Final Report: Massachusetts Department of Agriculture Resources
Title: Assessing European Honeybee Health in Massachusetts
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Focus: Determine the health of managed honeybee (*Apis mellifera*) hives throughout Massachusetts in 2016. We hoped to have hives of differing management types and grouped into three categories: migratory, commercial and backyard, but the samples were largely taken from backyard operations.

We used the following definitions:
Migratory- hives are moved to different apiary locations throughout the season to facilitate pollination of food crops and to take advantage of nectar flows
Commercial- beekeepers who sell products from honeybees such as honey, beeswax, pollen, propolis and royal jelly, along with beekeepers who also raise queens and bees to sell to other farmers, and to satisfy scientific curiosity.
Backyard- hobbyist beekeepers.

Factors Considered: Varroa mite count control total, *Nosema apis* count total, and tracheal mite count total.

Background:
Varroa destructor is an external parasitic mite that attacks honeybees such as *Apis cerana* and *Apis mellifera*. The disease caused by the mites is called varroosis. Varroa mites can only reproduce in honeybee colonies and develop on brood (larvae); they highly prefer drone cells due to the length of time it takes for a drone to develop. The mites feed and develop on the maturing bee larva by sucking the bees' bodily fluids that can lead to a shortened life for the bee. A colony's numbers will dwindle until Varroa mites ultimately break down the colony. When managing a hive, it is essential to perform a mite test such as an alcohol wash to determine the potential number of mites present in the hive. A typical threshold for determining whether or not to manage mites after performing an alcohol wash would be to find approximately 5 mites per approximately 300 bees sampled. Recommended management can include a powdered sugar wash where powdered sugar is shifted over the bees and with their hygienic behavior, the mites are cleaned off of the bees and fall through a screen bottom board. Another method could include placing a half sized frame in the brood chamber so the bees will draw out deep comb and the queen will lay unfertilized eggs in the cells. Mites prefer being in drone comb because drones stay in the comb for a longer period of time and the mites are able to lay more eggs. By simply scraping off the drone comb, one could manage Varroa mites. Finally, the use of chemicals is an option for Varroa mite management, such as Mite-away strips that contain the active ingredient formic acid.

Honey bees are infected with two species of microsporidia: *Nosema apis* and *Nosema ceranae.* A microsporidian is a small, unicellular parasite recently reclassified as a fungus. It causes nosemosis, also called nosema, which is the most common and widespread of adult European honeybee diseases and more specifically, infects epithelial cells that line the bee gut. This disease will cause a colony's population to dwindle. Recommended management includes antibiotic treatment and the sterilization of contaminated combs.

Acarapis woodi, also known as tracheal mite, is a microscopic internal mite that clogs the breathing tubes of adult bees, blocking oxygen flow and eventually killing them. The mites appear in adult bees and the females lay eggs in the trachea. This makes bees' wings degenerate and may cause an inability to fly. Infected bees are known to have higher bacterial counts. Acarine disease could persist in the colony for years causing little damage, but combined with other diseases or unfavorable conditions, the disease increases the mortality of colonies.

Methods:

When sampling managed honeybee hives across different counties in Massachusetts, we did not use a spread sample but an availability sample. Through an Internet search we found random backyard beekeepers in each county for our sample; once we had three backyard keepers we would consider the sample size large enough to represent the county based on beekeepers' availability and our availability. The Massachusetts Department of Agricultural Resources Apiary Inspector provided a list of potentially interested participants for the study whose honeybee hive management uses would qualify them as migratory and commercial beekeepers. The list of migratory and commercial beekeepers is small and participation of these beekeepers depended on their availability. Massachusetts state honeybee inspectors typically inspect 10% of a beekeeper's hives and we followed the same protocol.

