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ARTICLE

Does sending honey bee, *Apis mellifera* (Hymenoptera: Apidae), colonies to lowbush blueberry, *Vaccinium angustifolium* (Ericaceae), for pollination increase *Nosema* spp. (Nosematidae) spore loads?

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Abstract

In the Canadian Maritimes, many beekeepers rent honey bee, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), hives to growers of lowbush blueberry, *Vaccinium angustifolium* (Ericaceae), for pollination services. Anecdotally, hives have less vigour following pollination, potentially due to higher *Nosema* spp. (Nosematidae) spore loads, the microsporidian causing nosemosis. We undertook a study to determine whether sending honey bee hives to lowbush blueberry fields for pollination (blueberry hives) results in higher *Nosema* spp. spore loads relative to hives remaining in apiaries (home hives). *Nosema* spp. spore loads were quantified using light microscopy. *Nosema apis* and *Nosema ceranae* were differentiated using polymerase chain reaction and sequencing. *Nosema* spp. spore loads were greatest in April and May and declined to low levels from June to September. Ninety-eight per cent of *Nosema* detections were positive for *N. ceranae*. In April, blueberry hives had a lower spore load than home hives did; however, in June, spore loads were significantly higher in blueberry hives. No other differences in *Nosema* spp. spore loads were observed between hive types. We conclude that *Nosema ceranae* is the dominant *Nosema* species in the Canadian Maritimes and that using hives for lowbush blueberry pollination does not appear to influence long-term *Nosema* spp. spore loads.

Introduction

Honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), are subject to injury from numerous diseases, pests, and parasites. A parasite of great concern is *Nosema* spp. (Nosematidae), a genus of globally distributed microsporidian parasites (Chen *et al.* 2008; Schwarz *et al.* 2015; Holt and Grozinger 2016). Based on recent molecular phylogenetic work, many species formerly classified as *Nosema* are being reassigned to the genus *Vairimorpha* (Tokarev *et al.* 2020), but herein they are referred to as *Nosema*. Two species of *Nosema* that commonly infect European honey bees are *N. apis* and *N. ceranae* (Higes *et al.* 2013; Schwarz *et al.* 2015). Infection of honey bees by *Nosema* spp. (herein referred to as *Nosema*) causes nosemosis, a disease resulting in hives with lower number of bees (Botías *et al.* 2013), impaired health (Mayack and Naug 2009), and poor colony performance (Higes *et al.* 2008; Goblirsch 2018). Nosemosis can be especially concerning during overwintering, where the

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additional stress can further increase the likelihood of colony loss *via* suppressing the honey bee immune system (Higes *et al.* 2008; Antúnez *et al.* 2009).

Stressful conditions such as transportation to the crop field and conditions experienced during pollination may exacerbate disease problems for honey bees (Zhu *et al.* 2014). The relationship between stress and honey bee disease has been well documented, although the impacts of using and moving honey bees for crop pollination are not always consistent amongst and within studies (Zhu *et al.* 2014; Alger *et al.* 2018; Dolezal and Toth 2018). For example, Cavigli *et al.* (2016) found that the prevalence of 16 pathogens was highest in honey bees immediately after they had been sent to almond pollination. In a second study, in which colonies were transported 8600 km across the contiguous United States of America, migratory colonies had approximately 20% fewer bees than did stationary colonies immediately after transport, with the effect persisting through at least one month following return (Alger *et al.* 2018). However, the same study revealed complexity amongst end points, finding no difference in the prevalence of deformed wing virus (Iflaviridae) between migratory and stationary bees and finding that varroa mite (Mesostigmata: Varroidae) loads were approximately 60% lower in migratory colonies compared to in control hives one month after return. A study examining hives transported for almond pollination in California, United States of America, found decreased lifespan in adult bees and increased oxidative stress compared to colonies that remained stationary (Simone-Finstrom *et al.* 2016).

