Observations of native bumble bees inside of commercial colonies of *Bombus impatiens* (Hymenoptera: Apidae) and the potential for pathogen spillover

B.J. Hicks,[1] B.L. Pilgrim, E. Perry, H.D. Marshall

Abstract—Many fruit producers use commercial colonies of *Bombus impatiens* Cresson (Hymenoptera: Apidae) to supplement crop pollination by native bees. A small number of Newfoundland (Newfoundland and Labrador, Canada) farmers forego purchasing new colonies and, instead, purchase previously used colonies from crops in other provinces. This practice has potentially dangerous implications that may adversely affect future native bee diversity in Newfoundland. This study is the first to record the presence of native bumble bee species inside the colonies of new and pre-used commercial *B. impatiens* and the first to look at diseases in native bumble bees from Newfoundland. Polymerase chain reaction and taxon-specific oligonucleotides were used to screen the commercial and native bumble bee species for pathogens. *Crithidia bombi* (Lipa and Triggiani), *Apicystis bombi* (Liu, Macfarlane, and Pengelly), *Nosema bombi* Fantham and Porter, *Nosema ceranae* Fries *et al.*, and species of *Ascosphaera* Olive and Spiltoir, were detected in native bumble bees that were collected from inside the new and pre-used commercial *B. impatiens*. *Crithidia bombi*, *A. bombi*, and *N. bombi* were also detected among native bees that were collected away from the commercial colonies. *Nosema apis* (Zander) and *Melissococcus plutonius* (White) were not detected in any of the bees tested. The mixing of native bumble bees in *B. impatiens* colonies increases the potential for pathogen spillover and spillback that may threaten the small and vulnerable island bee fauna.

Résumé—De nombreux producteurs de fruits utilisent les colonies commerciales de *Bombus impatiens* Cresson (Hymenoptera: Apidae) pour compléter la pollinisation des cultures par les abeilles indigènes. Un petit nombre d'agriculteurs de Terre-Neuve (Terre-Neuve-et-Labrador, Canada) renoncent à l'achat de nouvelles colonies et, au lieu de cela, achètent des colonies déjà utilisées dans les cultures d'autres provinces. Cette pratique a des implications potentiellement dangereuses qui pourraient nuire à la diversité future des abeilles indigènes à Terre-Neuve. Cette étude est la première à signaler la présence d'espèces de bourdons indigènes à l'intérieur des colonies de *B. impatiens* commerciales nouvelles et pré-utilisées et la première à examiner les maladies chez les bourdons indigènes de Terre-Neuve. La réaction en chaîne par polymérase et les oligonucléotides spécifiques du taxon ont été utilisés pour cribler les espèces commerciales de bourdons indigènes et les agents pathogènes. *Crithidia bombi* (Lipa et Triggiani), *Apicystis bombi* (Liu, Macfarlane et Pengelly), *Nosema bombi* Fantham et Porter, *Nosema ceranae* Fries *et al.*, et les espèces d'*Ascosphaera* Olive et Spiltoir, ont été détectés chez des bourdons indigènes qui ont été recueillis à l'intérieur du *B. impatiens* commercial nouveau et pré-utilisé. *Crithidia bombi*, *A. bombi* et *N. bombi* ont également été détectés parmi les abeilles indigènes qui ont été recueillies loin des colonies commerciales. *Nosema apis* (Zander) et *Melissococcus plutonius* (White) n'ont été détectés chez aucune des abeilles testées. Le mélange de bourdons indigènes dans les colonies de *B. impatiens* augmente le risque de débordements et de retombées pathogènes qui pourraient menacer une petite population vulnérable d'abeilles insulaires.

