Parasitic mites and microsporidians in managed western honey bee colonies on the island of Newfoundland, Canada

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Abstract—Western honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), occur in nearly every region inhabited by man because they provide valuable honey, wax, and pollination services. Many commercial honey bee operations are plagued by economically important parasites; however, beekeepers on the island of Newfoundland, Canada, are in a unique position because of the province of Newfoundland and Labrador's strict import regulations and geographic isolation. We surveyed about 25% of the island's approximately 100 managed honey bee colonies. The parasitic mites *Varroa destructor* Anderson and Trueman (Acari: Varroidae) and *Acarapis woodi* (Rennie) (Acari: Tarsonemidae) were not detected, whereas *Nosema* spp. microsporidia were detected in two of four beekeeping operations and in 11 of 23 (48%) colonies (intensity = 482 609 ± 1 199 489 (mean ± SD); median intensity = 0). Because *V. destructor* and *A. woodi* are important pests that typically require chemical treatments, beekeepers on the island of Newfoundland may be uniquely positioned to market organic honey bee products from colonies that could also be a source of mite-naïve bees for research.

Résumé—L'abeille domestique occidentale, *Apis mellifera* L. (Hymnenoptera : Apidae), se retrouve dans pratiquement toutes les régions habités par les humains parce qu'elle fournit du miel, de la cire et des services de pollinisation précieux. Plusieurs entreprises commerciales d'apiculture sont gênées par la présence de parasites d'importance économique; cependant, les apiculteurs de l'île de Terre-Neuve sont en position enviable à cause des règlements stricts d'importation de la Province de Terre-Neuve et du Labrador et de l'isolement géographique. Nous avons inventorié environ 25 % de la centaine de colonies d'abeilles élevées commercialement. Aucun acarien parasite *Varroa destructor* Anderson et Trueman (Acari: Varroidae) ni *Acarapis woodi* (Rennie) (Acari: Tarsonemidae) n'a été décelé; alors que les microsporidies *Nosema* spp. ont été trouvées dans 2 de 4 apicultures et 11 de 23 (48 %) colonies (intensité moyenne ± ET = 482 609 ± 1 199 489; intensité médiane = 0), Parce que *V. destructor* et *A. woodi* sont des ravageurs importants qui nécessitent généralement des traitements chimiques, les apiculteurs de l'île de Terre-Neuve sont en position unique pour mettre sur le marché des produits de l'abeille organiques provenant de colonies qui pourraient aussi être une source d'abeilles jamais exposées aux acariens pour la recherche scientifique.

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Recent large-scale die-offs of western honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), around the world are of serious concern because of humanity's increasing dependence on

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insect-pollinated 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 died annually from 2006 to 2009 in Canada and the United States of America (Canadian Association of Professional Apiculturists (CAPA) 2008; vanEngelsdorp *et al.* 2009*b*).

Because they are in great demand, western honey bees, along with their parasites, are transported (legally and illegally) around the world. Accidental introduction of the parasitic mites *Varroa destructor* Anderson and Trueman (Acari: Varroidae) and *Acarapis woodi* (Rennie) (Acari: Tarsonemidae), and their associated viruses, to Canada in the early 1990s resulted in an increase of approximately 50% in colony overwintering mortality (CAPA 2008), with *V. destructor* suspected to be the main contributor to recent losses (Currie *et al.* 2010; Guzmán-Novoa *et al.* 2010). An additional contributor may also be the microsporidian *Nosema ceranae* Fries *et al.* (Fries 2010), which was first confirmed in Canada in 2007 (Williams *et al.* 2008*b*) but is believed to have been present since at least 1994 (Currie *et al.* 2010). Preliminary studies suggest that *N. ceranae* can be controlled using the antibiotic Fumagilin-B® and that this parasite is less pathogenic in Canada than in other areas of the world (Williams *et al.* 2008*a*, 2010*a*, 2010*b*).

Despite federal and provincial import regulations, exotic honey bee parasites occur in nearly all areas of Canada where honey 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 swarming (Fries and Camazine 2001) or from bees encountering disease agents on plants (Higes *et al.* 2008) or in neighbouring colonies' food stores (Fries and Camazine 2001). In the province of Newfoundland and Labrador, five beekeeping operations currently manage approximately 100 western honey bee colonies for honey production and pollination, although none of the operations are in Labrador, the mainland portion of the province. The island of Newfoundland, which at its closest point is about 15 km from the coast of Labrador, has received bee imports three times from *V. destructor*-free areas: 4 honey bee packages from Nova Scotia in 1997; 6 colonies from Nova Scotia in 1998; and 10 queens from Hawai′i in 2008. In previous informal surveys (R.E.L. Rogers, unpublished data), the American and European foulbrood bacteria *Paenibacillus larvae* (White) and *Melissococcus plutonius* (White), respectively, as well as the chalkbrood fungus, *Ascosphaera apis* (Maasen *ex* Claussen), were detected, but not *V. destructor* or *A. woodi*. In fall 2007, *Nosema apis* Zander, the historical microsporidian parasite of western honey bees, was detected in 14 of 23 (61%) colonies (intensity = 1 589 130 ± 2 054 870 (mean ± SD); median intensity = 700 000) using molecular and microscopic techniques (G.R. Williams and R.E.L. Rogers, unpublished data). Infection by *N. apis* often results in high colony mortality in winter or a slow buildup of surviving colonies during spring (Fries 1993), but how *N. apis* affects colonies in Newfoundland is not known. As part of a continuing program of monitoring western honey bee parasites in Newfoundland, we surveyed approximately 25% of colonies for *V. destructor*, *A. woodi*, and *Nosema* spp.