Many of the studies that explored the impacts of pollination stress on honey bees occurred in production systems where honey bees are transported hundreds to thousands of kilometres for pollination services (*e.g.,* Cavigli *et al.* 2016; Alger *et al.* 2018). In the Canadian Maritime provinces of New Brunswick, Nova Scotia, and Prince Edward Island, honey bees are primarily used for pollinating lowbush blueberry, *Vaccinium angustifolium* (Ericaceae), although many beekeepers also sell bee products (*e.g.,* wax and honey). Hives in this region are typically moved relatively short distances (less than 100 km) from the apiary and remain in blueberry fields for only 2–3 weeks before returning to noncrop apiary settings (McCallum and Cutler, unpublished data). The negative consequences for overall colony health may be less severe in the case of lowbush blueberry pollination compared to other pollinator-dependent crops in North America due to the shorter distance travelled and the shorter time period spent in blueberry fields.

At least two studies specifically examined the impacts of blueberry (*Vaccinium* spp.) pollination on honey bee health. Grant *et al.* (2021) found that sending bees to blueberry pollination increased the occurrence of European foulbrood disease by 41% and 53% during two field seasons. A second study, based in Québec, found that sending bees to pollinate lowbush blueberry significantly reduced brood production, which was potentially related to increased *N. ceranae* (Dufour *et al.* 2020). Such effects may be due to the low nutritional quality of *Vaccinium* pollen (14.9% crude protein; Somerville *et al.* 2006), poor weather conditions sometimes experienced during blueberry bloom (Tuell and Isaacs 2010), environment–pathogen interactions (Dufour *et al.* 2020), or exposure to pesticide residues (Drummond *et al.* 2021).

Understanding how pollination practices affect *Nosema* prevalence could be useful in helping beekeepers make informed management decisions. In this study, we investigated whether sending hives to lowbush blueberry pollination would increase *Nosema* spore loads in honey bees. We predicted increased *Nosema* spore loads in spring and autumn, due to the natural life cycle of *Nosema*, where spore loads tend to be higher in the colder months when bees cannot leave the hive for cleansing flights and where older, infected bees die during the summer months and do not transmit spores to newly developed bees (Bailey 1955). We also predicted that *Nosema* spore loads would increase following movement of hives into blueberry fields for pollination. Based on recent studies documenting proliferation of *N. ceranae* across Canada (Williams *et al.* 2008; Emsen *et al.* 2016), we anticipated *N. ceranae* would be more prevalent than *N. apis* in our samples.

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Material and methods

Data collection

The study was conducted from April to September 2020. Eleven beekeepers participated in the study, managing 12 beekeeping operations throughout the Canadian provinces of New Brunswick, Nova Scotia, and Prince Edward Island. Beekeepers were selected owing to their adherence to best management practices (*e.g.,* for feeding, overwintering, and disease and pest management) and research experience. All beekeepers had previously partnered with the research team on applied research projects, and all hives included in the study met the regional pollination standard for colony strength (*i.e.,* eight frames of bees, four frames of brood with 100% coverage, two frames of honey, and one laying queen; Atlantic Tech Transfer Team for Apiculture 2020). Due to restrictions posed by the COVID-19 pandemic, we were not able to standardise hives for similar levels of *Nosema* infection at the onset of the study.

Apiaries were distributed across two different treatments. In the first treatment, or "blueberry hives," all hives within the apiary ($n = 8$) were rented out for lowbush blueberry pollination (June) before being returned to the home apiary for the remainder of the study (until September). In the second treatment, or "home hives," all hives within the apiary ($n = 4$) remained at the beekeepers' home apiaries for the duration of the study. In the case of "home hives," bees could freely forage on floral resources within the surrounding landscape that would have included wild plants (*e.g.*, goldenrod (Asteraceae), brambles, rhodora (Ericaceae)), agricultural crops such as forage feed (*e.g.,* clover (Fabaceae), corn (Poaceae), and soybeans (Fabaceae)), and residential gardens. These home apiaries were at least 10 km away from commercial blueberry fields – outside of the foraging range of honey bees. None of the studied hives were treated with Fumagilin-B®, a registered chemical treatment for nosemosis, for at least 12 months before the study began. Otherwise, the studied hives were subject to standard best management practices, including optimal overwintering protection and preparation, spring feeding (*i.e.,* sugar syrup and pollen patties), varroa mite management, and young, healthy, and vigorous queens. The economic disadvantage of keeping honey bee colonies "home" from pollination meant we could only secure four apiaries for the home hive treatment.

