Nosema epidemiology and control in honey bees (*Apis mellifera*) under Canadian Prairie conditions

by

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Dedication

To my partner, Trevor Glover,

for his untiring support and encouragement.

I could not have done this without you.

And to my parents, Lorna and William Punko,

who would do anything to help me achieve my goals,

including moving to Winnipeg in 30 below.

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List of Abbreviations

DNA – deoxyribonucleic acid

PCR – polymerase chain reaction

qPCR – quantitative polymerase chain reaction

Abstract

Honey bees (*Apis mellifera*) are vulnerable to many diseases, including two species of the fungus *Nosema*, namely *Nosema apis* and *Nosema ceranae*. *Nosema ceranae* appears to be replacing *Nosema apis*, but its epidemiology, responses to drug treatments, climate, and wintering methods, and impact on colony productivity and survival are poorly understood. This study aimed to determine the seasonal pattern of *Nosema* abundance in two Alberta locations using indoor and outdoor wintering methods and the impact of *Nosema* on honey bee colony population and survival. This study also assessed the effects of spring and fall fumagillin treatment on spore abundance and colony productivity and mortality. Colonies that were naturally infected with *Nosema* had predominantly *N. ceranae*, but some had both *N. ceranae* and *N. apis*. *Nosema ceranae* had high spore abundance in spring, declining to low levels in the summer and fall. There was no clear pattern for differences in *Nosema* abundance between locations. Colonies that were wintered indoors had one-fifth the probability of mortality at similar *Nosema* abundance and more rapid spring population build-up than outdoor-wintered colonies. This suggests that the mitigation of temperature stress associated with indoor wintering reduced the impact of *Nosema* infections on colonies more than any potential benefits associated with late winter cleansing flights. Consequently, the existing *Nosema* threshold should be lower for outdoor-wintered colonies than those wintered indoors. Average *Nosema* abundance in the spring was a significant predictor of end-of-study winter colony mortality, highlighting the importance of spring *Nosema* monitoring and treatments. Fumagillin treatment in the spring and/or fall reduced *Nosema* abundance but did not eliminate the infection, making continued monitoring necessary. Honey bee colony population was improved by spring treatment, but not consistently between locations and years, possibly due to the late treatment application at a lower

dosage than the label recommends. Previous spring and/or fall fumagillin treatment did not reduce spring *Nosema* abundance or increase colony population in the following spring. Therefore, to maintain low spring *N. ceranae* abundance, colonies should be treated in the spring even if treated in the previous fall. Treating with fumagillin in both the spring and fall increased colony survival in one of two years.

General Introduction

The Western honey bee, *Apis mellifera* Linnaeus, is economically important as honey bees provide pollination services to many agricultural crops. In Canada, there are 750,000 managed honey bee colonies contributing an estimated 4 to 5.5 billion CAD in pollination services (Mukezangango and Page 2017). Honey bee colonies are subject to a wide variety of parasites and pathogens, many of which can cause colony mortality. While some mortality is financially tolerable, consistently high mortality (above 15% in Canada) will become unsustainable due to the costs associated with replacing the colonies and the immediate production losses. Therefore, beekeepers need effective pest management strategies to keep colonies healthy and productive.

Nosema infection in *A. mellifera* is caused by the midgut parasites, *Nosema apis* Zander and *Nosema ceranae* Fries et al., and has consistently been among the top four factors causing colony mortality in Alberta, according to surveyed beekeepers (Canadian Association of Professional Apiculturists 2014, 2015, 2016, 2017, 2018). *Nosema apis* is a long-time parasite of *A. mellifera* and has been effectively managed using the antifungal treatment, fumagillin (Bailey 1953a; Furgala et al. 1973; Webster 1994). *Nosema ceranae* was recently discovered in *A. mellifera* and can now be found worldwide (Higes et al. 2006; Klee et al. 2007). With the emergence of *N. ceranae*, information is needed on how this species differs from *N. apis* and whether the same treatment is effective.

Most colony mortality occurs over the winter as the cold temperatures confine bees to their hive, forcing them to survive on only stored food. In combination with winter stresses, honey bee diseases can become particularly detrimental to colonies (Currie et al. 2010). The

epidemiology of *Nosema* may be affected by regional differences in winter conditions as cold temperatures and differing wintering management methods (indoor and outdoor) provide varying levels of temperature stress and defecation flight opportunities. Also, winter climate varies regionally in Alberta, with some areas experiencing longer periods of cold temperatures and others having occasional warm periods that provide defecation flight opportunities.

The research reported in this thesis increases our knowledge of epidemiology, impact, and treatment of *Nosema* under Canadian Prairie apicultural management. This thesis is written in a manuscript style with a general literature review (Chapter 1), two manuscripts (Chapter 2 and 3), and a general discussion (Chapter 4). In Chapter 1, the literature review introduces the honey bee and the biology and management of *Nosema* and its interactions with the honey bee colony and Canadian Prairie winter environment. Chapter 2 reports the results of a long-term study aiming to determine the seasonal pattern of *Nosema* abundance in two Alberta locations using indoor and outdoor wintering methods and its impact on honey bee population, survival, and economic viability. Chapter 3 reports the outcomes of a study that assessed the effects of spring and fall fumagillin treatment on *Nosema* abundance and colony strength, honey production, and mortality within Alberta using indoor or outdoor wintering management. Chapter 4 is a general discussion of the research contained in this thesis.

Chapter 1: Literature Review

The Honey Bee, *Apis mellifera*

Western honey bees (*Apis mellifera* Linnaeus) are holometabolous, social insects belonging to the order Hymenoptera that live in colonies of 10,000 to 60,000 individuals. Managed colonies reside within a box or boxes referred to as "a hive," which contain movable frames with sections of hexagonal cells made of wax called comb. Though it has many functions, the most important uses of comb are for brood rearing and food storage. 'Brood' collectively refers to all the immature stages of the honey bee (eggs, larvae, and pupae). To efficiently carry out the many tasks needed for the colony to survive, worker honey bees specialize in different tasks throughout their lifetime (temporal polyethism).

Life Cycle and Caste Differences

Within the colony, there are male "drone" bees and two female castes: consisting of many workers and a single queen. The queen is central to the survival of the colony as she is usually the sole egg-layer and the only female capable of mating. Honey bees have a haplodiploid sex-determination system that allows the queen to control the sex of her offspring either by fertilizing the egg, producing female offspring, or laying an unfertilized egg, producing male offspring (Schneider 2015). A single queen-laid egg is placed in a cell where the developing honey bee will spend its entire immature life. When the egg hatches, the larva is regularly provided with food by adult workers, called 'nurse' bees, and molts five times. Depending on the type and amount of food given, female larvae will either develop into either a fertile queen or sterile worker. At the last larval stage, the cell entrance is covered with an air-permeable wax/pollen

capping, and the larva begins to spin a cocoon and pupate (Schneider 2015). Finally, the young adult honey bee chews through the capping, ready to become an active member of the colony. Newly emerged workers will immediately contribute to maintenance tasks within the hive and eventually transition through a range of age-related behavioural tasks necessary for colony function. Newly emerged drones take ten days after emergence to reach sexual maturity and begin to take mating flights (Gary 2015). The male drones do not perform any hive duties; their behaviours focus on locating and mating with queens.

The vast majority of individuals in the colony are non-reproductive female workers. For the first three weeks of their life, worker bees remain inside the hive doing various duties, including cleaning and building comb, nursing brood, and temperature regulation (Huang and Wang 2015). The remainder of their life is spent making trips outside the colony foraging for nectar, pollen, water, and plant resins and doing guarding tasks to defend the colony. Although the changing of worker tasks usually progresses in a particular sequence, the workers are capable of switching tasks to adjust for changes in the needs of the colony (Huang and Wang 2015).

Colony Communication

Honey bees must be able to effectively communicate and share information with the many individuals of the colony. One of the most well-known ways honey bees communicate is through the waggle dance, which is used to recruit and direct other workers to a new food source over 100 metres from the hive (Gary 2015). This 'dance' involves performing a figure-eight with a straight portion where the bee will vigorously shake its abdomen (waggle phase). The angle of the waggle phase relative to the vertical of the comb represents the angle between the current position of the sun and the direction of the food source (Gary 2015). Additionally, the duration of

the waggle phase indicates the distance to the food source. For food sources under 100 metres, the round dance is used, which involves the bee moving in a circle, turning at the end to following the circle back to the beginning (Gary 2015).

Another way honey bees communicate is through pheromones, a chemical substance released by an individual to elicit a behavioural or physiological response by other members of the same species, as reviewed in Grozinger (2015). The main pheromone produced by the queen is the queen mandibular pheromone, which maintains worker cohesion, suppresses queen rearing and swarming, and regulates worker behavioural development. Workers can release several different kinds of pheromones related to recruitment, defence, and developmental regulation. For example, ethyl oleate is produced by foragers and is transmitted by close contact and in-hive evaporation to inhibit the transition of house bees to foragers. If there are not enough foragers present, the pheromone inhibition will fail, allowing housekeeping bees to transition into foragers. Larvae influence adult worker bee physiology and behaviour through brood ester pheromone. Brood ester pheromone increases pollen foraging, alters the protein levels in brood food, prevents worker ovary development, and stimulates the capping of mature larvae cells. The chemical composition of brood ester pheromone differs by larvae age and caste so that appropriate care can be provided by worker bees.

Seasonal Patterns and Behaviour

In temperate climates, honey bee colony populations change dramatically in response to seasonal changes. In the spring, colonies are small and typically consist of about 10,000 workers. Brood production will rapidly increase the worker population during May and June, causing the population to peak at 40,000 to 60,000 workers during the mid-summer period in Canada (Currie

et al. 1998). The high population is maintained for several months during the honey flow until winter approaches, and brood production slows until the colony eventually becomes broodless and the population of workers declines. The workers emerging in the late summer to fall are known as winter bees and can survive the entire winter under the right conditions.