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Introduction

Pollinating insects are inarguably important for humans (see Potts *et al*. 2016). Bees (Hymenoptera: Apidae), in general, are considered the most important pollinating insects (Klein *et al*. 2007) and the abundance of wild bees, specifically, is more correlated to crop yields than the abundance of honey bees (Breeze *et al*. 2011; Garibaldi *et al*. 2013; Mallinger and Gratton 2015). However, the abundance and diversity of wild pollinators has decreased worldwide due to a number of factors (Biesmeijer *et al*. 2006; Colla and Packer 2008; Grixti *et al*. 2009; Committee on the Status of Endangered Wildlife in Canada 2010; Potts *et al*. 2010; Bommarco *et al*. 2012; Szabo *et al.* 2012; Burkle *et al*. 2013; Carvalheiro *et al*. 2013; Committee on the Status of Endangered Wildlife in Canada 2014a, 2014b; Committee on the Status of Endangered Wildlife in Canada 2015; Goulson and Hughes 2015; Koh *et al*. 2016), resulting in the ongoing search for and development of additional commercially managed pollinators. The global transportation of bees for commercial pollination has been, and continues to be a mechanism contributing to widespread introduction and establishment of non-native species and bee diseases. These diseases threaten native bee diversity and the vital ecosystem service that these pollinators provide to crops and wildflowers worldwide (Goulson and Hughes 2015; Potts *et al*. 2016).

The unique climate and isolation from mainland areas experienced by the island portion of the Canadian province of Newfoundland and Labrador have resulted in a bee fauna that is much less diverse than other areas of North America (Sheffield *et al*. 2017). Some growers in Newfoundland import commercially available colonies of the non-native bumble bee *Bombus impatiens* Cresson (Hymenoptera: Apidae) to supplement pollination of their crops, even though native Newfoundland bee species provide important pollination services to small fruit producers (Hicks 2011). Hicks and Sircom (2016) determined that such importations do not necessarily increase pollination on Newfoundland cranberry farms and this practice should be reconsidered. Furthermore, as some crops flower significantly later on the island than on the mainland of eastern Canada, some farmers purchase pre-used commercial *B. impatiens* colonies from different Maritime Provinces to supplement pollination, a practice that is not known from other areas.

It has been documented that commercially supplied bumble bees carry diseases that are transmitted to native bumble bees (Niwa *et al*. 2004; Otterstatter and Thomson 2008; Arbetman *et al*. 2013; Murray *et al*. 2013; Graystock *et al*. 2013b; Graystock *et al*. 2015). In North America, *B. impatiens* is commercially supplied and is also known to transmit several diseases to native bumble bees (Colla *et al*. 2006; Sachman-Ruiz *et al.* 2015; Cameron *et al*. 2016). The main mechanism of transmission is contact with infected nest mates, nest material, or flowers (Schmid-Hempel and Tognazzo 2010; Graystock *et al*. 2013a, 2015).

Bees that enter non-natal colonies are known as drifters (Free 1958). The term “drifting” is believed to be attributed to honey robbing or the result of orientation errors (Free 1958; Jay 1966; Pfeiffer and Crailsheim 1998; Neumann *et al*. 2000). Supersedure is different than drifting; here, one queen enters a nest and, if successful, will kill the resident queen and take over (Alford 1975). Drifting in bumble bees is likely a temporary phenomenon where foraging worker bees enter non-natal nests to rob honey (Alford 1975; Genersch *et al*. 2006), or in some cases to lay eggs (Lopez-Vaamonde *et al*. 2004; O’Connor *et al*. 2013). Where commercially reared bumble bees are used in agricultural settings and greenhouses, intraspecific drifting commonly occurs (Birmingham and Winston 2004; Birmingham *et al*. 2004; Lefebvre and Pierre 2007). In addition, Hobbs (1966, 1967) observed both intraspecific and interspecific supersedure of queen bumble bees using artificial domiciles placed in natural habitats. The only evidence of intraspecific drifting in natural colonies comes from Takahashi *et al.* (2010) where they used molecular analysis to show non-natal males of *Bombus deuteronymus* Schulz reared by unrelated workers.