Planned monthly data collection visits at each site were not possible due to COVID-19 pandemic travel restrictions. Therefore, participating beekeepers collected monthly samples using sampling kits that we provided with detailed sampling and shipping instructions (Supplementary material, Instructions provided to participating beekeepers). Beekeepers were asked to collect one-quarter cup (approximately 60–65 mL) of bees from the inner cover (or outer frames, if an inadequate number of bees was found on the inner cover) of each labelled hive in their yard monthly. These samples were stored at –18 °C until shipping and at the same temperature upon receipt at our laboratory until processing could occur.

Twelve hives were labelled within each apiary, and data were collected repeatedly from each hive monthly from April to September. We considered each hive as an independent experimental unit. Over the six-month data collection period, we anticipated 48 monthly samples from home hives and 96 monthly samples from blueberry hives, resulting in a total of 288 samples collected from the home hives and 576 samples collected from the blueberry hives. Hives in the blueberry treatment were sent to lowbush blueberry pollination on different dates across the Maritimes, due to differences in bloom period across the region (4–30 June 2020) and remained in the field for 2–3 weeks.

Quantifying *Nosema* spores

We used standardised methods previously described in McCallum *et al.* (2020) to quantify *Nosema* spores in bees from each hive. To release contents from guts of bees, 30 bees were crushed within a sealable plastic bag. Using an eyedropper, 5 µL aliquots of the resulting

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liquid were deposited into each well of a standard haemocytometer (Reichert Bright-Line, Improved Neubauer, 0.1 mm depth; Hausser Scientific, Horsham, Pennsylvania, United States of America), and the eyedropper was thoroughly cleaned between samples. The eyedropper was used to collect 5 µL of clean water and was observed for any possible spore carryover every few samples as an extra precaution to avoid spore contamination between samples.

The spores were counted under 400× magnification. In each well of the haemocytometer, total number of spores in each corner and the centre square was counted. The mean spore load was calculated following Cantwell (1970).

Nosema identification using polymerase chain reaction

After quantifying *Nosema* spore loads, the remaining bees from the original sample were then prepared and processed for a second test. We removed 50 bees (sample size recommended by the National Bee Diagnostic Centre, Beaverlodge, Alberta, Canada) from the samples with the highest spore loads to ensure ample spore abundance for optimal *Nosema* species representation (*Nosema apis versus Nosema ceranae*). These samples were shipped frozen on wet ice to the National Bee Diagnostic Centre.

Nosema species identification performed by the National Bee Diagnostic Centre followed protocols developed by Hamiduzzaman *et al.* (2010) and Gisder and Genersch (2013). *Nosema* species were identified using polymerase chain reaction with a Multiplex Supermix (Bio-Rad, Hercules, California, United States of America). Briefly, amplification assays were executed by engaging 60 ng of genomic DNA and 0.4 ng of each primer in a Veriti thermal cycler (Thermo Fisher Scientific, Waltham, Massachusetts, United States of America). The protein-coding gene *RPS*5 was used as a reference housekeeping gene. The polymerase chain reaction conditions were as follows: 95 °C for 5 minutes, followed by 35 cycles of 1 minute at 94 °C, 1 minute at 58 °C, 1 minute at 72 °C, and 7 minutes at 72 °C. Amplification products were separated by 1% agarose gel stained with SYBR Safe (Thermo Fisher Scientific) and were finally observed under ultraviolet and blue light illumination.

Statistical analysis

We used a completely randomised design, with the factor of interest being pollination with two levels – blueberry and home – and with hives being the experimental units. The number of replications for blueberry was $n_1 = 96$ and for home was $n_2 = 48$. Because the response values (*Nosema* spores in millions) were measured repeatedly (monthly), repeated measures analysis was completed to determine the effect of pollination on *Nosema* spores and how the effect changed during the six months. Akaike information criterion (Littell *et al.* 1998) was used to determine the most appropriate co-variance structure to be "unstructured." The repeated measures analysis was completed using the "mixed procedure" of SAS (SAS Institute, Inc. 2014). The validity of model assumptions (normal distribution and constant variance of the error terms) was verified by examining the residuals as described in Montgomery (2020), which showed the normal distribution assumption was violated in the original data. However, a fourth root transformation that was applied to *Nosema* spore values met the assumption. The *P*-value for the interaction between pollination and month effect was 0.006; therefore, multiple means comparison was conducted using the "lsmeans" statement of Proc Mixed at the 5% level of significance to generate letter groupings. The means reported in Fig. 1 are back-transformed to the original scale.