Honey bees are able to survive cold winter temperatures through clustering behaviour. The cluster has two distinct layers: the core and mantle (outer layer; Currie et al. 2015). In the core, bees contract their flight muscles to maintain the core at 20°C. The bees in the mantle form an insulating barrier from the cold by positioning their heads towards the core. The heat radiating from the core warms the mantle to 8°C, just above the temperature at which bees enter a chill coma. Periodically, the bees will rotate from the mantle to the core to warm their bodies and feed on honey stores. The cluster can accommodate changes in ambient temperature by expanding or contracting the cluster. A colony's ability to regulate its temperature depends on its surface area to volume ratio, with larger clusters being more efficient than smaller clusters. Additionally, adequate honey stores are necessary to provide the energy required for generating heat (Currie et al. 2015). As honey stores are depleted, the cluster will migrate around the hive to access new honey stores. During warmer daytime temperatures (>10°C), the cluster may move towards the entrance to allow for flight.

Honey bee flight activity is highly dependent on temperature, with flight only occurring above 10°C (Retschnig et al. 2017). In temperate climates, warm weather allows foragers to gather the pollen, nectar, and other resources necessary for growing and sustaining the colony. Additionally, honey bees usually only defecate while flying, with these defecation events known as cleansing flights (Winston 1987). Under low temperatures that prevent flight, bees are

confined inside the hive, and feces can be held within their rectum until favourable weather conditions return, which can take several months.

Colony Losses Caused by Pests and Disease

Honey bee colonies are subject to a wide variety of health issues caused by bacteria, fungi, viruses, predators, and parasites, many of which can cause colony mortality. While some winter colony losses are expected (~15% in Canada), consistently high mortality will become unsustainable due to the costs associated with replacing dead colonies and the immediate production losses (Canadian Association of Professional Apiculturists 2015). Both *Nosema* and *Varroa* (*Varroa destructor* Anderson and Trueman) have consistently been among the top four factors causing colony mortality in Alberta, according to surveyed beekeepers (Canadian Association of Professional Apiculturists 2014, 2015, 2016, 2017, 2018). Therefore, beekeepers must be equipped with the most up-to-date knowledge of pests and diseases so they can make integrated pest management decisions.

Varroa mites require the parasitism of both brood and adult bees to complete their life cycle. They feed on the fat bodies and hemolymph of their host, which has a wide range of consequences for the bee (Ramsey et al. 2019). Additionally, *Varroa* mites are a known vector for several viruses (Bailey and Ball 1991; Chen et al. 2004) and have been found to reduce colony population and cause colony death (Guzmán-Novoa et al. 2010). Fortunately, *Varroa* infestations can be monitored easily through field alcohol washes and sticky boards and controlled with chemical acaricides such as Apivar®.

Nosema

The genus *Nosema*, belonging to the phylum Microsporidia, has over 150 described species that infect 12 orders of insects (Bencel and Andreadis 2014). Of particular significance are those that infect bees, such as *Nosema bombis* Fantham & Porter in bumble bees, as they provide essential pollination services. *Nosema apis* Zander and *Nosema ceranae* Fries et al. are obligate intracellular spore-forming parasites of *A. mellifera*, collectively referred to as nosema in honey bees. Nosema in the European honey bee was initially believed to be restricted to one species, *N. apis*, but shortly after its discovery in *Apis cerana* Fabricius, *N. ceranae* was also found to infect *A. mellifera* (Higes et al. 2006). Later, it was determined that *N. ceranae* had been present in *A. mellifera* many years earlier than its first detection (Paxton et al. 2007; Chen et al. 2008). Both *Nosema* species are now prevalent in *A. mellifera* worldwide (Klee et al. 2007), with *N. ceranae* being present in Canada since at least 1994 (Currie et al. 2010).

In Europe, the proportion of *N. ceranae* infections appears to be greater in warmer climates than temperate climates (Fries 2010). Regional differences in species predominance may be due to climatic differences as *N. apis* is more successful than *N. ceranae* at lower temperatures (Burnside and Revell 1948; Woyciechowski and Czekonska 1999; Martín-Hernández et al. 2009; Higes et al. 2010). However, *N. ceranae* has since become the most prevalent species in Canada, which experiences colder weather suggesting temperature is not as important as previously thought (Williams et al. 2008a, 2010; Currie et al. 2010; Copley et al. 2012; Emsen et al. 2016). Alternatively, Canada may have a different haplotype of the pathogen than those in Spain and Germany (Williams et al. 2008b); however, differences between haplotypes have not been confirmed.

Life Cycle

There are three distinct stages in the *Nosema* life cycle: the reproductive stage (merogony), the spore-forming stage (sporogony), and the infective stage (spores). Adult workers become infected from consuming spores through cleaning comb infected by feces (Bailey 1953b) or through trophallaxis of contaminated food (Higes et al. 2009a; Smith 2012). The life cycle of *N. apis* and *N. ceranae* documented by Fries et al. (1992) and Gisder et al. (2011) is as follows (Fig. 1.1). The spores germinate upon entering the bee gut lumen, everting their polar filament to form a hollow tube. The filament penetrates a midgut epithelial cell and injects its sporoplasm, which will mature into a meront. During merogony, the meront will undergo binary fission to produce several daughter merozoites. It has been established that *N. apis* has up to two cycles of merogony (Gray et al. 1969), whereas *N. ceranae* may have more than two (Gisder et al. 2011). The merozoite subsequently will begin sporogony, where it becomes a sporont that will typically produce two sporoblasts, which will mature into spores. Additionally, mature spores within the host cell can germinate, reinfecting the same cell or other adjacent cells (Fries et al. 1992; Higes et al. 2007; Gisder et al. 2011). Eventually, the epithelial cell is shed or ruptured, releasing the spores into the gut lumen, where they will either infect other cells or be excreted to infect other bees.

The entire life cycle of a single *N. ceranae* spore is complete within three days post-infection (Higes et al. 2007; Gisder et al. 2011), whereas *N. apis* can produce spores one day sooner (Fries 1993). In both species, a fully developed infection occurs at 10-12 days post-infection with between 20 to 30 million spores per bee (Paxton et al. 2007; Forsgren and Fries 2010). *Nosema apis* is unable to infect bee larvae as the spores do not germinate in the larval gut

(Hassanein 1951). *Nosema ceranae* was also thought only to infect adults but has now been shown to infect larva under laboratory conditions (Eiri et al. 2015).

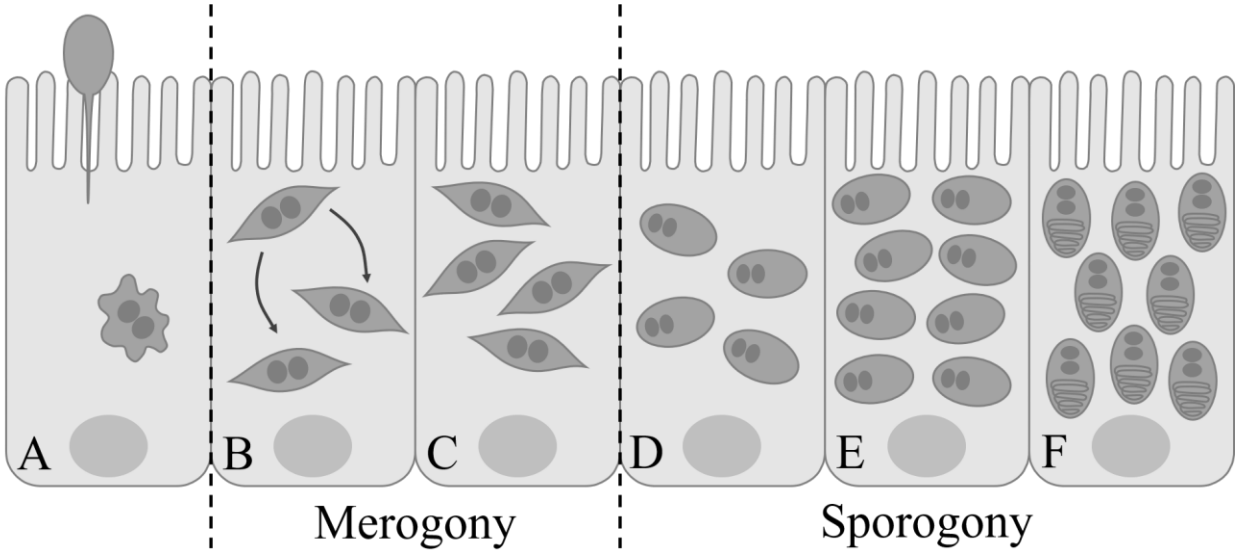

Fig. 1.1 – Life cycle of *Nosema* within the honey bee midgut. (A) The spore's polar filament penetrates an epithelial cell and injects its sporoplasm. (B) The sporoplasm matures into meront and begins binary fission to produce several daughter merozoites (C). (D) The merozoite becomes a sporont and begins sporogony. (E) The sporonts divide once into sporoblasts. (F) The sporoblast matures into spores, which can survive externally. Figure adapted from Gisder, S., Mockel, N., Linde, A., and Genersch, E. 2011. A cell culture model for *Nosema ceranae* and *Nosema apis* allows new insights into the life cycle of these important honey bee-pathogenic microsporidia. Environmental Microbiology, **13**: 404–413.

Symptoms and Detection

When a parasite can not be visually detected in the colony, beekeepers usually rely on morphological or behavioural symptoms caused by the parasite to determine their presence. Dysentery is often cited as a symptom of *N. apis*; however, it is not the only cause of dysentery (Bailey 1967), so it cannot be considered as definitive proof of infection. *Nosema ceranae* has been described as asymptomatic, with infection only being noticed as the colony's population begins to decline (Higes et al. 2008). As colony decline can be associated with many pests and diseases, *Nosema* infections should be diagnosed by light microscopy to examine spore loads or by molecular methods such as PCR.

