A survey of *Nosema* spp. and *Varroa destructor* mites in honey bee (*Apis mellifera*) colonies throughout Western Dominica Apiaries

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ABSTRACT

Honey bees (*Apis mellifera*) are arguably the most beneficial insects to agriculture worldwide, as they contribute over $200 billion annually to the global economy, particularly through crop pollination. Despite their importance, the number of managed honey bee colonies available for pollination has steadily decreased in the last decade due to several biotic and environmental factors including pests, pathogens, poor nutrition, poor queen quality, pesticide exposure, among others. The most severe pest of honey bees is the ectoparasitic mite, *Varroa destructor,* which, when found in high numbers, can cause colonies to collapse and die. Other problematic pathogens include the microsporidia *Nosema ceranae* and *N. apis*, which cause gastrointestinal problems and malnutrition. In this study, we collected honey bees from eight apiaries around the Caribbean island of Dominica. These were tested on site (using powder sugar shakes) and then in the laboratory for the presence and abundance of *Varroa* mites and *Nosema* spp. spores. We found *Varroa* mites in every colony, often at numbers higher than the recommended maximum incidence levels. *Nosema* spp. spores were found in almost every apiary, but at relatively low number of spores per bee.

INTRODUCTION

Honey bees (*Apis mellifera*) are arguably the most beneficial insects to agriculture worldwide, as they contribute over $200 billion annually to the global economy, particularly through crop pollination (Gallai et al. 2009). Despite their importance, the number of managed honey bee colonies available for pollination has steadily decreased in the last decade due to several biotic

and environmental factors including pests, pathogens, poor nutrition, poor queen quality, pesticide exposure, among others, which can have devastating consequences to the productivity and longevity of the hive. Honey bees serve as vitally important pollinators on the island of Dominica, a Caribbean island that has a thriving beekeeper community organized by an active member cooperative.

Nosema ceranae and *N. apis* are microsporidian gut pathogens that affects the digestive system of honey bees. *Nosema* spp. spores are ingested by adult bees when they clean their combs, and are transmitted between individuals through the oral-fecal route (Huang 2016). When ingested, *Nosema* spores enter the midgut of a bee, affecting epithelial cells and causing a variety of symptoms. *Nosema apis* is known to cause dysentery, which results in fecal streaking both on the outside of the hive walls and inside the frame area. *Nosema ceranae* infection is harder to identify, as dysentery is often not a symptom. Both species of *Nosema* are widespread and cause weak immune systems, protein deficiencies, different foraging behaviors, and energy stress in the hive, which all lend toward a lack of overall colony productivity (Huang 2011).

The mite *Varroa destructor* is an ectoparasite of honey bee brood and adults. When left untreated, high levels of *Varroa* mites can cause colonies to collapse or die (vanEngelsdorp et al., 2008; 2010). *Varroa* mites use adult honey bees as phoretic hosts to move to and from the brood nest of female worker and male drone pupae to partake in blood meals. Bees that emerge as adults after being parasitized by these mites may be heavily deformed with missing legs or destroyed wings, a symptom of high levels of one of the more than a dozen viruses transmitted by *Varroa* (vanEngelsdorp et al., 2008; 2010). *Varroa* mites can spread very easily from host to host and travel between colonies and apiaries throughout an area causing heavy damage to entire beekeeping operations (Bessin 2013).

In this study, we surveyed the presence and number of *Nosema* spp. spores and *Varroa* mite infections in apiaries across the island of Dominica. Camp (2015) conducted a survey of Dominican apiaries on the Western side of the island and discovered that the highest prevalence of *V. destructor* was found on the Northwestern region of the island. She also found that there was no *Nosema* infections in any of the apiaries sampled. We sampled additional apiaries to the ones that were sampled in 2015 to determine if *Nosema* is present in any colonies, and if so at what levels, and if *Varroa* mites have spread further throughout different regions around the island.

MATERIALS AND METHODS

Eight apiary sites were sampled along the Western coast of Dominica (Figure 1). At each site, a bee suit was used for protection from stings while sampling occurred. Both a smoker and hive tools were used to access a frame near the bottom of the hive that held a large quantity of bees. Multiple colonies were sampled per site, if possible.