First hive visit: May 13- July 26

Backyard Beekeeper County and Migratory and Commercial	**Number of sites visited**
Barnstable	3
Berkshire	3
Bristol	3
Essex	3
Franklin	1

Hampden	3
Hampshire	2
Middlesex	1
Norfolk	2
Plymouth	3
Suffolk	1
Worcester	3
Migratory	2
Commercial	1

Second hive visit: July 26- September 18

Backyard Beekeeper County and Migratory	**Number of sites visited**
Barnstable	3
Berkshire	2
Bristol	2
Essex	3
Franklin	1
Hampden	2
Hampshire	2
Middlesex	1
Norfolk	2
Plymouth	1
Suffolk	1
Worcester	1
Migratory	1

Varroa mite count-

To get a Varroa mite count we used the standard alcohol wash approach. We filled our filtration system with 70% isopropyl alcohol. The filtration system was a container with a screen constructed by two empty plastic containers; one of the containers is cut and the bottom is removed and replaced with a screen, this container is placed inside the other container and when the isopropyl alcohol is added, the screen will be completely submerged and the whole system will be filled ¾ of the way. To get an accurate representation of the number of mites in the hive, we took a frame that had mostly brood present with some drone comb, as this is the location that Varroa mites are most likely to be present. We then examined the frame for the queen, and if she was not present we shook the frame into a bucket and promptly scooped ½ cup of bees (~300 individual bees) using a baker's cup, and poured them into the isopropyl alcohol within the filtration container. We then shifted the bees around in the alcohol, causing the Varroa mites to detach from the bees and fall through the screen to the bottom of our filtration system where they could be counted. At that time, it is possible to count the number of mites that fall from the bees collected. Typically, if 5 mites are found per 300 bees, a colony is deemed 'treatable'. The numbers of Varroa mites were recorded per sample.

Nosema apis count-

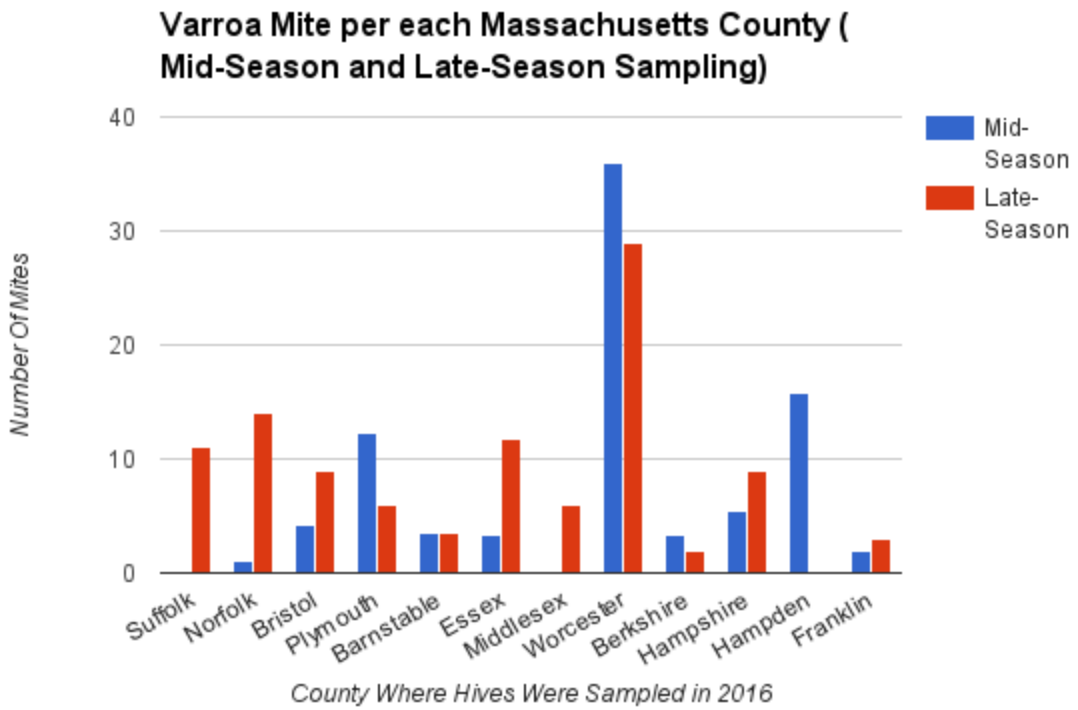
To count the number of Nosema spores, we collected 30 bees and mashed them up using a mortar and pestle with an initial 10 mL of water. Another 20 mL of water is added after 1 minute of mashing and the mixture is combined further until each individual bee is mashed and not in its original form. After that, we used a pipette to extract 500 micrometers of our solution. Then, we placed 2 drops of the solution onto a hemocytometer and placed a glass cover over the slide. Using a phase contrast microscope, we set the lighting to phase 2, which makes the pill- shaped nosema spores shine brighter and be more visible. Using the grids from the hemocytometer, we found a 4x4 grid and counted the number of spores in each of the four corners from this grid. This number gave us a representation of how many Nosema spores could be found per bee by multiplying the number of spores we counted by 50,000 as done by Jamie Ellis from the University of Florida's honeybee research and extension lab. The number of Nosema spores found per bee was then recorded per sample.