Parasite level estimations can be accomplished through different sampling and quantification techniques, each with its own advantages and disadvantages. Samples should target individuals or areas that are more likely to be infected as this allows for earlier detection. Generally, older bees are sampled as they are more frequently infected with *Nosema* than younger bees (Meana et al. 2010; Smart and Sheppard 2012). Samples of older bees can be obtained from outside the hive at the entrance or from within the hive on peripheral frames in the brood chamber or in the honey supers (Fries et al. 2013). Although entrance samples are likely to have more old bees, they are more time-consuming than in-hive sampling as foragers must be aspirated from the entrance. Additionally, entrance sampling is not possible during conditions where foragers are not flying, such as cold or rainy weather. Collecting bees from the outer frames is a generally accepted alternative to entrance sampling (Fries et al. 2013). To quantify *Nosema* infection, the sampled bees can be analyzed individually to determine the proportion of infected individuals (prevalence) and intensity (spores per infected bee) or pooled and described in terms of spores per bee (mean abundance). Individual analysis is thought to be somewhat

better at predicting the health of the colony but is more labour intensive than pooled samples (Higes et al. 2008). While there is a correlation between the proportion of infected individuals and average spores per bee (Smart and Sheppard 2012; McGowan et al. 2016), it is not always consistent (Higes et al. 2008; Jack et al. 2016a). When choosing a sampling and quantification technique, it is important to consider the accuracy, time, and cost of different techniques and what information beekeepers will use when making management decisions.

Species determination is potentially valuable as there are several pathological differences between the two species (see following sections). The spores can be distinguished based on size with spores of *N. apis* averaging 6.0 x 3.0 µm, which is slightly larger than *N. ceranae* spores at 4.7 x 2.7 µm (Fries et al. 1996). However, due to their overlapping size ranges, the two species can not be easily differentiated by spore size alone (Fries et al. 2013). The number of polar filament coils can be used to distinguish the two species as *N. ceranae* has 18-23 coils (Fries et al. 1996; Chen et al. 2009), and *N. apis* has over 30 coils (Liu 1973; Liu and Liu 1974). However, the coils can only be seen using a transmission electron microscope. The most accurate way to determine species is through molecular techniques such as PCR using primers unique to each species (see Martín-Hernández et al. 2007, Chen et al. 2008).

Pathology at the Individual Worker Level

Microsporidia are not capable of producing energy themselves and, therefore, likely obtain their energy from their host. In cage trials, both *Nosema* species cause increased sugar syrup consumption in infected honey bees, with *N. ceranae*-infected bees having higher and more prolonged consumption than *N. apis*-infected bees (Mayack and Naug 2009; Martín-Hernández et al. 2011). Despite higher sugar consumption, *N. ceranae*-infected workers still

have lower trehalose levels than uninfected workers suggesting that they are unable to compensate for the energetic stress (Mayack and Naug 2010). This energetic stress is likely a primary cause of death by *N. ceranae* as infected bees have similar mortality to healthy bees when feeding *ad libitum* (Mayack and Naug 2009). *Nosema ceranae* causes higher bee mortality at seven days post-infection than *N. apis* at the same spore dose, with mortality positively correlated with spore dose (10^3-10^5 spores per bee; Martín-Hernández et al. 2011). Furthermore, *N. apis*-infected workers only have higher mortality than healthy bees when fed initial doses above 5 x 10^4 spores per bee (Martín-Hernández et al. 2011). Overall, workers infected with either species have a shorter lifespan than healthy workers (Hassanein 1953; Malone et al. 1995; Malone and Gatehouse 1998; Woyciechowski and Moroń 2009; Dussaubat et al. 2013; Goblirsch et al. 2013).

Hypopharyngeal glands, used by workers to supply proteins and lipids to other individuals in the colony, are also affected by *Nosema* infection. Newly emerged workers must consume large amounts of protein to properly form their hypopharyngeal glands, which are used to produce larval food (sometimes called royal jelly or worker jelly). *Nosema apis* also causes a reduction in midgut proteolytic enzymes in infected workers, likely due to the lysis of infected midgut epithelial cells (Liu 1984; Malone and Gatehouse 1998). Thus, infected workers have a reduced ability to digest protein, which may explain the malformation of their hypopharyngeal glands. *Nosema*-infected workers have significantly smaller hypopharyngeal glands that are partial to non-functioning (Wang and Moeller 1969, 1971; Liu 1990; Alaux et al. 2010; Jack et al. 2016b). *Nosema apis*-infected workers also perform less larval and queen feeding duties than healthy workers (Wang and Moeller 1970).

Honey bee foraging behaviour is also affected with individuals infected by *Nosema* beginning foraging at an earlier age (referred to as precocious foraging), possibly because they are unable to perform nursing duties (Hassanein 1953; Wang and Moeller 1970; Goblirsch et al. 2013; Natsopoulou et al. 2016). These infected bees are also more likely to get lost returning to the colony from foraging trips (Kralj and Fuchs 2009). *Nosema ceranae*-infected foragers take more flights in a day than uninfected bees, possibly because they are inefficient foragers, to satisfy their hunger, or perform cleansing flights (Dussaubat et al. 2013). *Nosema apis*-infected bees are more likely to undertake risky behaviour such as foraging in poor weather (Woyciechowski and Kozłowski 1998).

Additionally, *Nosema* infection causes other physiological changes to the immune system and pheromone production. While *N. apis* causes an increased immune response in infected bees, *N. ceranae* causes a decrease in the transcription of several antimicrobial peptides during the first several days post-infection (Antúnez et al. 2009; Chaimanee et al. 2012). *Nosema*-infected bees have higher ethyl oleate levels that are positively correlated with the number of spores (Dussaubat et al. 2010). Ethyl oleate is produced by foragers and inhibits the transition of house bees to foragers (Grozinger 2015). Therefore, infected bees may be reducing the flight activity of healthy bees (Dussaubat et al. 2013). The suppression of immune response by *N. ceranae* may also increase infected bees' susceptibility to other diseases and viruses.

Pathology at the Colony Level

If the individual pathological effects caused by *Nosema* are large enough, the infection can affect the functioning of the colony as a whole. For example, *N. apis* has been associated with reduced colony populations and brood production (Farrar 1947; Hassanein 1953; Fries

1988; Anderson and Giacon 1992). Additionally, heavily infected colonies are more likely to have infected queens that lay non-hatching eggs before they stop laying altogether. Eventually, these queens are superseded, and occasionally, when there are no suitable eggs or larvae to develop into queens, the colony could remain queenless and subsequently perish (Farrar 1947). Poor spring build-up of colony populations leads to lower worker populations, which can impact colony productivity and performance. *Nosema apis* has been correlated with lower honey production and pollen collection as well as increased winter losses (Farrar 1947; Fries 1988; Anderson and Giacon 1992).

The colony-level effects of *N. ceranae* appear to vary between study locations. For example, in Spain, the proportion of infected foragers was negatively correlated with brood production and worker bee population (Higes et al. 2008; Botías et al. 2013a). Also, when infected by *N. ceranae*, untreated colonies produced significantly less honey than treated colonies (Botías et al. 2013a). *Nosema ceranae* was also associated with sudden depopulation and death in some regions (Higes et al. 2008, 2009b; Botías et al. 2013a). In contrast, a long-term study in Germany found that *N. ceranae* infections in the spring or fall were not correlated with colony losses during the following summer or winter (Gisder et al. 2010). A similar lack of effects on colony strength or winter mortality were reported in Canada (Williams et al. 2011; Desai and Currie 2016). However, a recent study from Ontario found that *N. ceranae* was negatively correlated with bee population and food stores, but not colony mortality (Emsen et al. 2020).

Seasonal Prevalence of Nosema

Awareness of the seasonal trends of parasites will inform the appropriate timing of treatments to prevent outbreaks (see below). *Nosema apis* has a well-established seasonal pattern in honey bees with the highest infection in the spring, a low summer infection, another smaller peak in the fall, and a slow increase over the winter (Fries 1993; Guzmán-Novoa et al. 2010; Copley et al. 2012). The high spring infection level is believed to be caused by old workers cleaning contaminated comb in preparation for increased brood rearing. The infection level drops in late spring as the winter bees die and are replaced by new workers (Bailey 1955). In contrast, *N. ceranae*, which has been less well studied, shows highly variable seasonality due to as yet undetermined factors. For instance, high levels of *N. ceranae* have been detected in the winter in Germany (Higes et al. 2008), in the summer in Quebec (Copley et al. 2012), and in the late spring in the United States (Traver et al. 2012). However, recent studies from eastern Canada have shown *N. ceranae* has a higher abundance in the spring and early summer and low fall levels (Emsen et al. 2020; McCallum et al. 2020). Data on long-term trends in the western prairie region of Canada are needed to inform the management of this pathogen.

Treatment

Fumagillin was determined to be an effective treatment against *N. apis* shortly after this drug was isolated from *Aspergillus fumigatus* Fresenius in the 1950s (Hanson and Elbe 1949; Elbe and Hanson 1951; Katzelson and Jamieson 1952; Bailey 1953a; Webster 1994). In Canada, it is registered as a treatment against nosema under the trade name Fumagilin-B® in the form of fumagillin dicyclohexylamine. Fumagillin acts against the MetAP-2 enzyme, which is found in both microsporidian and honey bee and only affects actively growing stages, not spores (Huang

et al. 2013). It is unclear whether fumagillin reduces *Nosema* infection by reducing infection within infected bees or killing infected bees, as N. apis-infected bees treated with fumagillin had higher mortality than healthy bees treated with fumagillin in cage trials (Furgala and Boch 1970). The label recommends using a maximum dose of 100 mg fumagillin/colony in the spring and 200 mg fumagillin/colony in the fall.

