Newfoundland Labrador

Forestry and Agrifoods Agency

2009 Newfoundland and Labrador Honey Bee Disease Survey*

Final Report
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Table of Contents

Abstract

Western honey bees (*Apis mellifera*) occur in nearly every region inhabited by man because they provide valuable honey, wax, and pollination services. Much of the world's commercial honey bee operations are plagued by economically important parasites; however, beekeepers in Newfoundland and Labrador, Canada, are uniquely positioned because of that province's strict importation regulations and geographic isolation. We surveyed ~25% of the province's ~100 managed honey bee colonies. The parasitic mites *V. destructor* and *A. woodii* were not detected in sampled colonies, whereas *Nosema* spp. microsporidia were detected in 2/4 beekeeping operations and in 11/23 colonies (mean ± SD intensity = 482,609 ± 1,199,489; median intensity = 0). Because *V. destructor* and *A. woodii* are economically important pests that typically require chemical treatments, it may be possible for Newfoundland and Labrador beekeepers to adopt organic beekeeping practices that produce products that can be marketed around the world.

Introduction

Recent large-scale die-offs of western honey bees (*Apis mellifera*) around the world are of extreme concern because of humanity's increasing dependence on pollinator-dependent crops (Aizen and Harder 2009). Although honey bee declines have occurred in the past, the magnitude and speed of recent declines are likely unprecedented. Approximately one third of all commercial colonies have died annually since 2006 in the United States (vanEngelsdorp et al. 2009), with similar numbers reported in Canada (CAPA 2009).

Because they are in great demand, western honey bees, along with their parasites, are transported both legally and illegally around the world. Accidental introduction of the parasitic mites *Varroa destructor* and *Acarapis woodii*, and their associated viruses, to Canada in the early 1990s resulted in a ~50% increase in winter colony mortality (CAPA 2009). An additional contributor may also be the recently detected microsporidian *Nosema ceranae* (Fries 2009); however, preliminary studies in Canada suggest that *N. ceranae* can be controlled using the antibiotic Fumagilin-B® and that this parasite is less pathogenic here than it is in other areas of the world (Williams et al. 2008; Williams et al. unpublished).

Despite strict federal and provincial importation regulations, exotic honey bee parasites occur in nearly all areas of Canada where bees are managed. Disease introductions are often the result of illegal human movement of bees across political boundaries, whereas subsequent spread of disease may result from bees encountering disease agents on plants (Higes et al. 2008), or from bees visiting infected colonies' food stores, or from swarming (Fries and Camazine 2001). Currently, five beekeeping operations manage ~100 western honey bee colonies for honey production and pollination in Newfoundland and Labrador (no operations occur in Labrador), which at its closest point is ~15 km offshore mainland Canada. Newfoundland and Labrador has received bee imports three times from *V. destructor*-free areas: in 1997, four honey bee packages from Nova Scotia; in 1998, 6 colonies from Nova Scotia; in 2008, 10 queens from Hawaii. Previous surveys have detected the bacteriae *Paenibacilus larvae* and *Melissococcus plutonius*, responsible for American and European foulbroods, respectively, as well as the fungus *Ascosphaera apis*, responsible for chalkbrood. *V. destructor* and *A. woodii* were not detected during a 2004 survey (Rogers unpublished); however, the microsporidian *Nosema apis* was detected in 2007 (Williams and Rogers unpublished). Infection by *N. apis*, the historical microsporidian parasite of western honey bees, often results in high winter colony mortality or

slow build-up of surviving colonies during spring (Fries 1993). As part of a continuing monitoring program of western honey bee parasites in Newfoundland and Labrador, we surveyed ~25 % of colonies for the mites *V. destructor* and *A. woodii*, and *Nosema* spp. microsporidians.

Methods

Between 27 July 2009 and 25 August 2009, approximately 400 worker honey bees were collected from each broodnest of 23 colonies belonging to 4 beekeeping operations (Table 1, Appendix 3). Samples were immediately stored at -20 °C, except when shipped overnight to Acadia University. For each colony, *V. destructor* intensity (mites per bee) was estimated by counting detached mites on a cotton sheet after collected bees were agitated in a stainless steel mesh strainer for ~3 minutes in a basin lined with a cotton sheet that contained windshield washer fluid (-40 °C formulation) (Currie 2008). *A. woodii* prevalence (% colonies infested) was estimated by examining tracheal tubes of 100 randomly-selected *V. destructor*-washed bees from each colony using a dissecting microscope. Tracheal tubes were exposed by cutting between the head and prothoracic segment, and immediately posterior to the point of wing attachment, and soaking these thoracic disks in 7.5 % potassium hydroxide for 6 hours at 75 °C (modified from Shimanuki and Knox, 2000). *Nosema* intensity (spores per bee) was estimated for each colony by crushing 30 abdomens in 30 ml distilled water and examining created suspensions for spores using a haemocytometer and light microscope (Williams et al., 2008). Molecular analyses were performed on all *Nosema* suspensions using duplex PCR and primers 321APIS-FOR and 321APIS-REV for *N. apis* and 218MITOC-FOR and 218MITOC-REV for *N. ceranae* (Martín-Hernández et al. 2007).