We also described trends in *Nosema* species identification using percentages of detections (*e.g.,* percentage of *N. ceranae* detected in total samples submitted) as a function of all samples submitted for detection by polymerase chain reaction.

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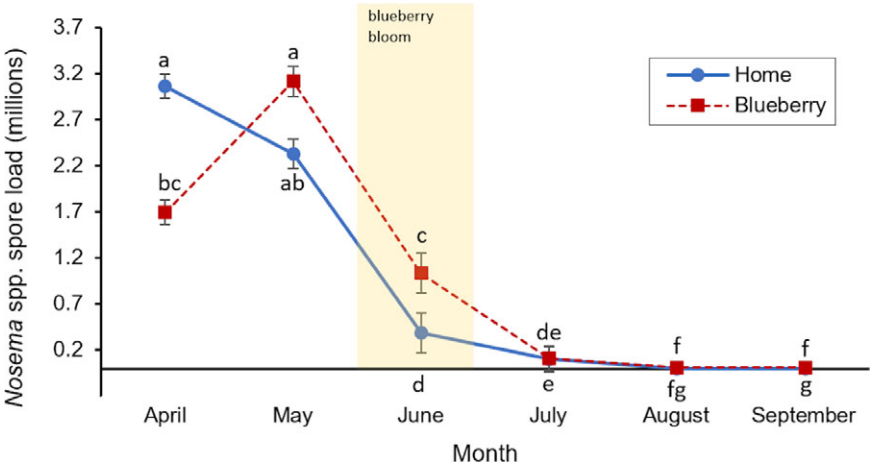

Fig. 1. Mean *Nosema* spp. spore loads in honey bee hives across three Maritime provinces, Canada, during spring and summer of 2020. Error bars represent standard deviation. Means sharing the same letter are not significantly different at the 5% level of significance. "Blueberry" hives were brought to lowbush blueberry fields during blueberry bloom, whereas "home" hives remained at an apiary. The area highlighted in yellow (blueberry bloom) represents the time when lowbush blueberry fields were flowering.

Results

Nosema spore loads in blueberry pollination *versus* home apiary treatments

We found a significant interaction ($F_{5,139} = 3.40$, $P = 0.006$; Supplementary material, Table S1) between pollination treatment and month. *Nosema* spore counts in home hives decreased sharply from April to June, whereas spore counts in blueberry hives increased from April to May and then decreased from May to June (Fig. 1). During the first sampling period (April), *Nosema* spore loads in prepollination blueberry hives (1.70 ± 0.12 million spores, mean ± standard deviation) were 44% lower than those in home hives (3.12 ± 0.09 million spores, mean ± standard deviation). Spore loads in prepollination blueberry hives more than doubled in May samples but did not significantly differ from spore loads from home hives during that period (Fig. 1). In June, blueberry hive *Nosema* spore loads (1.04 ± 0.15 million spores, mean ± standard deviation) were 170% greater than spore loads in home hives (0.39 ± 0.22 million spores, mean ± standard deviation; $P < 0.05$; Supplementary material, Table S2). Spore loads through the remainder of the year remained low and consistent (range 0–0.112 million spores) among all hives (Fig. 1).

Nosema species identification

We had anticipated submitting 72 samples to the National Bee Diagnostic Centre for *Nosema* species identification, but only 54 samples across the six collection periods were submitted by beekeepers (36/54 = blueberry hives, 18/54 = home hives). Of the samples analysed, 76% (41/54) tested positive for *N. ceranae* (Fig. 2). Only 2% of samples (1/54) tested positive for *N. apis*. One sample (1/54) was positive for both *N. apis* and *N. ceranae*. Twenty per cent of samples (11/54) were negative for *Nosema*.