Colonies treated with fumagillin in the fall had lower *N. apis* abundance in the following spring than untreated colonies (Furgala 1962; Furgala and Boch 1970; Furgala et al. 1973). In comparison, fall fumagillin treatment suppressed *N. ceranae* in the following spring, but within a few months post-treatment, spore abundance was similar between treated and control colonies (Williams et al. 2008a, 2011; Higes et al. 2011; Mendoza et al. 2017). Alternative chemotherapies have been investigated, but none have been as effective as fumagillin (Botías et al. 2013b; van den Heever et al. 2016). There are other management options; for example, fecal-covered equipment can be disinfected effectively through irradiation (Pernal et al. 2010). However, this procedure is expensive and requires sending equipment to a specialized facility. Heat treatment and fumigation with acetic acid of infected comb have also shown some success (Pernal et al. 2010). Currently, in Alberta, it is recommended to apply fumagillin treatment in the spring and/or fall when the generally accepted threshold of one million spores per bee is exceeded (Alberta Agriculture and Forestry 2020a). However, this nominal threshold, used throughout North America, was established for *N. apis* infections and thus needs updating.

The Relationship between *Nosema* and Winter

Alberta and Winter Colony Management

Alberta has a wide range of bioregions across the province due to its vast area and geographical features. Approximately 68% of managed colonies in Alberta are located in the Grassland and Parkland bioregions (Alberta Agriculture and Forestry 2020b). The Grassland bioregion of southern Alberta is warm and dry with milder winters due in part to the winter Chinook winds that extend as far north as Red Deer, AB (Natural Regions Committee 2006). These Chinook winds occur when cool, moist air from British Columbia travels over the Rocky Mountain range and becomes hot, dry air as it enters southern Alberta, creating significantly milder winter conditions when they occur. The Parkland bioregions in central Alberta have a shorter plant growing season and a lower mean annual temperature by 1.7℃ than the Grasslands (Natural Regions Committee 2006).

An important aspect of beekeeping management in Canada relates to preparing colonies for overwintering. In order to survive the winter, colonies must have adequate food stores, large bee populations in fall, low levels of pests and diseases, and additional beekeeper-provided protection from low temperatures. Beekeepers can choose to winter their colonies outdoors or indoors. Colonies that are overwintered outdoors are wrapped in insulated winter wraps to protect them from the elements and trap heat, though bees can still enter and exit the hive. Colonies that are overwintered indoors are moved to a wintering facility maintained between 4-5°C, with proper ventilation, and kept dark to prevent bee flight (Currie et al. 2015). Beekeepers account for cost, convenience, and risk when choosing a wintering method. In Alberta, 81% of colonies are wintered outdoors, and the remaining 19% are wintered indoors (Canadian Association of Professional Apiculturists 2020).

Nosema Effects in Different Wintering Environments

Winter management methods have significant potential to alter the dynamics of honey bee disease epidemiology. Bees typically defecate while flying when the ambient temperature is above 10℃ (Retschnig et al. 2017). Temperatures colder than 10℃ prevent bees from defecating through "cleansing" flights, and consequently, bees hold their feces within their rectum. The inability of bees to take cleansing flights increases *Nosema* spore abundance in the gut (Retschnig et al. 2017). Indoor-wintered colonies cannot perform cleansing flights due to lack of light; however, the stable, cool temperatures maintain optimal cluster conditions, which could reduce stress, a factor that often exacerbates disease. Conversely, outdoor-wintered colonies can be exposed to extreme temperature fluctuations, which may increase stress, but allow for occasional cleansing flights during short periods of warm weather in the mid or late winter.

Canadian studies have suggested that wintering environment does not influence *Nosema*. Williams et al. (2010) found wintering method did not affect *N. ceranae* abundance when treated with fumagillin in Nova Scotia. Additionally, studies from eastern Canada show *Nosema* does not cause winter mortality in Ontario (Guzmán-Novoa et al. 2010) and Nova Scotia (Williams et al. 2010). However, it should be noted that these study locations (southern Ontario and the Maritime provinces) experience somewhat milder winters with more chances for bees to perform cleansing flights than the Canadian Prairies. Desai and Currie (2016) found that *Nosema* abundance increased in colonies wintered outdoors but decreased in colonies wintered indoors when colonies were managed in different regional beekeeping zones in Manitoba. Similarly, lengthy Alberta winters (5-6 months) could have a greater impact on *Nosema* and colony health due to the long period of winter stress. It is anticipated that long winters with consistently cold

temperatures could increase spore build-up within the bees in central Alberta, forcing them to defecate within the hive, which in turn could increase the spread of infection. However, short periods of warm weather that more commonly occur in southern Alberta due to Chinook winds could provide more opportunities for winter defecation flights than in central Alberta, which tends to have consistently cold temperatures throughout the winter.

Rationale for Research

Beekeepers need to suppress most parasites and pathogens to low levels for the colonies to be productive. Implementing an effective pest management strategy relies on having accurate information on pests and their treatments. With the emergence of *N. ceranae*, more information is needed on how this species affects colony performance and whether fumagillin is an effective treatment. Current research on *N. ceranae* has shown highly variable results, possibly due to regional differences. Additionally, the epidemiology of *Nosema* may be affected by winter conditions as cold temperatures and different wintering methods (indoor and outdoor) provide varying levels of temperature stress and defecation flight opportunities. The goal of this study was to increase knowledge of the epidemiology, impact, and control of *Nosema* under Canadian Prairie apicultural management. More specifically, this study aimed to characterize the seasonal pattern of *Nosema* abundance and its impacts on honey bee colonies and assess the effectiveness of fumagillin treatment at reducing *Nosema* abundance and increasing colony productivity and survival. Furthermore, this study examined possible differences in *Nosema* abundance, *Nosema* species, and fumagillin effectiveness in different wintering environments using indoor and outdoor wintering management in two different climate zones in Alberta.

Chapter 2: Epidemiology of *Nosema* in untreated honey bee colonies and its effect on colony size and survival under indoor and outdoor wintering in the Canadian Prairies

Abstract

The epidemiology of *Nosema* may be affected by winter conditions as cold temperatures and differing wintering methods (indoor and outdoor) provide varying levels of temperature stress and defecation flight opportunities. Across the Canadian Prairies, including Alberta, the length and severity of winter vary among geographic locations. This study investigates the seasonal pattern of *Nosema* abundance in two Alberta locations using indoor and outdoor wintering methods and its impact on bee population, survival, and economic viability. This study found that *N. ceranae* had a distinct seasonal pattern in Alberta, with high spore abundance in spring, declining to low levels in the summer and fall. The results showed that fall *Nosema* monitoring might not be the best indicator of treatment needs or future colony health outcomes. There was no clear pattern for differences in *Nosema* abundance by location or wintering method. However, wintering method affected survival under pressure from the pathogen. Colonies wintered indoors had one-fifth the mortality at similar *Nosema* abundance and more rapid spring population build-up than outdoor-wintered colonies. Consequently, the existing *Nosema* threshold should be lower for outdoor-wintered colonies than those wintered indoors to reflect this difference. Furthermore, the results suggest that mitigation of temperature stress associated with indoor wintering decreased the impact of *Nosema* infections on colonies more than any potential benefits associated with weather allowing late winter cleansing flights.

Average *Nosema* abundance in the spring was a significant predictor of end-of-study winter colony mortality, highlighting the importance of spring *Nosema* monitoring and treatments.

Introduction

Nosema apis Zander and *Nosema ceranae* Fries et al. are spore-forming obligate parasites of the midgut epithelial cells of honey bees. Initially, *Nosema* infection in *Apis mellifera* Linnaeus was only caused by *N. apis*, but in 2006, *N. ceranae* was also identified in *A. mellifera* (Higes et al. 2006), and now both species are prevalent in honey bees worldwide (Klee et al. 2007). *Nosema ceranae* has become the predominant species in many regions, suggesting that it is replacing *N. apis* (Klee et al. 2007; Paxton et al. 2007; Chen et al. 2008; Martín-Hernández et al. 2012). In Europe, the proportion of *N. ceranae* infections appears to be greater in warmer climates than temperate climates (Fries 2010). Regional differences in the relative dominance of *N. ceranae* may be due to *N. ceranae* tolerating higher temperatures than *N. apis*, whereas *N. apis* is more cold-tolerant (Burnside and Revell 1948; Woyciechowski and Czekonska 1999; Martín-Hernández et al. 2009; Fries 2010). However, *N. ceranae* is also successful in cold climates, having become the most prevalent species in Canada and Siberia, suggesting temperature is not as important as previously thought (Williams et al. 2008a, 2010; Currie et al. 2010; Copley et al. 2012; Emsen et al. 2016; Ostroverkhova 2020; MacInnis et al. 2020).

Nosema infection has been shown to affect colony strength and productivity, with *N. apis* associated with reduced bee populations and brood and honey production as well as increased winter losses (Farrar 1947; Hassanein 1953; Fries 1988; Anderson and Giacon 1992). However, the effect of *N. ceranae* on the colony is less clear. Higher proportions of infected foragers have been negatively correlated with brood production and worker bee population in Spain (Higes et

al. 2008; Botías et al. 2013a). Multiple studies have found *N. ceranae* to be associated with sudden depopulation and colony death (Higes et al. 2008, 2009b; Botías et al. 2013a). In contrast, a long-term study in Germany found that *N. ceranae* infections in the spring or fall were not correlated with colony losses in the following summer or winter (Gisder et al. 2010). A similar lack of effects on colony strength or winter mortality was reported in Manitoba (Desai and Currie 2016) and Nova Scotia, Canada (Williams et al. 2011). However, a recent study from Ontario found that *N. ceranae* was negatively correlated with bee population and food stores, but not colony mortality (Emsen et al. 2020). More research is needed to understand the effect of *N. ceranae* on honey bee colonies under various regional climatic and seasonal conditions and the variability observed in colony-level effects.