On site, bees were sampled for *Varroa* mites using the "powder sugar shake" method. To do this, a frame was selected from the brood nest area and after it was confirmed that the queen was not on that frame, adult workers were shaken off the frame and knocked onto a deep plastic container. A half-cup of bees, which is the equivalent to approximately 300 individuals, was placed into a mason jar with wire gauze on the lid (Reuter and Spivak 2016). Two tablespoons of powdered sugar were placed into the jar through the lid and the jar was then rotated for approximately one minute to loosen the mites from the bees, and then mites were shaken into a white tray and counted on site. Approximately 100 bees from each colony were collected in vials

containing 75% ethyl alcohol for further testing in the laboratory at Archbold Tropical Research and Education Center, whereby bees from every colony were counted and checked for further mites not collected by hand on site.

To determine the number of *Nosema* spp. spores per bee, we removed the abdomens from 25 randomly selected bees from a colony using forceps and place them into a mortar with 12.5 mL of water. A pestle was used to grind up the 25 abdomens into a solution. We then loaded a subsample from the mixture and loaded it onto a slide on a Hemocytometer to observe the number of *Nosema* spores found in the sample. We then extrapolate these values to estimate the total number of spores per bee from each sampled colony. Briefly, both sides of a 0.1 mm deep hemocytometer were covered with a glass cover slip and a pipette was used to fill both chambers for two trials per set of abdomens. The microscope was first set to 100X magnification to help find the grid on the hemocytometer and then switched to 450X magnification. Within the grid, five designated squares were checked for *Nosema* spores. To calculate estimated number of *Nosema* spores per bee, we counted the sum of the spores in the five squares and multiplied it by 25,000, as recommended by Reuter and Spivak (2016b).

We analyzed the data by taking the average number of *Varroa* mites per 100 bees counted with the powder sugar shake method in each apiary. We also found the average number of *Varroa* mites present in the subsample of bees that was placed in alcohol from each colony. Finally, we extrapolated the number of *Nosema* spores per bee and averaged the number found per bee from the two chambers analyzed per colony. We plotted these data in scattered line graphs showing average values per apiary +/- the standard error of the mean (SEM) whenever there were more than two colonies per apiary sampled.

RESULTS

The eight different sites sampled along the Western coast of Dominica are shown in Figure 1. The average number of mites per 100 bees collected on site through the powder sugar shake is shown in Figure 2. Apiary 5, which corresponds to the one Colihaut (#1), had the highest incidence of *Varroa* mites compared to the overall eight-site average. In contrast, apiary 2, which corresponds to the one in Portsmouth, had the lowest number of *Varroa* mites per 100 bees collected. Apiaries 3 and 6 did not show any *Varroa* mites on site.

The average number of mites per 100 bees collected by hand in alcohol and tested in the laboratory is shown in Figure 3. Apiary 4, which corresponds to the one in Bornes, had the highest incidence of *Varroa* mites compared to the overall average, while apiary 1, which corresponds to the one in Caneville, had the lowest number of *Varroa* mites per 100 bees collected in the laboratory.

Finally, the average number of *Nosema* spores found per bee in the laboratory is shown in Figure 4. The overall average estimated number of spores per bee across all apiaries was 13,151 spores per bee. Apiary 3 had the highest average value at 29,167 spores per bee, while apiary 4 had the lowest with zero spores per bee.

DISCUSSION

The Dominica / China Agriculture Department in Portsmouth (site 3), Bornes (site 4), and Portsmouth (site 2) were new apiaries sampled this year that were not sampled in 2015. A different apiary at Portsmouth sampled in the previous year was not sampled in 2016.

Varroa mites were found in almost every colony when sampled on site using the powder sugar shake method. The Dominica / China Agriculture Department bees (site 3) and the Portsmouth bees (site 2) were not sampled for mites on site because of either aggression or weather. The feral bee colony at the Botanical Gardens (site 6) was also not tested for *Varroa* on site as it was unfeasible to collect half a cup of bees for sampling. After checking for them in the laboratory, *Varroa* mites were found in every apiary in increased amounts compared to that found by Camp's survey (2015) last year.

