082824D	dead		8/28	-	-	-	-	-	-	-	-	-
	DC090324D	dead		9/3	-	-	-	-	-	-	-	+	-

	GH082724L	live		8/27	-	-	-	-	+	-	-	-	-
	GH082824L	live		8/28	-	-	-	-	+	-	-	-	-
	GH090324L	live		9/3	-	-	-	-	+	-	-	-	-
	GH090324D	dead		9/3	-	-	-	-	-	-	-	-	-
	JP082724L	live		8/27	-	-	-	-	-	-	-	-	-
	JP082824L	live		8/28	-	-	-	-	+	-	+	-	-
	JP090324L	live		9/3	-	-	-	-	+	-	+	-	-
treatment	LR082724L	live	Plymouth	8/27	-	+	-	-	-	-	-	-	-
	LR082824L	live		8/28	-	-	-	-	+	-	-	-	-
	LR090324L	live		9/3	-	+	-	-	+	-	-	+	-
	LR082824D	dead		8/28	-	-	-	-	+	-	-	+	-
	CS082724L	live		8/27	-	-	-	-	+	-	-	-	-
	CS082824L	live		8/28	-	-	-	-	+	-	-	-	-
	CS090324L	live		9/3	-	-	-	-	+	-	+	-	-
	CS082824D	dead		8/28	-	-	-	-	-	-	-	-	-
			Total Samples	38	0	3	1	0	22	0	10	10	0
			Prevalence (%)		0	7.89	2.63	0	57.89	0	26.32	26.32	0

(+) Indicates that the pathogen or parasite was detected in the sample. (-) Indicates that the pathogen or parasite was not detected in the sample.