Awareness of the seasonal trends of parasites informs the appropriate timing of treatments to prevent outbreaks. *Nosema apis* has a well-established seasonal pattern in honey bees with the highest spore abundance in the spring, lower spore abundance in summer, and typically another smaller peak in the fall followed by a slow increase over the winter in colonies wintered outdoors (Fries 1993; Guzmán-Novoa et al. 2010; Copley et al. 2012). In contrast, studies to date on *N. ceranae* have shown high variability and seasonality that is difficult to predict (Higes et al. 2008; Copley et al. 2012; Traver et al. 2012; Emsen et al. 2020; McCallum et al. 2020). Data on *Nosema* seasonality in Canada's prairie region are needed to inform the management of this pathogen.

Nosema can be controlled using fumagillin, a product registered in Canada under the trade name, Fumagilin-B. Alberta's current control recommendations are to apply fumagillin in the spring and fall when spore abundance is above one million spores per bee (Alberta Agriculture and Forestry 2020a). However, this nominal threshold used throughout North

America was established for *N. apis* infections and has not been appropriately validated for either *N. apis* or *N. ceranae* under different beekeeping winter management and climatic conditions.

Due to its vast area and geographical features, Alberta has a wide range of bioregions across the province that differ in climate and vegetation. The majority of managed colonies in Alberta are located in the Parkland and Grassland bioregions (Alberta Agriculture and Forestry 2020b). The Grassland bioregion in southern Alberta is the hottest and driest region and experiences milder winters due in part to the winter Chinook (warming) winds that extend as far north as Red Deer, AB (Natural Regions Committee 2006). The Parkland bioregion in central Alberta has a shorter growing season and a lower mean annual temperature by 1.7°C than the Grassland (Natural Regions Committee 2006). The warmer temperatures in the Grassland may reduce *Nosema* abundance due to decreased winter temperature stress on the honey bees. Additionally, both the Grassland and Parkland are heavily cultivated, with natural vegetation dominated by grasses in the Grassland and aspen trees in the Parkland zone (Natural Regions Committee 2006). The lack of tree windbreaks in the Grassland may affect outdoor wintering survival, and beekeepers often add a windbreak if shelter is not available.

Honey bee diseases can become particularly detrimental to colonies in winter (Currie et al. 2010). Bees typically defecate while flying when the ambient temperature is above 10°C (Retschnig et al. 2017). Winter temperatures prevent bees from defecating through such "cleansing" flights, causing bees to hold their feces within their rectum for long periods of cold weather, increasing *Nosema* spore abundance in the gut (Retschnig et al. 2017). Eventually, bees may be forced to defecate within the hive, which could increase the spread of infection (Retschnig et al. 2017). In Canada, colonies can be either wintered outdoors or indoors. Colonies

that overwinter outdoors are wrapped with insulating covers to protect them from the elements and trap heat, but bees can still enter and exit the hive on warmer days. In contrast, colonies that overwinter indoors are moved in the autumn (late October) into buildings that are temperature regulated at 4-5°C with constant air exchange and air remixing where the colonies are always kept in the dark to prevent bee flight (Currie et al. 2015). Therefore, different wintering management options provide varied defecation potential and exposure to a range of temperatures during the long Canadian Prairie winters.

How these differences in climate and management may affect *Nosema* is poorly understood. Outdoor wintering may allow for defecation flights, thus reducing their *Nosema* spore load. Additionally, the intermittent short periods of warm weather brought to southern Alberta by Chinook winds could provide more opportunities for winter defecation events than in central Alberta, which typically has consistently cold temperatures throughout the winter. However, indoor wintering may reduce stress, a factor that often exacerbates disease, by avoiding extreme temperature fluctuations.

The objectives of this study were to: (1) characterize the patterns of seasonal variation in *Nosema ceranae* abundance in honey bee colonies over two years; (2) assess the pattern of *Nosema* abundance in different climatic zones within Alberta (Parkland and Grassland bioregions); (3) assess the impact of different wintering management methods (indoors versus outdoors) on *Nosema*, and (4) characterize the impact of variation in *Nosema* in these environments on honey bee colony population, survival, and economic viability.

Methods

Experimental design

The study ran continuously from June 2017 to April 2019 and spanned two winters. Honey bee colonies borrowed from local beekeepers were located in two apiaries near Edmonton, Alberta (53°38'49.5 "N 113°21'25.5 "W and 53°39'32.2 "N 112°38'38.2 "W), and in two apiaries near Rainier, Alberta (50°22'33.6 "N 112°05'19.3"W and 50°23'50.0"N 112°06'41.6"W), which are within the Parkland and Grassland bioregions of Alberta, respectively (Natural Regions Committee 2006). Hereafter, the apiaries near Edmonton will be referred to as "North" (as it is North of Rainier) and the Rainier apiaries as "South". These locations were separated by 370 km. Within regions, North apiaries were 47 km apart, and South apiaries were 3 km apart. In each apiary, of approximately 40 colonies, eight colonies were randomly selected for this study.

At the beginning of the study, the adult bee and brood population were equalized for all 40 colonies in each apiary to make them as similar in size as possible. This equalization occurred from May 29-30 in the North apiaries and June 7-8 in the South apiaries. Several days before equalization, existing queens were removed from the colonies. On the day of equalization, adult bees from all colonies were shaken into a large, screened box (52" L x 24" W x 28.5" H). Next, all available brood, pollen, and honey frames were shared equally among the colonies. Then, a scoop was used to distribute the bees equally among the colonies. The colonies were fed 3.8 L of 2:1 sugar syrup from in-hive feeders, and the entrances were screened for 2-3 days to ensure the bees were retained in their new hives. All colonies were given new mated queens (Kona Queen Hawaii, USA) 1-3 days post-equalization, all of which were marked with a paint dot on their thorax. After equalization, each apiary had 40 equivalent single chamber colonies with newly

mated queens. Additional brood chambers containing empty frames and honey and pollen frames were added when the bee population became too large for a single brood chamber.

In each apiary, eight colonies were selected for this longitudinal study, for a total of 32 colonies. One colony was removed from the study entirely as the colony never accepted the introduced queen, and the colony population collapsed before the second sampling date. Therefore, the study started with 31 colonies. Colonies were not treated with fumagillin for the entirety of the study. For winter 2017-2018, one apiary was wintered outdoors, and the other apiary was wintered indoors at each of the two locations. In mid to late October, the outdoor-wintered colonies were wrapped with a commercial western insulated wrap with a top pillow, usually in groups of four on a pallet as per standard practice for the region (Currie et al. 1998). When only three colonies were on a pallet, a stack of empty boxes was used to stand in for the fourth colony so that the wrap fits properly. Colonies were provided with a top entrance hole in the front for ventilation, which allowed the bees to exit and re-enter the hive. Pallets of colonies that were wintered indoors were transported approximately 13 km from the apiary site to a wintering building in each location and stored in stacks of five high in several rows, along with non-experimental colonies from the beekeeping operation. The wintering buildings were maintained at 4-5℃ with a ventilation rate of 0.25 L/s per colony (in the wintering building) and were kept dark to prevent bee flight (Currie et al. 2015). For winter 2018-2019, each remaining live colony received the same wintering treatment that it had previously. Additionally, if a colony became queenless during the study, this was noted and it was given a mated replacement queen (Kona Queen Hawaii, USA).

Due to the distance between locations, the North and South were sampled/evaluated on alternating weeks with sampling periods ranked in ascending order within locations (e.g. first

sample taken =1, second sample taken =2, etc.). Sampling periods in different locations with the same rank were considered sampled at the same time. For example, North sample 1 on June 12-13, 2017 and South sample 1 on June 15-16, 2017 were grouped. Within a location, apiaries were sampled on the same day or one day later. For ease of reference and analysis, dates are presented as the average date of the sampling periods within the same rank (e.g. sample 1 for both locations is dated June 14, 2017). Table 2.1 shows the timeline for colony sampling and bee population evaluations.

Varroa *monitoring and control*

Varroa populations were monitored throughout the study and maintained below the economic threshold (3%). Samples of approximately 300 bees were collected from the interior brood frames approximately every two weeks beginning in June 2017 and continuing until colonies were prepared for winter. Sampling resumed in the spring once colonies were taken out of the wintering building and winter wraps were removed. Samples were stored in 70% ethanol at room temperature until further processing. *Varroa* infestation level (recorded as mites per 100 bees) was estimated using an alcohol wash. To dislodge the mites from the bees, the sample jars were placed on an orbital shaker for 10 minutes at 300 rpm. Next, the bee sample was poured into a strainer above a basin and rinsed with a sink sprayer for 1-2 min to separate the mites from the bees. The number of mites in the basin was recorded. The number of bees in the sample was estimated by dividing the wet weight of the bees by the average weight from three samples of ten bees. *Varroa* was controlled in all colonies with Apivar® (500 mg Amitraz/strip) at the beginning of September in 2017 and 2018 as some colonies had infestation levels above the 3 mites per 100 bees fall threshold (Nasr and Muirhead 2017).

Nosema *abundance*

Worker bees were also collected approximately every two weeks for *Nosema* analysis, beginning in June 2017 until colonies were wintered and the same sampling regime resuming following the winter. For these samples, approximately 100 adult bees were collected from either the outer honey frames in the brood chamber or from honey supers (Fries et al. 2013). For the first sample following winter, colonies that died over the winter were also sampled for *Nosema* but by collecting dead bees from the bottom boards. Bee samples were stored in 70% ethanol at room temperature until further processing. Samples were prepared for analysis by grinding the abdomens of 30 bees with 5 mL water in a 35 mL conical tissue grinder. The solution was then poured into a new 50 mL conical tube. An additional 10 mL of water was used to rinse the grinder and tube separately, then poured into the same conical tube. The total amount of water used was 15 mL (0.5 mL/abdomen), allowing for a minimum detection level of 25,000 spores/bee. Samples were vortexed before being pipetted onto both sides of the hemocytometer to ensure an even distribution of spores. The samples were allowed to settle for 1 minute after being loaded into the hemocytometer, and both sides were counted (Cantwell 1970). After conversion to account for dilution, values were averaged to produce a unit of spores/bee. Samples that contained more than approximately 100 spores per square were further diluted to ensure accurate counting.