The threshold for treatment is 3 *Varroa* mites per 100 bees (Berry 2015). The total average number of mites collected per 100 bees was significantly high for six of the eight apiaries sampled. These apiaries are: Portsmouth (3.9), the Dominica / China Agriculture Department (3.3), Bornes (8.6), Colihaut #1 (6.5), the Roseau Prison (5.6), and Colihaut #2 (5.2). Only Caneville (1.9) and the feral colony at the Botanical Gardens (2.7) were under the threshold. The implications for not treating for *Varroa* are severe as brood that emerge as adults after being parasitized by these mites may be heavily deformed with missing legs or destroyed wings, which is a symptom of high levels of one of the more than a dozen viruses transmitted by *Varroa* (vanEngelsdorp et al., 2008; 2010). While surveying we noticed that some of the bees at certain apiaries were highly aggressive as noted above for Portsmouth. There is the possibility that this aggressive behavior might be a deterrent against *Varroa* mites. The bees rubbing mites off themselves and fighting back against their parasitism might be a strategy that enables these bees in Dominica to withstand higher-than-recommended levels of *Varroa*.

Nosema spores were found in the gut contents of bees from most of the apiaries. Bornes (site 4) was the only site that did not contain any *Nosema,* at least not within the five squares checked. This is an increase from the survey done in 2015, which found no *Nosema* in any of the

apiaries sampled. The *Nosema* levels found in the bees sampled on Dominica are still considered very low compared to the millions of spores per bee that can be found in heavily infected colonies (Mussen 2011).

There are relatively few studies documenting the *Varroa* and *Nosema* levels for Dominica and the Caribbean in general. As these two pests are devastating to the vitality of honey bee colonies, it is important to continue to monitor these levels annually in areas with high quantities of man-made apiaries. It is even more important to monitor in areas where treatment is not readily available, such as Dominica.

ACKNOWLEDGEMENTS

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FIGURES AND TABLES

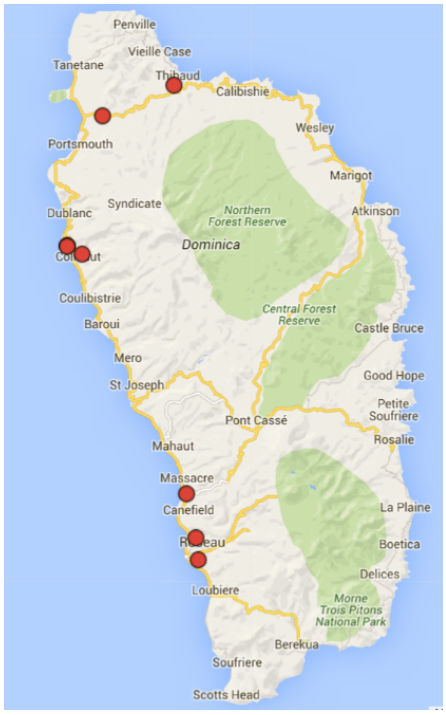

Figure 1. Map of Dominican apiaries sampled for *Nosema* spp. spores and *Varroa destructor* mites. A total of 8 apiaries were sampled across the west coast of the country.