Here we follow up on questions we had following our previous study (Hicks and Sircom 2016) where *B. impatiens* colonies were initially purchased to look at the efficiency of these bees as pollinators in Newfoundland cranberry farms. As Hicks and Sircom (2016) determined that commercial colonies are likely not needed in Newfoundland, their continued importation

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(either new or pre-used) puts the native bumble bee species of the island at an undue risk. The questions we address include: (1) Will bumble bees in Newfoundland enter commercial *Bombus* Latreille colonies? If so, (2) what is the pathogen profile of selected bee diseases in the introduced bees and native bees that may enter and/or leave these colonies? (3) What is the significance of potential pathogen spillover to Newfoundland native bees?

Methods

To help answer the questions, we examined commercial *B. impatiens* colonies for the presence of native bumble bee species after the colonies were removed from the field. In addition, we used the polymerase chain reaction with taxon-specific oligonucleotides to screen native and commercial bees for pathogens and address the potential of pathogen spillover from commercial bumble bee colonies to the native bees of Newfoundland. As per Graystock *et al.* (2013b), bees were tested for seven pathogens: *Crithidia bombi* (Lipa and Triggiani) (Kinetoplastea: Trypanosomatidae), *Apicystis bombi* (Liu, Macfarlane, and Pengelly) (Neogregarinorida: Lipotrophidae), *Nosema bombi* Fantham and Porter (Dissociodihaplophasida: Nosematidae), *Nosema apis* (Zander) (Dissociodihaplophasida: Nosematidae), *Nosema ceranae* Fries *et al.* (Dissociodihaplophasida: Nosematidae), *Melissococcus plutonius* (White) (Lactobacillales: Enterococcaceae), and *Ascosphaera* Olive and Spiltoir (Ascomycota: Onygenales: Ascosphaeraceae) species.

Bee sampling

On 15 July 2013, six quads (*i.e.*, each quad is four externally connected colonies, each with a queen and its own foragers) of *B. impatiens* that were previously used for lowbush blueberry pollination in New Brunswick, Canada (hereafter, called "pre-used quads") were obtained privately by a local cranberry farmer. An additional four quads (hereafter, called "new quads") of *B. impatiens* were purchased new on 31 July 2013 from Biobest Canada (Leamington, Ontario, Canada) and placed on the same cranberry (*Vaccinium* Linnaeus; Ericaceae) field. The cranberry farm was located near Stephenville, Newfoundland and Labrador (48°27'13"N, 58°24'25"W). At the end of the pollination season (14 August 2013) the new quads and one pre-used quad were taken off the field and frozen, their contents later examined. All specimens of *B. impatiens* located inside the colonies of the quads were collected and frozen (−20 °C) and specimens of native species found inside the colonies were frozen separately. To determine the disease presence in free-living native bumble bees, we sampled bees that were pan-trapped on the field where the commercial bees were located, and from one other area away from the field. That area had bees pan-trapped from a commercial cranberry farm located 17 km northwest of the study field (48°34'17"N, 58°31'27"W) for a different study (but during the same time). These native specimens had been pinned and air dried for the other study (Hicks and Sircom 2016). Identification of native bumble bee species were done using the key of Laverty and Harder (1988). Voucher specimens from this study were deposited in the general collection of the College of the North Atlantic, Carbonear, Newfoundland and Labrador, Canada.

Molecular analysis

Table 1 summarises the 440 bee specimens that were used in the molecular analysis. DNA was extracted from individual bees with the exception of the *B. impatiens* specimens sampled at the end of the pollination season from the new and pre-used quads for which five to six specimens were pooled before extraction. DNA was extracted from frozen or dried bee abdomens, using the Qiagen DNeasy blood and tissue kit (Qiagen, Toronto, Ontario, Canada) following the tissue protocol. Abdomens were minced before extraction, and lysed overnight. Due to the increased amount of starting material for the pooled *B. impatiens*, specimens were lysed in twice the volume of buffer ATL and proteinase K, mixed with twice the volume of 95% ethanol and buffer AL, and added to the spin column in two separate volumes. DNA was eluted with two consecutive 75 μL volumes of AE buffer.