Determining Nosema species

To determine which *Nosema* species were infecting the colonies, composite apiary samples from June 12-16, 2017, and April 24 and 26, 2018, were analyzed (8 samples total). All colonies from within the same apiary had *Nosema* samples of 30 bees frozen using liquid nitrogen and crushed in a mortar and pestle. An equal portion (measured in g) of the crushed sample from each colony was mixed to create an equivalent 30 bee sample. Crushed bee samples were stored at -80℃ until DNA extraction.

DNA was extracted from ~100 µL of homogenized bee using the DNeasy Blood and Tissue Kit (Qiagen, Mississauga, ON, Canada) with the QIAcube (Qiagen) automatic DNA extraction instrument along with its associated protocol. The lysing step with proteinase-K and Buffer ATL was done overnight using a thermomixer set to mix at 500 rpm for 15 s every 30 min. DNA concentration and purity were determined using a NanoDrop Lite spectrophotometer (Thermo Scientific, Wilmington, DE, USA). DNA samples were stored at -20℃ until further processing.

DNA samples were quantified for *N. apis* and *N. ceranae* using an Applied Biosystems Quantstudio 6 Flex (Thermo Scientific, Wilmington, DE, USA) qPCR instrument. Each 20 µL reaction contained 10 µL SsoFast Evagreen Supermix (Bio-Rad, Hercules, CA, USA), 1 µL primer (F + R), 5 µL DNA template, and 4 µL nuclease-free water. For *N. apis* and *N. ceranae*, the primers Na-321 (Martín-Hernández et al. 2007) and Nc-104 (Bourgeois et al. 2010) were used, respectively (both primers were obtained from Integrated DNA Technologies, Coralville, IA, USA). The primer Actin181 (Chen et al. 2005) was used for detecting bee actin (Integrated DNA Technologies). For each sample, the *Nosema* reactions were run in triplicate with a positive bee actin control. Each plate had a *Nosema* standard curve from 10^4 to 10^8 of synthesized target

sequences (GBLOCK; Integrated DNA Technologies) as well as negative primer controls and no-template controls. All qPCR protocols were performed using under the following conditions: initial denaturation of 95℃ for 3 min, PCR cycling (40 cycles of 95℃ for 15s, 55℃ for 30s, and 72℃ for 30s), and melt curve analysis (raising the temperature from 65℃ to 95℃ in 0.5℃ increments with a hold of 5 seconds for each increment).

Estimating adult bee population

The population of adult bees was estimated approximately once each month through the active beekeeping season (April-September). Depending on weather conditions, one of two methods was used to estimate bee population. The first method for measuring bee population during warm weather involved recording the percent (to the nearest 25%) of bees covering each surface (front and back) of every Langstroth frame (48 x 23 cm for one side) in the hive and multiplying by the known number of bees to cover a frame. It was assumed that 2430 bees fully cover both sides of a frame (Burgett and Burikam 1985). The second method for assessing the bee population was done by determining the colony cluster size to approximate bee population when inclement weather prevented a full assessment (late fall and early spring). This was done by viewing the top of the cluster from the top and bottom brood chambers and counting the number of frames covered by bees (adapted from Nasr et al. 1990). A cluster size estimate was used in April 2018, September 2018, and April 2019. Adult bee population was expressed as number of bees, whereas cluster size uses number of frames with bees. A colony was considered dead when no live queen or bees were left in the hive and recorded at the time of sampling. In the spring (April 2018, April 2019), colony viability was also assessed, with colonies being considered non-viable from a commercial standpoint when there were fewer than four frames of

bees in the colony (Canadian Association of Professional Apiculturists 2020). Queen presence, supersedure, and acceptance were recorded when observed throughout the study. When colonies became queenless, a new marked queen was introduced.

Statistical Analysis

The effects of sampling date, location, and wintering method on *Nosema* abundance, adult bee population, and cluster size were analyzed with PROC MIXED (SAS v.9.4) using a repeated measures design with colonies nested within apiary as the subject and sampling date as the repeated measure using the REML statement (restricted maximum likelihood). *Nosema* abundance was logarithmically transformed to meet the assumption of normality. The Kenward-Roger Degrees of Freedom Approximation was used to adjust for issues with homogeneity of variance. The following covariance structures were found to have the best fit; heterogeneous first-order autoregressive for *Nosema* abundance and first-order autoregressive for adult bee population. When significant interactions occurred, the Slice option in the LSMEANS statement was used to partition the effects by location and wintering method to compare the differences between means within date. Only live colonies were used in the adult bee population and cluster size analyses. Additionally, contrasts were used to find differences in *Nosema* abundance, adult bee population, and cluster size before and after winter by location and wintering method. Contrasts were also performed to determine if any changes over winter were different between locations or wintering methods. Analyses were performed on the transformed data but are presented as untransformed means.

A multivariate analysis of the effects of average *Nosema* abundance, location, and wintering method on colony mortality and non-viability after winter was performed using a

binary logistic regression with backward elimination (PROC LOGISTIC, SAS v.9.4). Analyses were conducted for June 2017-April 2018 and April 2018-2019 as well as for the whole study (2017-2019). *Nosema* abundance was included in the analysis as either individual sampling dates or averages. Average *Nosema* abundance was calculated for the spring (April-June), summer (July-August), and fall (September), as well as the maximum *Nosema* spores/bee and average for the period leading up to winter. The whole study analyses included *Nosema* samples taken from dead bees (first sample after winter) that were collected separately. One colony was removed from the 2018-2019 logistic analysis due to having extremely high mite levels despite *Varroa* treatment. It should be noted that PROC LOGISTIC removes missing values for the response or explanatory variables from the analysis. This resulted in the removal of one dead colony from the first year and whole study analyses. The colony died early in the first year and would likely not change the results of the analyses. In the second year, only spring *Nosema* abundance (along with location and wintering method) was included in the analyses as most dead colonies died during the summer and fall.

Results

The study began with 31 colonies; however, by autumn, only 29 colonies were alive (Fig. 2.1). Six colonies (20.7%) died over winter 2017-2018, leaving 23 live colonies. Of these, an additional six colonies (26.1%) died during the summer of 2018. By winter 2018-2019, only 17 colonies were overwintered. One colony (5.9%) died over winter, leaving 16 of the 31 original colonies alive after 23 months.

All eight composite apiary bee samples that were analyzed using qPCR were infected with *Nosema*. In June 2017, all four apiaries were infected with only *N. ceranae*. In April 2018,

three of the apiaries were infected with only *N. ceranae*, whereas one North apiary was infected with both *N. ceranae* and *N. apis*. In the mixed infection, *N. apis* had approximately four times the number of copies as *N. ceranae*.

Nosema abundance varied over time (F=13.69, df=19, 100, P<.0001). In general, *Nosema* abundance was low in the late summer and fall and highest in the spring. *Nosema* abundance was significantly higher after winter than in samples taken before winter in September for both years (2017-2018: F=28.85, df= 1, 43.6, P<0.0001; 2018-2019: F=7.04, df=1, 23.1, P=0.014, Contrast). Furthermore, there was a significant interaction between location and date on *Nosema* abundance (F=1.81, df=18, 103, P=0.033; Fig. 2.2). The North colonies had significantly higher *Nosema* abundance than the South on July 28, 2017, and April 28, 2018 (P<0.05, Slice). Whereas the colonies in the North had lower *Nosema* abundance than the South on June 5 and 16, 2018 (P<0.05, Slice). *Nosema* abundance increased overwinter at both locations in 2017-2018 (P<0.05, Contrast). In 2018-2019, *Nosema* abundance increased overwinter only for North colonies (F=8.95, df=1, 23.8, P=0.0064, Contrast), whereas the South colonies had similar levels before and after winter (F=1.31, df=1, 22.5, P=0.26, Contrast). The relative change in *Nosema* abundance overwinter in North apiaries compared to South apiaries was not significantly different between locations for either winter (P>0.05, Contrast).

Wintering method had effects on *Nosema* spore abundance, but only in one year. After the first winter, outdoor-wintered colonies had a higher average *Nosema* abundance than indoor-wintered colonies, with the opposite occurring after the second winter (Fig. 2.3). However, the difference in either year was not significant (wintering method*sampling date: F=0.04, df=1, 100, P=0.47). In 2017-2018, *Nosema* abundance increased over winter for both wintering methods (P<0.05, Contrast). In 2018-2019, *Nosema* abundance increased overwinter for indoor-

wintered colonies (F=4.86, df=1, 23.8, P=0.037, Contrast) but did not change significantly for outdoor-wintered colonies (F=3.20, df=1, 22.5, P=0.087, Contrast). The relative change in *Nosema* abundance over winter was not significantly different between wintering methods for either winter (P>0.05, Contrast).

In this study, location did not affect adult bee population (live colonies) over time (F=1.07, df=8, 153, P=0.38). In contrast, there was a significant interaction among wintering method and sampling date on adult bee population (F=2.01, df=8, 153, P=0.048). Indoor-wintered colonies built up larger bee populations than outdoor-wintered colonies during the subsequent period of population growth in spring and summer, from June to July 2018 (P<0.05, Slice; Fig. 2.4). Over winter 2017-2018, adult bee population was significantly lower after winter than before winter (F=123.72, df=1, 158, P<0.0001, Contrast). The change in adult bee population over winter was not significantly different between locations or wintering methods (P>0.05, Contrast). Neither location nor wintering method affected cluster size (number of frames with bees) in September 2018-April 2019 (F=0.33, df=1, 11.8, P=0.58, and F=1.85, df=1, 12.4, P=0.20, respectively). Cluster size decreased overwinter 2018-2019 in both locations (P<0.05, Slice). Over the winter of 2018-2019, the cluster size of surviving colonies decreased for indoor-wintered colonies (F=27.36, df=1, 11.2, P=0.0003, Slice), whereas outdoor-wintered colonies that survived had similar cluster sizes before and after winter (F=2.01, df=1, 11.8, P=0.18, Slice). The change in cluster size overwinter in 2017-2018 did not differ among locations or wintering methods (P>0.05, Contrast).