Results and Discussion

V. destructor and *A. woodii* were not detected in sampled colonies; *Nosema* spores were observed in 2/4 beekeeping operations and in 11/23 colonies (Table 1, Appendices 5-8). Overall mean ± SD and median *Nosema* intensity was 482,609 ± 1,199,489 and 0, respectively; only 2 colonies were infected with *Nosema* above the threshold of 1,000,000 spores per bee for suggested Fumagilin-B®-treatment (Table 2). Unfortunately, we failed to amplify targeted 16S rRNA genes used for *Nosema* species identification. This was likely due to poor sample storage conditions. Size and shape of spores observed using light microscopy support unpublished work by Williams and Rogers that only *N. apis* persists in Newfoundland and Labrador; future surveys will confirm this.

Although many challenges are associated with beekeeping on an island exposed to cool short temperate summers, beekeepers on Newfoundland currently have a unique opportunity to manage their bees under relatively parasite-free conditions. *V. destructor* is widely considered to be the single most devastating pest to honey bees in the world because of the physical damage it inflicts and the viruses it vectors (Sammataro et al. 2000; Kevan et al. 2006). Because of this, beekeepers often rely on a number of costly and time-consuming chemical treatments to prevent colony mortality (Sammataro et al. 2000); these chemicals can also contaminate honey, pollen, and wax (e.g. Frazier et al. 2008; vanEngelsdorp et al. 2009; Rogers and Williams unpublished). Without a need for these chemicals, it may be possible for Newfoundland and Labrador beekeepers to adopt organic beekeeping practices that produce products that can be marketed

around the world. Creating and maintaining a sustainable beekeeping industry without a need for imports would be Newfoundland's best option for preventing colonization of *V. destructor*, *A. woodii*, and *N. ceranae*, as well as other damaging pests that threaten the Canadian beekeeping industry, such as the small hive beetle, *Aethina tumida*. To make this possible, strict provincial importation regulations must be upheld (Whitney and Jennings 2005). Moreover, continual and dynamic monitoring must be enforced to prevent future introductions of exotic pests, as well as to maintain current pests at acceptable levels.

Table 1. Summary of sampling regime and western honey bee (*Apis mellifera*) parasites surveyed for in 23 colonies from 4 beekeeping operations and 9 bee yards during summer 2009 on Newfoundland. *Varroa destructor* and *Nosema* spp. intensities = number parasites / bee; *Acarapis woodii* and *Nosema* spp. prevalences = % colonies infected.

Operation #	Apiary #	Collection Date	n	*V. destructor* intensity	*A. woodii* prevalence	*Nosema* spp. intensity		*Nosema* spp. prevalance
						Mean	SD	
1	1	27 July	4	0	0	25,000	28,868	50
	2	27 July	4	0	0	0	0	0
	3	3 August	5	0	0	1,920,000	2,123,558	100
2	1	29 July	1	0	0	450,000	n/a	100
	2	29 July	2	0	0	225,000	318,198	50
	3	29 July	1	0	0	0	n/a	0
	4	29 July	2	0	0	250,000	70,711	100
3	1	25 August	3	0	0	0	0	0
4	1	9 August	1	0	0	0	n/a	0

Table 2. Individual western honey bee (*Apis mellifera*) colony survey results from 4 beekeeping operations and 9 bee yards for the parasitic mites *Varroa destructor* and *Acarapis woodii*, and the microsporidia *Nosema* spp. *V. destructor* and *Nosema* spp. intensities = number parasites / bee / colony; *A. woodii* prevalence = % bees infested / colony. Each row represents one colony.