DNA extractions were tested for seven pathogens by taxon-specific polymerase chain reactions, as detailed in Graystock *et al.* (2013b). The Apidae 18S rRNA gene was also amplified from each DNA extraction to confirm that the quality of DNA was sufficient for polymerase chain reaction. Reactions contained 1× Qiagen Type-it Master Mix, 0.2–0.4 μM each forward and

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Table 1. The number of specimens (*n*) used in in the molecular analysis with species identification, stage, provenance, and sample date.

Species	Stage	Provenance	Date sampled	*n*
B. impatiens	Worker	New	End of season	247
	Worker	Pre-used	End of season	84
B. ternarius	Worker	Inside new	End of season	14
	Worker	Inside pre-used	End of season	10
	Worker	Away from field	Throughout season	15
	Worker	On field	Throughout season	15
B. terricola	Worker	Inside new	End of season	4
	Worker	Inside pre-used	End of season	1
	Worker	Away from field	Throughout season	3
	Worker	On field	Throughout season	9
B. vagans bolsteri	Worker	Inside new	End of season	2
	Worker	Inside pre-used	End of season	2
	Worker	Away from field	Throughout season	20
	Worker	On field	Throughout season	6
B. frigidus	Worker	Inside new	End of season	3
	Worker	Inside pre-used	End of season	5

Note: The *Bombus impatiens* numbers were pooled in groups of either five or six specimens for the analysis.

reverse primer, and 3 μL of DNA. Polymerase chain reaction profiles consisted of an initial denaturation at 95 °C for five minutes, 35 cycles at 95 °C for 30 seconds, annealing temperature for 45 seconds, and 72 °C for 30–45 seconds, with a final elongation at 72 °C for 10 minutes (see Table 2 for pathogen-specific profile parameters). Polymerase chain reaction products were stained with EZ-vision three DNA dye (Amresco LLC, Cleveland, Ohio, United States of America) and visualised on a 1.5% agarose gel by electrophoresis. Samples that produced a single amplicon of expected size (Table 2) were identified as positive for the pathogen. Samples with no amplicons were considered to be free from the pathogen. Some samples resulted in either multiple amplicons or an amplicon of unexpected size; the result for these samples was identified as unknown. Positive (known infected specimens) and negative (no-template) controls were included for each set of reactions.

We were unable to secure bee specimens with known *N. bombi* infections or *N. bombi* DNA to test as positive controls, so instead we sequenced the amplicons to confirm their provenance. Polymerase chain reaction products were purified for cycle sequencing using a 100 K Pall AcroPrep 96 Multi-Well filter plate (Pall Life Sciences, Port Washington, New York, United States of America) according to the instructions of the manufacturer. Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, United States of America) following the protocol of the manufacturer, and purified via ethanol precipitation. Sequencing products were electrophoresed in an Applied Biosystems 3730 DNA analyser using Sequencing Analysis v5.2 Software. Sequences were edited, aligned, and compared to those available in GenBank (www.ncbi.nlm.nih.gov/genbank) using the BLAST sequence alignment software (www.blast.ncbi.nlm.nih.gov; Altschul *et al*. 1990, 1997), to confirm that amplicons resulted from the targeted pathogen.

Statistical analysis

Minitab version 15 was use to perform *post-hoc* χ^2 tests (after a Bonferroni correction was applied) for sample independence to determine differences in presence and absence of the specific pathogens between the native bumble bees found inside the new colonies and pre-used colonies as well as natives collected away from the field and ones collected on the field. Pairwise comparisons were done using Fisher's exact tests and statistical significance was determined after a Bonferroni correction was applied.