Multivariate analyses showed that colonies with higher *Nosema* abundance in summer 2017 (July-August) were more likely to die by the following spring (April 25, 2018) (χ^2=3.9486, df=1, P=0.047; Fig. 2.5). When the average summer *Nosema* abundance was lower than the one

million spores/bee threshold, there was less than a 20.5% chance of mortality. In the second year, higher spring 2018 (April-June) *Nosema* abundance significantly increased the probability of mortality in the following spring (April 5, 2019) (χ^2=4.8374, df=1 P=0.028; Fig. 2.6). There was less than a 3.1% chance of mortality when the average spring *Nosema* abundance below the one million spores/bee threshold. Over both years, colonies with a higher two-year average *Nosema* abundance (June 2017-September 2018) were more likely to die by the end of the second winter (χ^2=4.9830, df=1, P=0.026; Fig. 2.7). Additionally, outdoor-wintered colonies were more likely to die at a lower *Nosema* abundance than indoor-wintered colonies. At the one million spores/bee threshold, outdoor-wintered colonies were almost five times as likely to die than indoor-wintered colonies (48.9% and 9.9%, respectively). More specifically, colonies with a higher two-year average *Nosema* abundance in the spring (June 2017, April-June 2018) were more likely to die by the end of the study (χ^2=4.8524, df=1, P=0.028; Fig. 2.8). There was less than a 16.7% chance of mortality by the end of the study when the two-year average spring *Nosema* abundance was below the one million spores/bee threshold. The probability of having non-viable surviving colonies in the spring was not predicted by *Nosema* abundance, location, or wintering method.

Discussion

This study demonstrates a distinct seasonal pattern of *N. ceranae* abundance in untreated colonies under Canadian Prairie conditions, specifically in Alberta, with high spore loads in spring declining to low levels in the summer. Unlike traditional patterns of *N. apis*, an increase in the fall period when beekeepers usually monitor for *Nosema* was not observed for *N. ceranae* in this study. This has implications for managing this pathogen as fall assessments may not be the best indicator of future colony health outcomes, *Nosema*-induced stress, or treatment

requirements. There was no consistent pattern for differences in *Nosema* abundance by location, possibly due to the highly variable nature of this pathogen or the small number of sites. Similarly, wintering method did not consistently affect *Nosema* abundance following winter. However, colonies that were wintered indoors had lower mortality at equivalent *Nosema* abundance and faster spring population build-up than outdoor-wintered colonies. Also, when averaged over the two-year study, *Nosema* abundance in the spring was a significant predictor of end-of-study colony mortality, highlighting the importance of spring *Nosema* monitoring and treatments.

In this study, apiaries were either infected predominately with *N. ceranae* or, to a lesser degree, co-infected with *N. ceranae* and *N. apis*. This represents an increasing trend of *N. ceranae* becoming the dominant form of this pathogen in Canada. In 2010, 41% of Alberta colonies had single *N. ceranae* infections, and 25% had infections containing both species (Emsen et al. 2016). Recent studies in Ontario, Quebec, and Maritime Provinces also show *N. ceranae* as the predominant species (Williams et al. 2008a, 2010; Currie et al. 2010; Copley et al. 2012; Emsen et al. 2016). While this study did not include only pure infections of *N. ceranae* throughout the study, it is reasonable to assume that *N. ceranae* is the species causing the seasonal patterns and impact on colonies observed in this study, given its numerical dominance.

Although several studies in Europe showed unpredictable seasonal patterns for *N. ceranae* (Martín-Hernández et al. 2007, 2012, Higes et al. 2008), our results from 4 sites over two years showed a consistent spring peak and low summer and fall levels, supporting the findings of previous North American studies (Pernal et al. 2010; Traver et al. 2012; Emsen et al. 2020; McCallum et al. 2020). In contrast, a 3-year study in Quebec, Canada, found *N. ceranae* infection peaks were at a different time for each year of the study, including the fall, summer,

and spring (Copley et al. 2012). High variation in their study could be due, in part, to the study's small sample size (8 colonies total). The seasonal pattern for *N. ceranae* seen in this study has some similarities to the established pattern for *N. apis*, which has a spring peak and low summer levels, but unlike *N. ceranae*, typically shows a prominent fall peak (Fries 1993; Guzmán-Novoa et al. 2010; Copley et al. 2012). It should be noted that it is possible that our sampling would have missed a potential late fall peak as sampling ended in September in both years. A recent study from Nova Scotia found *N. ceranae* had a small peak in October though it did not exceed the 1 million spores/bee threshold (McCallum et al. 2020). Typically, beekeepers monitor for *Nosema* in the early fall (September) to determine if treatment is needed. However, if the fall peak is detected in October based on the current threshold, it would be too late to treat for the coming winter under Alberta conditions. The difference in fall *Nosema* abundance for these two species further demonstrates that sampling timing when using the nominal one million spores/bee threshold needs to be reassessed in order to make sound control decisions and prevent economic losses.

This study found there was no consistent pattern for differences in *Nosema* abundance by location within Alberta. It was predicted that *Nosema* abundance would be lower in the South than in the North, where the average annual temperature is higher by 2.1℃ and colonies are often exposed to warm periods in winter that allow for defecation flights (Appendix 1). During *Nosema* sampling, the South was consistently warmer than the North (Appendix 2). However, at most sampling dates, there was no difference in *Nosema* abundance between the two locations. When differences did occur, sometimes the North had higher *Nosema*, and sometimes it was the South. Additionally, *Nosema* abundance increased in both locations overwinter in 2017-2018, but in 2018-2019, only colonies in the North saw an increase in *Nosema* over winter. The winters

in the South varied substantially between the two years, and abnormally low temperatures late in the first winter may explain the relatively high winter *Nosema* abundance. March 2018 had no days above 10°C, whereas March 2019 had 11 days, which is more consistent with the 20-year average (Appendix 1, 2). Although bees performing cleansing flights during warm periods near the end of the second winter may have allowed them to reduce their spore load, it should be noted that the change in *Nosema* abundance overwinter was not different between the North and South for either winter. Also, the small number of sites in this study may not adequately represent Alberta's full climatic variation.

In this study, the effects of wintering method on *Nosema* abundance in fumagillin-free colonies sampled after winter were not consistent. After the first winter, outdoor-wintered colonies had numerically greater *Nosema* abundance than indoor-wintered colonies. In contrast, *Nosema* abundance increased over the second winter for indoor-wintered colonies, while outdoor-wintered colonies had similar abundance before and after winter. It appears that factors from before winter caused differences in wintering treatment. A similar lack of consistency was seen in Nova Scotia where overall there was no effect of wintering method on *Nosema* abundance in fumagillin-treated colonies, except for one beekeeping operation where outdoor wintered colonies had higher abundance than indoor-wintered colonies (Williams et al. 2010). In Manitoba, Desai and Currie (2016) found that *Nosema* abundance decreased in indoor-wintered colonies but increased in outdoor colonies. It should be noted that both treated and untreated colonies were included in their analysis.

However, wintering method had a significant impact on colony performance related to *Nosema* infection level. Indoor-wintered colonies were less likely to die from *Nosema* infections and had larger bee populations in the following summer than outdoor-wintered colonies. This

supports other Canadian studies that have demonstrated that colonies infected with high levels of parasites and/or pathogens were more likely to survive when wintered indoors than when wintered outdoors (Williams et al. 2010; Bahreini and Currie 2015; Desai and Currie 2016). The difference in colony mortality between wintering methods at the same *Nosema* abundance suggests that the threshold for damage tolerated by beekeepers from *Nosema* infection should be lower for colonies that will be wintered outdoors to have survival similar to indoor-wintered colonies. The reduced population growth of outdoor-wintered colonies may also be due to *Nosema*. Although not significantly different, *Nosema* abundance in outdoor-wintered colonies trended higher than indoor-wintered colonies from May to mid-July. *Nosema ceranae* infection has been shown to reduce colony population growth in other studies (Higes et al. 2008; Botías et al. 2013a; Villa et al. 2013; Emsen et al. 2020). These results showed that indoor wintering is an attractive option for beekeepers looking to reduce mortality due to *Nosema* and increase summer colony population size.

It was predicted that temperature stress and cleansing flight opportunities could impact *Nosema* abundance, but the relative importance of these factors was unknown. Indoor-wintered colonies experience lower temperature stress and no cleansing flights, whereas outdoor-wintered colonies would have greater temperature stress with cleansing flight opportunities varying with the local climate. The results of this study show that the wintering method (indoor versus outdoor) affected the impact of *Nosema* on colony performance. This study found that colonies with similar *Nosema* abundance performed better when wintered indoors than wintered outdoors. These results suggest that mitigation of temperature stress during indoor wintering affected the impact of *Nosema* infections on colonies more than any benefits associated with the availability of late winter cleansing flights. This may be due to cleansing flight opportunities being

unpredictable and short-lived, while reduced temperature stress can occur all winter. These results do not preclude the possibility that other factors, such as variation in relative humidity in the different environments or mid-winter brood-rearing levels, could have also affected the results.