Operation	Apiary	*V. destructor* intensity	*A. woodii* prevalence	*Nosema* spp. intensity
1	1	0	0	50,000
		0	0	50,000
		0	0	0
		0	0	0
	2	0	0	0
		0	0	0
		0	0	0
		0	0	0
	3	0	0	5,550,000
		0	0	450,000
		0	0	600,000
		0	0	950,000
		0	0	2,050,000
2	1	0	0	450,000
	2	0	0	450,000
		0	0	0
	3	0	0	0
	4	0	0	200,000
		0	0	300,000
3	1	0	0	0
		0	0	0
		0	0	0
4	1	0	0	0

Acknowledgements

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Appendix 1. Summer 2009 Newfoundland Honey Bee Disease Survey Protocols

Summer 2009 Newfoundland Honey Bee Disease Survey Protocols

The objective of this survey is to quantify *Varroa* mites, tracheal mites, *Nosema apis*, and *Nosema ceranae* in approximately 40% of Newfoundland's honey bee colonies.

Following this collection protocol and providing accurate colony management information will ensure data collected are consistent among colonies and beekeepers, and allow for a better interpretation of results.

In addition to normal beekeeping tools/clothing, large (~ 25 x 25 cm) Ziploc bags, a black permanent marker, and a cooler with ice or ice packs will be required during sampling.

Worker bee collection from the brood nest

1. Randomly select **x** number of colonies to be sampled from operation/yard. Using a permanent black marker, label each colony with your initials and hive number. For example, three colonies belonging to John Doe would be marked JD01, JD02, and JD03.

For each colony sampled:

2. Label a Ziploc bag with the hive number and collection date.
3. Search through the colony for a frame that contains 25-50% open brood and 25-50% capped brood, and is covered by more than 50% bees. In other words, a frame that has lots of bees and is mostly filled with all stages of brood. If bees cannot be collected from this type of frame, please note this on the "Collection Log" form, which is included.
4. Scan both sides of the frame for a queen. If she is present, return the frame to the hive and select a new frame, or carefully move her from the frame to another frame in the hive.
5. Rotate the frame 90° and tilt the upper edge forward approximately 45°. Collect approximately 300 bees (about 2 cups worth) in the Ziploc bag by scraping the edge of the bag's opening in short bursts along the entire length of the frame, or until enough bees are collected; flip the frame and repeat if not enough bees can be collected from one side of the frame.
6. Reassemble the hive.
7. Immediately place the Ziploc bag containing bees in a cooler with ice or ice packs, and place into a freezer as soon as possible.
8. For each colony bees are collected from, record sampling date, hive number, and hive location on "Collection Log" form.

Management info

Please provide as much detail as possible regarding management, including treatments, for each colony bees are sampled from, beginning August 2008 and ending the date bees are collected. Included are two "NF Survey Hive Management Log forms." One form contains fictitious information for two colonies owned by a beekeeper named John Doe. The second form is blank and can be used to provide your management information.

For more information, please contact the following:

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Appendix 2. 2009 NF Survey Hive Management Log

2009 NF Survey Hive Management Log:

Page:___ of ___

Record of hive treatments

Beekeeper:_____ Beekeeper initials: _____

Date	Hive Number	Treatment	Other (e.g. fall *Varroa* counts, splits removed)
Notes:			

Appendix 3 - 2009 NF Survey Collection Log

2009 NF Survey Collection Log Page:___ of ___

Beekeeper:_____ Beekeeper initials: _____

Hive Number	Collection Date	Collection Location	Other (e.g. location of sampling within the hive)
Notes:			

Appendix 4 - Results – Collection Log

Results - Collection Log			
	Beekeeper	Collection date	Hive number
1	1	27-Jul-09	87
2	1	27-Jul-09	139
3	1	27-Jul-09	48
4	1	27-Jul-09	122
5	1	27-Jul-09	141
6	1	27-Jul-09	54
7	1	27-Jul-09	11
8	1	27-Jul-09	95
9	2	29-Jul-09	2
10	2	29-Jul-09	11
11	2	29-Jul-09	18
12	2	29-Jul-09	16
13	2	29-Jul-09	22
14	2	29-Jul-09	25
15	1	03-Aug-09	72
16	1	03-Aug-09	100
17	1	03-Aug-09	115
18	1	03-Aug-09	131
19	1	03-Aug-09	145
20	3	25-Aug-09	1
21	3	25-Aug-09	2
22	3	25-Aug-09	3
23	4	09-Aug-09	1

Appendix 5 - Results – Varroa

Results - Varroa					
	Beekeeper	Hive number	Bees	Mites	Mites per 100 bees
1	1	87	402	0	0
2	1	139	524	0	0
3	1	48	516	0	0
4	1	122	340	0	0
5	1	141	445	0	0
6	1	54	404	0	0
7	1	11	580	0	0
8	1	95	810	0	0
9	2	2	402	0	0
10	2	11	389	0	0
11	2	18	368	0	0
12	2	16	390	0	0
13	2	22	338	0	0
14	2	25	362	0	0
15	1	72	501	0	0
16	1	100	454	0	0
17	1	115	611	0	0
18	1	131	506	0	0
19	1	145	405	0	0
20	3	1	235	0	0
21	3	2	283	0	0
22	3	3	296	0	0
23	4	1	489	0	0