Tracheal mite count-

To count the number of tracheal mites, we collected 10 live bees and froze them to be sure to preserve their structure. Using two forceps we would separate the legs of the honeybee lying on its back and hold the thorax just in front of the second set of legs. With the other forceps we would grab the head on top of the collar, and tear off the head, front legs, and first part of the thorax (the collar). The honeybee was then ready to be placed on a glass slide and looked at under a compound microscope. The microscope was on a 40x magnification because the condition of the trachea was obvious as long as it was well lit. To determine if the trachea were infested with mites or not we looked for their trachea to be clear/slight cream color in

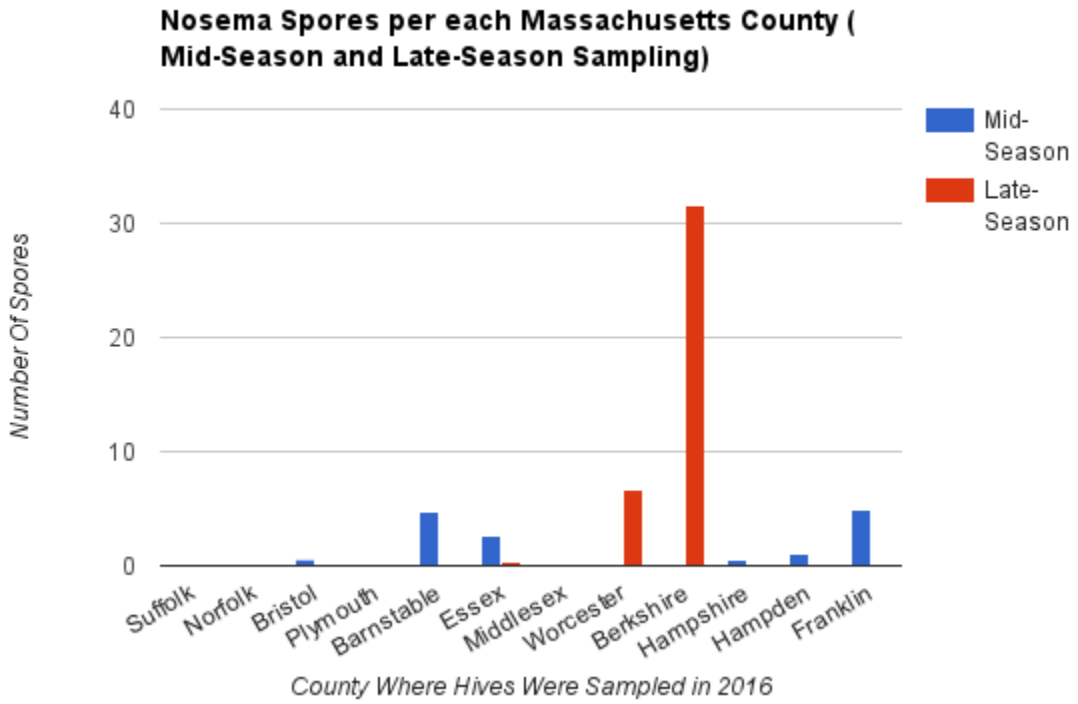
comparison to black and or spotted/speckled, which would represent tracheal mite damage. We did not find any tracheal mites in our samples, however, if we did we would record whether the mites were present or not. The presence of tracheal mites found per bee was then recorded per sample.