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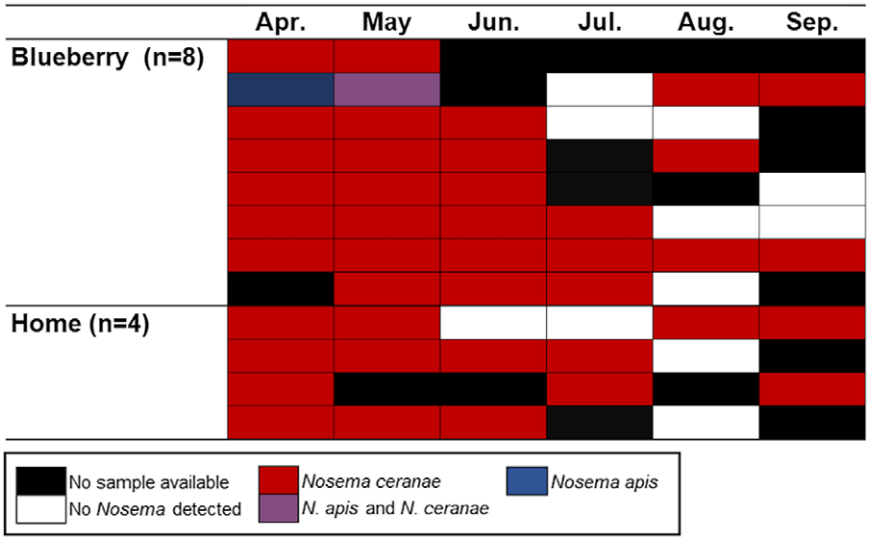

Fig. 2. *Nosema* spp. detection *via* polymerase chain reaction from honey bees from hives used in blueberry pollination "Blueberry" and hives that remained at a home apiary, "Home," in the Canadian Maritime provinces during spring and summer 2020.

Discussion

Consistent with our predictions and with other published studies (*e.g.,* Traver *et al.* 2012; Dufour *at al.* 2020; McCallum *et al.* 2020), *Nosema* spore loads were highest in the spring (April and May) and decreased over the summer months. Bees leave their hive more often as seasonal temperatures rise, resulting in more cleansing flights and less faecal matter in the hive (Winston 1987; Retschnig *et al.* 2017). With less infected faecal matter around the colony, uninfected bees have reduced exposure and probability of *Nosema* infection. Spore loads in April–May frequently exceeded the economic threshold of 1 million spores per bee, reaching levels above 25 million spores per bee, a level nearly 25 times higher than untreated hives in a regional study conducted in 2018–2019 (McCallum *et al.* 2020). Differences between spore loads in these studies could be due to a variety of biotic (*e.g.,* genetic differences in brood stock) or abiotic (*e.g.*, weather differences) factors.

By July, the mean spore load was below the economic threshold and remained low until September, which is consistent with many other Canadian studies (*e.g.,* Copley *et al.* 2012; McCallum *et al.* 2020). Contrary to our expectations, we did not detect an increase in *Nosema* spore loads during the September collections, which is consistent with results by Punko *et al.* (2021). This may be because September 2020 was warmer than historical temperature averages for the region. Data from three weather stations close to the apiaries (Kentville, Nova Scotia; Mactaquac Provincial Park, New Brunswick; and New Glasgow, Prince Edward Island) show that the mean temperatures during September 2020 were 1.0 °C higher than the 1981–2010 mean (Supplementary material, Table S3). In warmer weather, continued regular cleansing flights may delay the onset of enhanced *Nosema* spore numbers.

We expected *Nosema* spore loads to be greater in blueberry hives than in home hives following blueberry pollination, but this was not observed. We did see a sharp increase in *Nosema* spore counts in blueberry hives in May (coinciding with a decrease in spore counts from home hives). However, this was well before the hives went to blueberry fields for pollination, and therefore, the act of hives being moved to or residing in blueberry fields did not cause this increase in spore counts. We have no quantitative or qualitative information pointing to

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differences in home hive *versus* blueberry hive management before movement that could explain the spike in *Nosema* spore counts in May. Beyond specific treatments for *Nosema*, the use of pollen substitutes has also been shown to potentially affect *Nosema* incidence. Pollen substitutes (*i.e.,* pollen patties) are protein-rich formulations used for stimulating brood production and colony growth, and use of these has been shown in some cases to correspond to increased *Nosema* incidence (DeGrandi-Hoffman *et al.* 2016; Jack *et al.* 2016). In our study, beekeepers were not asked to provide information regarding the details of supplemental feeding, but blueberry hives and home hives both would have received pollen patties of varying origin and composition. Further exploration of the interaction between early-season supplemental feeding and *Nosema* infections would be useful in understanding how *Nosema* infections are affected by management.