Appendix 6 - Results – Nosema

Results - Nosema									
	Beekeeper	Hive number	Block 1	Block 2	Block 3	Block 4	Block 5	Total	Mean spores per bee
1	1	87	0	0	0	0	1	1	50,000
2	1	139	0	0	1	0	0	1	50,000
3	1	48	0	0	0	0	0	0	0
4	1	122	0	0	0	0	0	0	0
5	1	141	0	0	0	0	0	0	0
6	1	54	0	0	0	0	0	0	0
7	1	11	0	0	0	0	0	0	0
8	1	95	0	0	0	0	0	0	0
9	2	2	2	1	4	1	1	9	450,000
10	2	11	4	2	2	1	0	9	450,000
11	2	18	0	0	0	0	0	0	0
12	2	16	0	0	0	0	0	0	0
13	2	22	0	0	2	0	2	4	200,000
14	2	25	1	1	1	1	2	6	300,000
15	1	72	25	17	23	25	21	111	5,550,000
16	1	100	5	2	0	2	0	9	450,000
17	1	115	4	2	2	3	1	12	600,000
18	1	131	6	4	4	3	2	19	950,000
19	1	145	6	12	9	9	5	41	2,050,000
20	3	1	0	0	0	0	0	0	0
21	3	2	0	0	0	0	0	0	0
22	3	3	0	0	0	0	0	0	0
23	4	1	0	0	0	0	0	0	0

Appendix 7 - Results – Tracheal Mites

Results - Tracheal Mites						
	Beekeeper	Date Col	Date Ch	No Ch	Pos	% Pos
1	1	27-Jul-09	22-Oct-09	100	0	0.00%
2	1	27-Jul-09	22-Oct-09	100	0	0.00%
3	1	27-Jul-09	22-Oct-09	100	0	0.00%
4	1	27-Jul-09	22-Oct-09	100	0	0.00%
5	1	27-Jul-09	22-Oct-09	100	0	0.00%
6	1	27-Jul-09	22-Oct-09	100	0	0.00%
7	1	27-Jul-09	22-Oct-09	100	0	0.00%
8	1	27-Jul-09	23-Oct-09	100	0	0.00%
9	2	28-Jul-09	23-Oct-09	100	0	0.00%
10	2	28-Jul-09	23-Oct-09	100	0	0.00%
11	2	28-Jul-09	23-Oct-09	100	0	0.00%
12	2	28-Jul-09	23-Oct-09	100	0	0.00%
13	2	28-Jul-09	23-Oct-09	100	0	0.00%
14	2	28-Jul-09	23-Oct-09	100	0	0.00%
15	1	03-Aug-09	26-Oct-09	100	0	0.00%
16	1	03-Aug-09	26-Oct-09	100	0	0.00%
17	1	03-Aug-09	26-Oct-09	100	0	0.00%
18	1	03-Aug-09	26-Oct-09	100	0	0.00%
19	1	03-Aug-09	26-Oct-09	100	0	0.00%
20	3	25-Aug-09	26-Oct-09	100	0	0.00%
21	3	25-Aug-09	26-Oct-09	100	0	0.00%
22	3	25-Aug-09	26-Oct-09	100	0	0.00%
23	4	09-Aug-09	26-Oct-09	100	0	0.00%

Appendix 8 - Results – Summary

Results - Summary					
	Beekeeper	Hive number	Varroa per 100 bees	Mean Nosema intensity	Tracheal mite prevalence
1	1	87	0	50,000	0
2	1	139	0	50,000	0
3	1	48	0	0	0
4	1	122	0	0	0
5	1	141	0	0	0
6	1	54	0	0	0
7	1	11	0	0	0
8	1	95	0	0	0
9	2	2	0	450,000	0
10	2	11	0	450,000	0
11	2	18	0	0	0
12	2	16	0	0	0
13	2	22	0	200,000	0
14	2	25	0	300,000	0
15	1	72	0	5,550,000	0
16	1	100	0	450,000	0
17	1	115	0	600,000	0
18	1	131	0	950,000	0
19	1	145	0	2,050,000	0
20	3	1	0	0	0
21	3	2	0	0	0
22	3	3	0	0	0
23	4	1	0	0	0