Results:



Late-season sampling Berkshire, Hampden, Plymouth, and Worcester counties; we were unable to resample all of the same hives, therefore the county value comparisons are based on incomplete data

From data we collected, on our first round of hive visits, 10/12 of the counties we visited had Varroa mites present. On our second round of hive visits, 12/12 of the counties we visited had Varroa mites present. In most cases (7/12 counties), the number of Varroa mites found increased later in the summer. The number of mites throughout each hive visit fluctuates but the trend shows that the average number of Varroa mites increase throughout the summer. For our first round of hive visits, one of two migratory beekeepers had Varroa mites present, the single commercial beekeeper that was sampled had Varroa mites present, and 21/31 backyard beekeepers had Varroa mites present. For our final round of hive visits, the single migratory beekeeper sampled had Varroa mites, and 20/25 backyard beekeepers had Varroa mites present.



Late-season sampling Berkshire, Hampden, Plymouth, and Worcester counties; we were unable to resample all of the same hives, so that the county value comparisons are based on incomplete data

The Nosema spore counts do not show a trend from the first round of hive visits to the second. In the first round of hive visits, Nosema was present in 6/12 counties. It was also present in both migratory beekeeper's sampled hives, and the single commercial beekeeper's sampled hives. For the second round of hive visits, Nosema was present in hives within 3/12 counties. It was also present in the single migratory beekeeper's sampled hives.

Tracheal mites were not found in any of the bees we examined.

Discussion:

In order to make a more informed claim about general hive health between migratory, commercial, and backyard beekeepers we would need a larger sample size for this study. Based on our discussions with beekeepers representing the different management types, the migratory and commercial beekeepers appear to be the most proactive with hive maintenance regarding treating for virus and diseases. The migratory and commercial beekeepers all inquired about their counts compared to the backyard beekeepers in which significantly fewer participants inquired about their counts. Since migratory and commercial beekeepers depend on their hives to make a living, it is crucial for the beekeepers to be thorough. Typically, migratory and commercial beekeepers feed their bees supplements when there is not a heavy nectar and pollen flow, such as in the beginning of the spring when the bees have already fed on their storage for the winter, or

during a drought. A common trend we saw for backyard beekeepers was that about a quarter of the packages were new, and the beekeepers were just starting to maintain hives. Around half of the backyard beekeepers said they use treatments or plan to and half do not do currently have management plans in place for their hives.

Presently, Varroa mites appear to be a major pest of managed backyard honeybee colonies in Massachusetts. These mites weaken the hive to the point where the hive is too weak and collapses. Mites are found sucking the bodily fluids of adults, and the brood- specifically found in drone brood. If the Varroa mite goes untreated and causes too much destruction, beekeepers often mistake this devastation for winter mortality or that the hive is queenless. As seen in our data, Varroa mites were present in almost every county. A domesticated colony's ability to survive depends on the diligence of the beekeeper. There are multitudes of reasons for hives to collapse so being able to detect virus and disease within a hive is crucial for their survival. This gives the beekeeper a chance to manage their hive properly, whether their management is cultural, mechanical, biological, or chemical. Based on our observations of this study, it would appear that Varroa mites could have an impact on the overall health of managed honeybee hive in Massachusetts in the summer of 2016. 42 out of 75 hives were found this summer to have 5+ Varroa mites per 300 bees sampled. This threshold is commonly accepted by the Massachusetts Department of Agricultural Resources as the number of Varroa mites present indicating that management for that hive pest is necessary to protect overall hive health.

Tracheal mites no longer seem to be much of an issue in Massachusetts. They were more common in the 80's-90's (Hood, 2016).

Nosema apis is a relatively benign parasite of honeybees. However, it makes the hive slightly less healthy (Bessin, 2016). It affects colonies that must winterize in order for survival. It can also be more of an issue when honeybees are not able to forage due to weather conditions, or if a package of bees is infested and confined in a small area. This could result in a supersedure of the queen. Honeybees should be able to tolerate Nosema infection when they receive good nutrition.

In order to prevent colony loss, beekeepers must recognize and monitor for the many issues hives may face. This means the beekeeper must also be aware of the options that are available to help manage each different pest or pathogen. Education provided to beekeepers through their local beekeeping association is a common way this information is disseminated across the state. Beekeepers benefit from presentations by knowledgeable speakers who can show them how to identify, monitor for, and manage pests and pathogens in honeybee hives. Providing a wide array of recommended management practices that are cultural, mechanical, and chemical while posing the least risk to human health and the environment is essential for aiding beekeepers navigating variable honeybee colony health.

Bibliography

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