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Table 2. Primers and polymerase chain reaction conditions used to screen seven pathogens in Newfoundland bees.

	Primers					
Pathogen	Name	Sequences (5'–3')	Reference	Primer concentration (μM)	T_A (°C) | Extention time (seconds)	Amplicon size (base pairs)
Apidae[A] and *Crithidia bombi*[Cb] (duplexed)	ApidaeF	AGATGGGGGCATTCGTATTG	Meeus *et al.* (2010)	0.2	56 | 45	130[A]
	ApidaeR	ATCTGATCGCCTTCGAACCT				
	SEF	CTTTTGGTCGGTGGAGTGAT		0.5		420[Cb]
	SER	GGACGTAATCGGCACAGTTT				
Nosema bombi	Nbombi-SSU-Jf1	CCATGCATGTTTTTGAAGATTATTAT	Klee *et al.* (2006)	0.4	50 | 45	323
	Nbombi-SSU-Jr1	CATATATTTTTAAAATATGAAACAATAA				
Apicystis bombi	NeoF	CCAGCATGGAATAACATGTAAGG	Meeus *et al.* (2010)	0.4	62 | 30	260
	NeoR	GACAGCTTCCAATCTCTAGTCG				
Nosema apis	NosaRNAPol-F2	AGCAAGAGACGTTTCTGGTACCTCA	Gisder and Genersch (2013)	0.4	60 | 30	297
	NosaRNAPol-R2	CCTTCACGACCACCCATGGCA				
Nosema ceranae	NoscRNAPol-F2	TGGGTTCCCTAAACCTGGTGGTTT	Gisder and Genersch (2013)	0.4	60 | 45	205
	NoscRNAPol-R2	TCACATGACCTGGTGCTCCTTCT				
Melisococcus plutonius	MP1	CTTTGAACGCCTTAGAGA	Djordjevic *et al.* (1998)	0.4	61 | 45	486
	MP2	ATCATCTGTCCCACCTTA				
Paenibacillus larvae (nested) external[Ex] and internal[In]	PleF	TCG AGC GGA CCT TGT GTT	Lauro *et al.* (2003)	0.4	N/A	969[Ex]
	PleR	CTA TCT CAA AAC CGG TCA GAG				
	PliF	CTT CGC ATG AAG AAG TCA TG		0.4	N/A	525[In]
	PliR	TCA GTT ATA GGC CAG AAA GC				
Ascosphaera	AscoAll1	GCA CTC CCA CCC TTG TCT A	James and Skinner (2005)	0.4	63 | 45	550
	AscoAll2	GAW CAC GAC GCC GTC ACT				

T_A refers to annealing temperature; [A] refers to Apidae; [Cb] refers to *Crithidia bombi*; [Ex] refers to external; [In] refers to internal.

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Results

A total of 23 specimens of native bumble bee were collected from inside the four new *B. impatiens* quads (14 *B. ternarius* Say; four *B. terricola* Kirby; two *B. vagans bolsteri* Franklin; three *B. frigidus* Smith) and 18 native specimens were observed in the one pre-used *B. impatiens* quad (10 *B. ternarius*; one *B. terricola*; two *B. vagans bolsteri*; five *B. frigidus*).

Five of the seven pathogens screened were detected among the native and imported bumble bees tested (Table 3) *Nosema apis* and *Melisococcus plutonis* were not detected. *Bombus impatiens* workers sampled from the new quads at the end of the pollination period tested positive for three out of the seven pathogens screened. While there was an observation of dysentery outside the entrances of some of the colonies, we did not observe any outward signs of disease in the bees.