Between 27 July and 25 August 2009, approximately 400 worker honey bees were collected from the brood nest of each of 23 colonies belonging to four beekeeping operations (Table 1). Samples were immediately stored at −20 °C, except when shipped overnight to Acadia University (Wolfville, Nova Scotia). For each colony, *V. destructor* intensity (number of 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 approximately 3 min in a basin lined with a cotton sheet containing windshield-washer fluid (−40 °C formulation) (Currie 2008). *Acarapis woodi* prevalence (percentage of colonies infested) was estimated by examining under a dissecting microscope the tracheal tubes of 100 bees washed free of *V. destructor* that were randomly selected from each colony. The tracheal tubes were exposed by cutting out the prothoracic segment from each bee and soaking the segments in 7.5% potassium hydroxide for 6 h at 75 °C (modified from Shimanuki and Knox 2000). *Nosema* spp. intensity (number of spores per bee) was estimated for each colony by crushing 30 abdomens in 30 mL

Table 1. Summary of the sampling regime and intensity and (or) prevalence of surveyed western honey bee (*Apis mellifera*) parasites in surveys of 23 colonies from four beekeeping operations and nine bee yards during summer 2009 on the island of Newfoundland.

Operation no.	Apiary no.	Collection date	*n*	*Varroa destructor* intensity*	*Acarapis woodi* prevalence†	*Nosema* spp. intensity*		*Nosema* spp. prevalence†
						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	na	100
	2	29 July	2	0	0	225 000	318 198	50
	3	29 July	1	0	0	0	na	0
	4	29 July	2	0	0	225 000	70 711	100
3	1	25 August	3	0	0	0	0	0
4	1	9 August	1	0	0	0	na	0

*Number of parasites per bee.
†Percentage of colonies infested.

distilled water and examining the created suspensions for spores using a haemocytometer and light microscope (Cantwell 1970; Rogers and Williams 2007).

Varroa destructor and *A. woodi* were not detected; *Nosema* spp. spores were observed in two of four beekeeping operations and in 11 of 23 (48%) colonies (Table 1). Overall mean $\pm$ SD and median *Nosema* spp. intensity were 482 609 $\pm$ 1 199 489 and 0, respectively; only 2 colonies were infected with *Nosema* spp. above the threshold of 1 000 000 spores per bee suggested for Fumagilin-B® treatment (Table 2), in contrast to 11 colonies that were above this threshold in 2007. This may have been due to the time of sampling (Fries 2010), to previous Fumagilin-B® treatment (Williams 2008*a*, 2010*a*), or to the particular operations sampled. The size and shape of spores observed using light microscopy support our previous finding (G.R. Williams, K. Head, R.E.L. Rogers, and D. Shutler, unpublished data) that only *N. apis* persists on the island of Newfoundland; to confirm this, surveys using molecular techniques are planned.

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. *Varroa destructor* is widely considered to be the single most devastating pest affecting 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; Currie *et al.* 2010; Guzmán-Novoa *et al.* 2010). Because of this, beekeepers often rely on a number of costly and time-consuming chemical treatments to prevent colony mortality (Sammataro *et al.* 2000); however, these chemicals can contaminate honey, pollen, and wax (Frazier *et al.* 2008; vanEngelsdorp *et al.* 2009*a*; Mullin *et al.* 2010). Without the need to control *V. destructor*, it may be possible for Newfoundland beekeepers to provide mite-free bees for research, as well as to adopt organic beekeeping practices (using antibiotics to control *Nosema* spp. (Williams 2008*a*, 2010*a*) and *P. larvae* (Genersch 2010) sparingly, if at all). Creating and maintaining a sustainable beekeeping industry without the need for imports would be Newfoundland's best option for preventing colonization by *V. destructor*, *A. woodi*, and possibly *N. ceranae*, as well as other damaging pests that threaten the Canadian beekeeping industry, such as the small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae). To make this possible, strict provincial importation regulations must be enforced (Whitney and Jennings 2005). Moreover, continual and dynamic monitoring must be practised to prevent future introductions of exotic pests, as well as to maintain current pests at acceptable levels.

Table 2. Results of surveys for *Varroa destructor*, *Acarapis woodi*, and *Nosema* spp. in individual western honey bee (*Apis mellifera*) colonies from four beekeeping operations and nine bee yards on the island of Newfoundland. Each row represents one colony.

Operation No.	Apiary no.	*V. destructor* intensity*	*A. woodi* 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
		0	0	0
		0	0	0
		0	0	0
4	1	0	0	0

*Number of parasites per bee.
†Percentage of bees infested per colony.

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