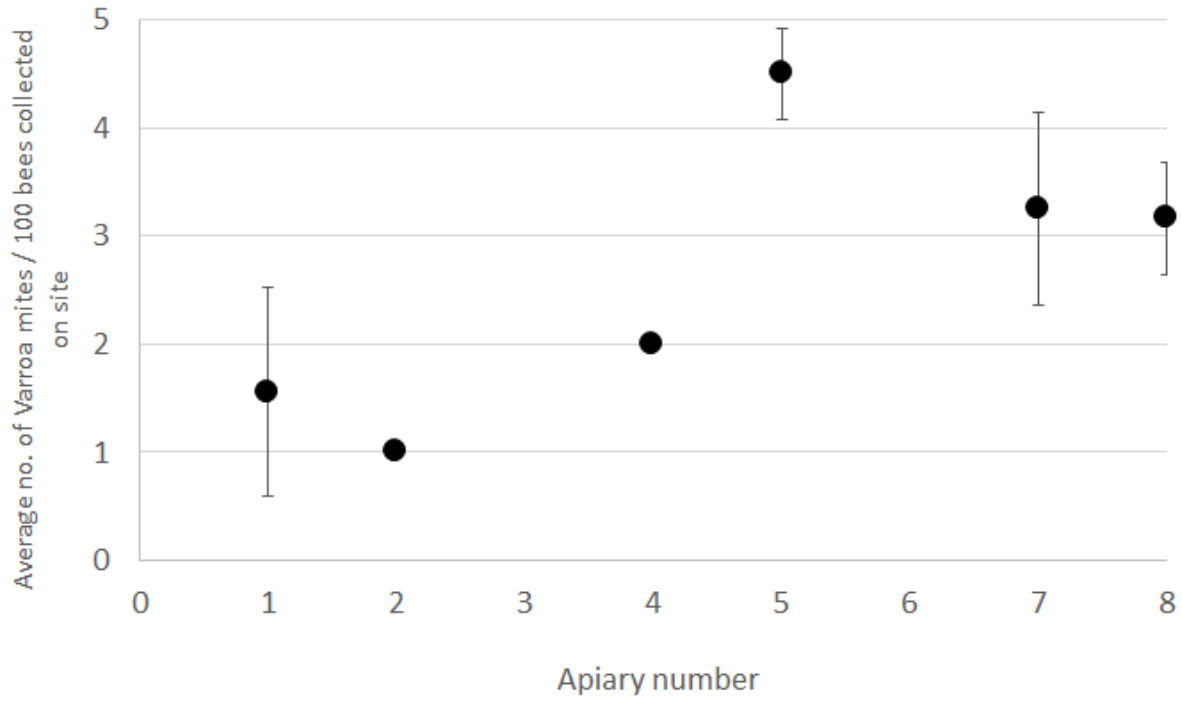

Figure 2. Average number of *Varroa* mites per 100 bees collected using the powder sugar shake method for Varroa estimation.