Crithidia bombi was detected most frequently in native Newfoundland bumble bee species. In native bees collected away from the study area (Table 3), *C. bombi* was present in 75.0% (21/28) of the bees sampled while only 53.3% (16/30) of bees sampled on the field-tested positive. By contrast, native bumble bees found inside the new quads tested positive with *C. bombi* at a rate of 81% (17/21), with all (18/18) native species inside the pre-used colonies were positive. A *post-hoc* χ^2 test showed a significant difference of the *C. bombi* rates among all of the native bumble bees collected (new quads, pre-used quads, away from field, and on field) (χ^2 (3, 97) = 13.60, $P = 0.004$). Only the bees on the field had a lower detection of pathogens than expected. Further pairwise comparisons using Fisher's exact test and a Bonferroni correction showed only the native bumble bees found inside the pre-used colonies and the bumble bees collected on the field were significantly different (Table 3).

Apicystis bombi was the next most prevalent pathogen detected. Among native bees, 50.0% (14/28) of the *Bombus* individuals away from the field and 20% (6/30) collected on the field-tested positive for *A. bombi*. *Apicystis bombi* was found in 38.1% (8/21) of native bees collected inside the new colonies and 72.2% (13/18) of native bees inside the pre-used colony tested positive for this pathogen (Table 3); these values were significantly different (χ^2 (3, 97) = 13.55, $P = 0.004$). In this case, the bees

Table 3. Percentage (prevalence) of seven pathogens screened from native and commercial bees in Newfoundland.

Species	Provenance	*Crithidia bombi*	*Apicystis bombi*	*Nosema bombi*	*Nosema apis*	*Nosema ceranae*	*Melisococcus plutonius*	*Ascosphaera* species
B. impatiens	New	40.4 (19/47)	18.2 (8/44)	0 (0/47)	0 (0/47)	0 (0/47)	0 (0/47)	41.3 (19/46)
	Pre-used	31.7 (11/15)	100 (16/16)	0 (0/16)	0 (0/16)	6.3 (1/16)	0 (0/16)	93.8 (15/16)
Native *Bombus**	Inside new	81.0 (17/21)	38.1 (8/21)	34.8 (8/23)a	0 (0/23)	0 (0/23)	0 (0/23)	18.2 (4/22)
	Inside pre-used	100 (18/18)a≅	72.2 (13/18)a	16.7 (3/18)	0 (0/10)	5.6 (1/18)	0 (0/18)	50 (9/18)ab
Native *Bombus**	Away field	75.0 (21/28)	50.0 (14/28)	3.6 (1/28)a	0 (0/28)	0 (0/28)	0 (0/28)	0 (0/28)a
	On field	53.3 (16/30)a	20 (6/30)a	6.7 (2/30)	0 (0/30)	0 (0/30)	0 (0/30)	0 (0/30)b

* The native species located in the quads and sampled freely include: *B. ternarius*, *B. terricola*, *B. vagans bolsteri*, and *B. frigidus*.
≅ Values of particular disease prevalence for native *Bombus* followed by the same letter signifies significant statistical difference at $P = 0.05$.

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from new quads and bees on the field had *A. bombi* detected at levels lower than the expected rate but native bees in the pre-used colonies were much higher than the expected rate. Plus, the pairwise comparisons again only showed a significant difference in pathogen detection between pre-used colonies and native bumble bees collected away from the field (Table 3).

The third most prevalent pathogen, *Nosema bombi*, was detected in 3.6% (1/28) of natives away from the field and 6.7% (2/30) on the field, 34.8% (8/23) of natives inside new colonies and 16.7% (3/18) of native bumble bees inside the pre-used colony tested positive for this pathogen. While the χ^2 indicates these values were significantly different (χ^2 (3, 99) = 12.12, $P = 0.007$), caution should be exercised as some of the expected counts were less than five. Only the native bumble bees inside the new quads were significantly different from the native bees collected away from the field after the pairwise comparisons (Table 3).