Numerous biological and management factors can affect disease pressures in honey bee colonies, and the results of studies examining the effects of hive transport for crop pollination have been variable. A study by Zhu *et al.* (2014) found that *Nosema* spore loads were 2.5-fold greater in hives that were transported 275 km for highbush blueberry pollination relative to hives that remained stationary. Migratory colonies transported between bee yards in Spain had greater *Varroa* and *N. ceranae* loads than stationary colonies did in the period following initial transportation but a lower viral load of deformed wing virus (Jara *et al.* 2020). Although Simone-Finstrom *et al.* (2016) did not examine specific diseases, these authors found transporting hives for pollination had a significant negative impact on adult bee lifespan. The results of our study, compared to others, suggest transportation or placement of honey bee hives in agricultural settings for pollination can have variable effects on pathogen loads.

More than three-quarters of our samples contained *N. ceranae*, with only two samples (4%) testing positive for *N. apis*. In our region (Maritime Canada), *N. ceranae* was first detected in samples collected in 2006 (Williams *et al.* 2008), but it is possible that *N. ceranae* has been established for a longer time, having been misidentified as *N. apis*. Dominance of *N. ceranae* has been observed in numerous other studies (*e.g.,* Klee *et al.* 2007; Chen *et al.* 2008; Williams *et al.* 2008; Emsen *et al.* 2016; McCallum *et al.* 2020) and has become a common honey bee pathogen around the world (Grupe and Quandt 2020). Although the global spread of *N. ceranae* was caused by human actions, the displacement of *N. apis* by *N. ceranae* may also be due to the ability of *N. ceranae*, but not of *N. apis*, to infect wild hosts (Martín-Hernández *et al.* 2011), a faster reproduction rate of *N. ceranae* compared to *N. apis* (Williams *et al.* 2014), combined with the ability of *N. ceranae* to thrive across a wide range of environmental conditions (Martín-Hernández *et al.* 2018). Given that only one antimicrobial product is currently available to treat nosemosis (Fumagilin-B®; Can-Vet Animal Health Supplies Ltd., Guelph, Ontario, Canada; DIN 02231180), it is important to know the identity and quantity of the parasite for effective treatment.

Due to COVID-19 restrictions, we relied on cooperating beekeepers to collect and send samples, using standardised sampling methods and frequent communication with beekeepers. This was largely effective, but we did experience some challenges. Some hives were excluded from the study throughout the season if they swarmed, became queenless, became weak, or died, or if the hive label was lost, and sometimes beekeepers did not submit records as to why a hive was removed from the study. This means we would not have known if a hive failed due to *Nosema* pressure. Inconsistency of sample submissions by beekeepers also would have led to some degree of variability in the seasonal variation in *Nosema* spore loads, although reporting was consistent from May to July, which marked the point before and after bees were moved to and from lowbush blueberry fields (Supplementary material, Table S2). In addition, due to shipping delays associated with COVID-19, receipt of some samples was delayed and not every sample had enough bees, precluding *Nosema* species diagnostics by the National Bee Diagnostic Centre; however, the planned large sample size helped to mitigate this challenge. Logistical issues associated with the COVID-19 lockdowns meant that samples

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could not be sent on dry ice, and we cannot rule out whether potential degradation of sample quality during shipping affected our results and interpretation.

We found no evidence that movement within Canada's Maritime provinces of honey bee colonies to lowbush blueberry fields for pollination affects *Nosema* spore loads. Future studies might consider how these relatively small-scale movements of hives from apiaries to lowbush blueberry fields, and associated management practices (*i.e.,* supplemental feeding), might affect other end points of honey bee health (e.*g.,* colony growth, brood and honey production, and disease and pest pressures). Because most research on movement and pollination stress focuses on higher-impact (*e.g.,* longer-distance) hive migrations, future research efforts could benefit from examining smaller-scale movements of bees for use as commercial pollinators. Improving understanding of the consequences that sending bees for pollination has on bee health, hive productivity (*e.g.,* honey production), and resilience (*e.g.,* overwintering success) could be useful for beekeepers when deciding whether to participate in pollination.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.4039/tce.2022.30.

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Competing interests. The authors declare no competing interests.

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