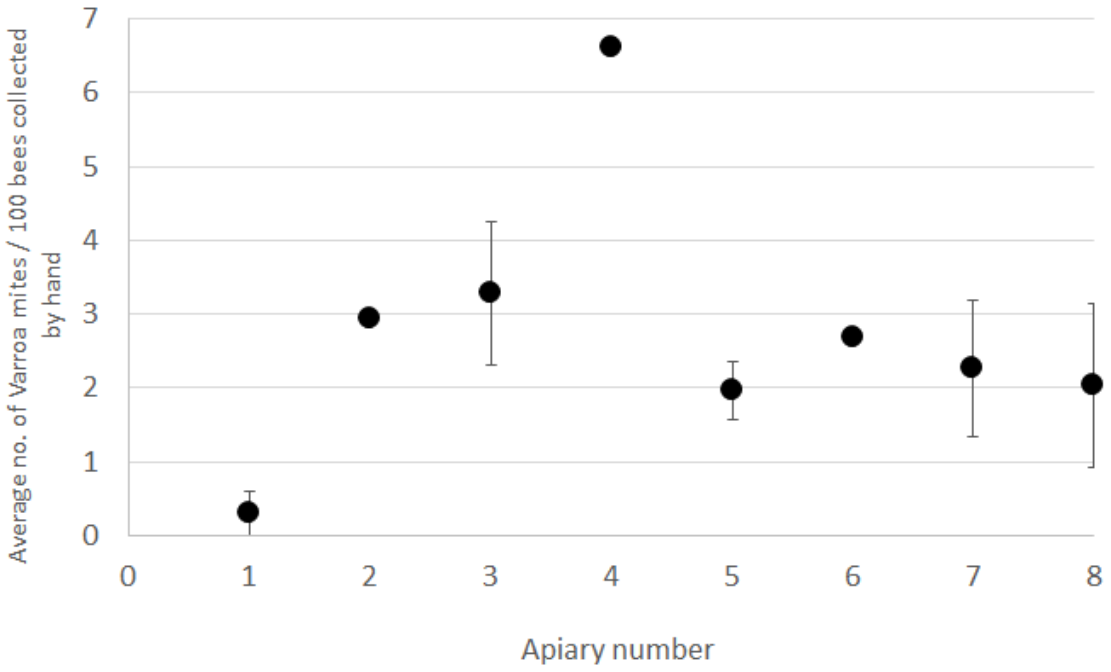

Figure 3. Average number of *Varroa* mites per 100 bees counted by hand in the laboratory.

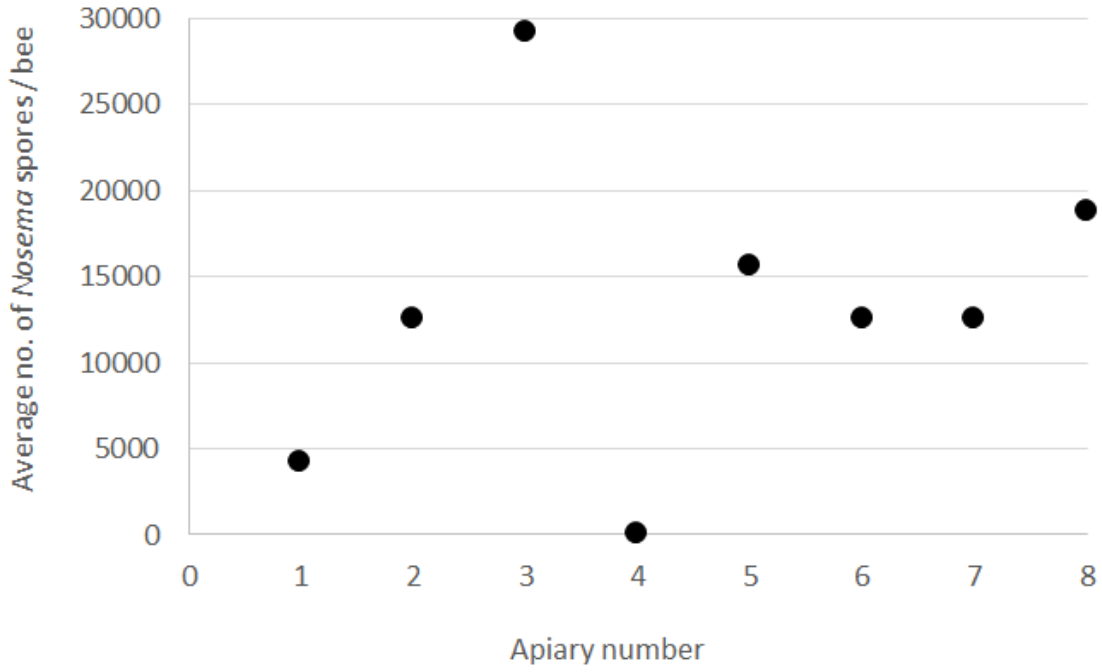

Figure 4. Estimated average number of *Nosema* spp. spores per bee using a hemocytometer. See *Materials and Methods* for more details.

Table 1. Locations of apiaries were honey bee colonies were sampled for *Nosema spp.* spores and *Varroa spp.* mites across Dominica in 2016.

Site #	Sample Date	Location	Beekeeper's name	# Colonies Sampled
1	17-Jun-16	Caneville	Garth Clark	3
2	21-Jun-16	Portsmouth	Mr. Laurance	2
3	21-Jun-16	Dom/China Ag Dept.	Bryan Bertrand	3
4	21-Jun-16	Borne	Bryan Bertrand	1
5	21-Jun-16	Colihaut #1	Mr. Busy	4
6	22-Jun-16	Botanical Gardens	Dr. Reginald Thomas	1
7	22-Jun-16	Roseau Prison	Garth Clark	4
8	22-Jun-16	Colihaut #2	Mr. Busy	4

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