The fourth most common pathogen detected was the fungus *Ascosphaera* species. While no native *Bombus* species living away from the study area or on the field-tested positive for this pathogen, native *Bombus* species located inside the new colonies were 18.2% (4/22) positive and native species located in the pre-used colony tested 50.0% (9/18) positive for this fungus (Table 3). While the χ^2 test showed that these values were significantly different (χ^2 (3, 98) = 30.44, $P < 0.001$), caution should be exercised as some expected counts had values lower than five. Pairwise comparisons of *Ascosphaera* species prevalence showed that the native bumble bees collected inside the pre-used colonies were significantly different than the native bumble bees collected away from the field and for bumble bees collected on the field (Table 3). There was no difference in pathogen detection between new and pre-used colonies. *Nosema ceranae* was only detected in one *B. impatiens* from a pre-used colony, with one native *B. ternarius* specimen testing positive from within the same colony box.

Discussion

This is the first documented report of interspecific drifting of native bumble bee species in commercial colonies of *Bombus impatiens*. Though the drifting of individuals of the same species (intraspecific) into foreign nests occurs frequently in bee species (Birmingham and Winston 2004; Birmingham *et al*. 2004; Lefebvre and Pierre 2007), interspecific movement of native bumble bees into commercial nests has not been previously recorded. Drifting can be the result of disorientation, or provision robbing (Pfeiffer and Crailsheim 1998; Neumann *et al*. 2000; Birmingham *et al*. 2004). However, it is unlikely that the observed drifting was caused by disorientation in the present study as the commercial nests were in very conspicuous boxes on cranberry fields. Therefore, it seems more likely that these drifters were attempting to steal provisions from the commercial colonies. We are unsure how long the native specimens were inside the *B. impatiens* nests or whether they may have returned repeatedly. Of more concern to us is the potential for disease transmission between the commercial bees and the native bees. Drifting bees can pick up pathogens from the host nest (spillover) or they may bring diseases to the nest where the host species can become infected. The diseases could spread rapidly because of close proximity of individuals and then the disease can be transmitted back to other drifting bees (spillback) which may go on to infect other native species. As we see from the analysis, in some instances the native bees away from the field had similar levels of disease detection as the bees in the new quads as no natural population will be diseases free. However, importing commercial bees into areas will exacerbate pathogen spillover and spillback with native bees. The mechanisms of spreading diseases among managed and wild bees include shared flower use, drifting, and honey robbing (see Goulson *et al*. 2012; Graystock *et al*. 2015). O'Connor *et al*. (2013) suggested that drifting by worker *Bombus* is important among intraspecific disease transmission, however, we think it is fair to speculate that interspecific disease transmission by drifting is very likely.

During this study we screened native Newfoundland bees sampled freely and from inside pre-used and new *B. impatiens* colonies for seven pathogens associated with serious bee diseases. The trypanosome *Crithidia bombi* was the most prevalent pathogen detected among Newfoundland bees (Table 3). The parasite resides in the

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hindgut of its host where it attaches to the gut wall and multiplies with transmission stages passing out in the faeces of the host (Schmid-Hempel 2001). This pathogen may be directly transmitted without any vector through contact with infected nest mates, nest material or via flowers (Schmid-Hempel and Tognazzo 2010). The prevalence of *C. bombi* in the native Newfoundland *Bombus* species no matter from where they were sampled was high and is consistent with other areas of North America (Colla *et al*. 2006; Otterstatter and Thomson 2008; Gillespie 2010).

The neogregarine, *Apicystis bombi*, was the second most prevalent pathogen in native *Bombus*. It infects the adipose tissue of bees (Lipa and Triggiani 1996) and causes mortality to bumble bees (Rutrecht and Brown 2008; Graystock *et al.* 2016). The spillover of *A. bombi* from commercial *B. terrestris* in Argentina played a role in the decline of at least one bumble bee species there (Arbetman *et al*. 2013). In Newfoundland, *A. bombi* prevalence was high in pre-used commercial colonies and there was a significant difference in prevalence in native bees located inside the pre-used colonies with those found outside.

The third most prevalent pathogen among Newfoundland bees was *Nosema bombi*, a fungal pathogen that has been implicated in the decline of bumble bees is North America (Gillespie 2010; Cameron *et al*. 2011; Bushmann *et al.* 2012; Malfi and Roulston 2014; Sachman-Ruiz *et al*. 2015). Spores are released into the environment by the host feces and are the likely mechanism of transmission when bees share flowers (Graystock *et al.* 2015). Laboratory experiments have demonstrated that *N. bombi* can be transmitted from commercial *B. terrestris* colonies to the native species in both Japan and the United Kingdom (Niwa *et al*. 2004; Murray *et al*. 2013; Graystock *et al*. 2013b). Otti and Schmid-Hempel (2007, 2008) indicated that *N. bombi* can deform wings, decrease the survival of workers and males and prevent queens from mating. The incidence of *N. bombi* close to greenhouses supplied with commercial *B. impatiens* rose to 15% in Ontario, Canada (Colla *et al*. 2006). Recently, Cameron *et al*. (2016) showed that while *N. bombi* was historically present and widespread in North American native *Bombus* species, it was the spillover from heavily affected commercial colonies in the mid-1990s that may have greatly increased the prevalence of this pathogen in some declining native species.

Nosema ceranae was originally thought of as a pathogen of honey bees (Higes *et al*. 2008) but recent studies have indicated that *Bombus* species are susceptible as well (Graystock *et al.* 2013a; Fürst *et al.* 2014; Graystock *et al*. 2015). In the present study, we found one specimen of *B. impatiens* and one native bumble bee (*B. ternarius*) located in the same colony box infected with *N. ceranae*. Shutler *et al*. (2014), using molecular techniques, found *N. ceranae* in two of 55 colonies of *Apis mellifera* Linnaeus in one large beekeeping operation in Newfoundland. Moreover, several unpublished records recently indicate that this species is now common in Newfoundland honey bees. As Graystock *et al.* (2013a, 2015) showed that honey bees and bumble bees can acquire *N. ceranae* from flowers visits by other infected bees, there is possibility that Newfoundland bumble bees and honey bees are significantly at risk for this pathogen.

Ascosphera is known to infect the larvae of honey bees and other native bees (Stephen *et al.* 1981; Evison *et al*. 2012; Wynns *et al*. 2013; Maxfield-Taylor *et al*. 2015). Hedtke *et al*. (2011) and Evison *et al*. (2012) have suggested that non-host organisms may vector the fungal spores. None of this fungus was detected in native bumble bees sampled freely but was found in native species located inside both new and used *B. impatiens* colonies. Therefore, it appears that *Ascosphera* can readily move to native species from commercial bumble bees because of the close proximity of bees inside the nests. We can only speculate that drifting bees have the potential to transmit the fungus to other native species outside of the host nest.

The contact between native bumble bees and bees inside the *B. impatiens* colony boxes increases the likelihood that the native bees will acquire the diseases by pathogen spillover or help propagate diseases by pathogen spillback. These bees then can spread the diseases further when they return to their own colonies or share flowers with other insects. Pathogen spillover from commercial *B. impatiens* has been documented in other places (see Colla *et al*. 2006; Szabo *et al.* 2012; Murray *et al*. 2013; Sachman-Ruiz *et al.* 2015) and it has been suggested that populations of native eastern North American bumble bee

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species have been negatively impacted by the commercial *B. impatiens*. In Canada, *B. terricola* is listed with "special concern" (Committee on the Status of Endangered Wildlife in Canada 2015) and its cuckoo *B.* (*Psithyrus*) *bohemicus* (Seidl) is listed as "endangered" by the Committee on the Status of Endangered Wildlife in Canada (2014b, 2015). These are two native bumble bee species in Newfoundland and a ban on the importation of new and especially pre-used commercial bumble bee species will be a major step in protecting native bee diversity.

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