Nosema abundance was found to be a significant predictor of colony mortality. At the end of the first year (April 25, 2018), the probability of colony mortality was predicted by summer *Nosema* abundance (July - August 2017). In contrast, spring *Nosema* abundance (April-June 2018) predicted colony mortality at the end of the second year (April 5, 2019). The difference between the first and second years may be due to missing the spring *Nosema* peak in 2017. The spring peak in the second year of this study occurred in April to May, but spring 2017 included only one *Nosema* sample taken in June. However, this appears to be accounted for by taking the two-year average as *Nosema* abundance in the spring (June 2017, April-June 2018) was a significant predictor of end-of-study colony mortality. While previous Canadian studies have found that *N. ceranae* was not correlated with mortality (Guzmán-Novoa et al. 2010; Williams et al. 2010, 2011; Desai and Currie 2016; Emsen et al. 2020), these studies restricted sampling to the fall through to the spring as it was assumed that fall *Nosema* abundance were most likely to predict winter mortality. Perhaps these studies would have found associations between *Nosema* and mortality if sampling had been carried out in the spring to early summer. Our study has shown that spring and summer *Nosema* abundance is a better predictor of mortality when colonies are not treated than early fall abundance. Coupled with the lack of a fall peak, this suggests that spring fumagillin treatments may be more important than fall fumagillin treatments in managing this species of *Nosema* in Alberta.

In conclusion, this study demonstrates the presence of a seasonal pattern of *N. ceranae* abundance in the Canadian Prairies, with higher *Nosema* abundance in the spring than in summer or early fall. This study found no consistent evidence that *Nosema* is a greater threat to colony health in the colder 'North' region of Alberta than the relatively warmer South. There was no consistent effect of wintering method on *Nosema* abundance; however, indoor wintering was more effective in mitigating the impact of *Nosema* than outdoor wintering under equivalent *Nosema* abundance. Also, indoor-wintered colonies had greater populations in the following spring than their outdoor-wintered counterparts. Therefore, to achieve similar mortality to indoor-wintered colonies, the one million spores/bee threshold as assessed in spring needs to be lowered for colonies being wintered outdoors. This study found clear evidence that spring *N. ceranae* impacts the health of the colony, as the probability of survival decreased with increasing *Nosema* loads above 1 million spores/bee. Further research is needed to determine appropriate seasonal thresholds for *N. ceranae* and how wintering method interacts with pests and disease to affect colony health and survival. It also needs to be determined if beekeepers could benefit more from treating *Nosema* in the spring than in the fall, as our results suggest it may have a greater impact in reducing colony mortality.

Tables and Figures

Table 2.1 – Timeline for honey bee sampling and bee population estimates from May 2017 to April 2019 in Alberta colonies. Dates are presented as the average date for the sampling period.

Date	Description
May 29-June 8, 2017	Colony Equalization
June 14, 2017	Sampling
July 15, 2017	Sampling & Bee Population
July 28, 2017	Sampling
August 12, 2017	Sampling & Bee Population
August 26, 2017	Sampling
September 10, 2017	Sampling & Bee Population
September 27, 2017	Sampling & Bee Population
April 25, 2018	Sampling & Cluster Size
May 8, 2018	Sampling & Bee Population
June 5, 2018	Sampling & Bee Population
June 16, 2018	Sampling
June 30, 2018	Sampling & Bee Population
July 14, 2018	Sampling
July 28, 2018	Sampling & Bee Population
August 12, 2018	Sampling
August 24, 2018	Sampling & Bee Population
September 8, 2018	Sampling
September 22, 2018	Sampling & Cluster Size
April 5, 2019	Sampling & Cluster Size
April 29, 2019	Sampling (only South apiaries)

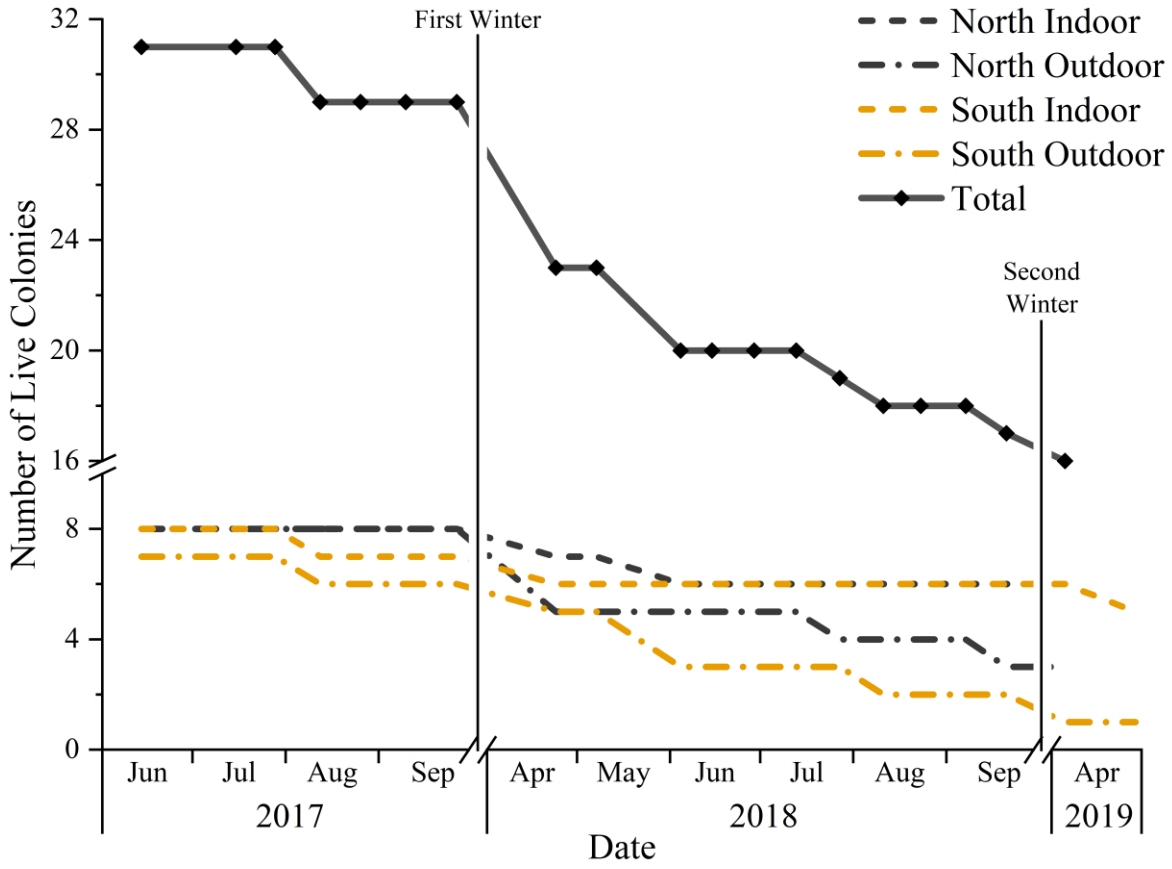

Fig. 2.1 – Number of live colonies in total and by apiary (location and wintering method) over 23 months with x-axis breaks with vertical lines indicating winter.

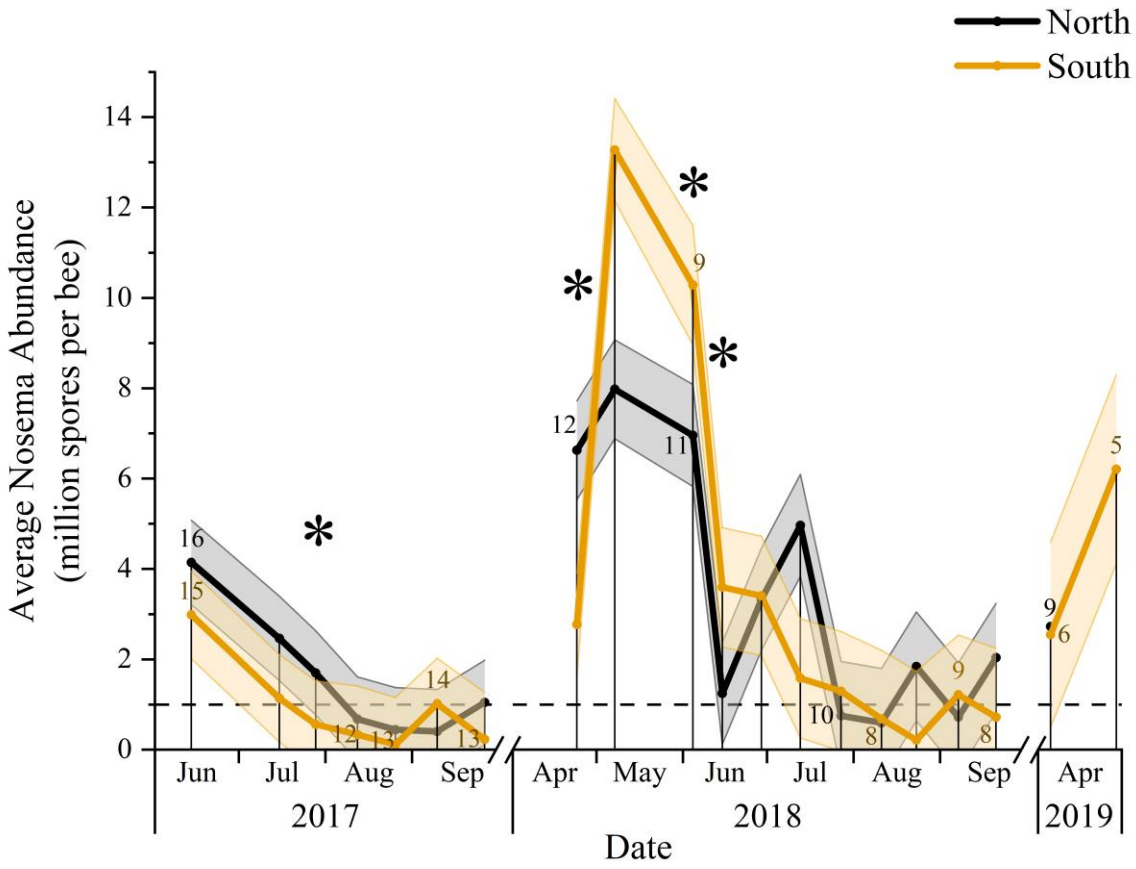

Fig. 2.2 – Influence of geographic location on average *Nosema* abundance over 23 months. North represents colonies near Edmonton and South represents colonies near Rainier. Data are plotted as untransformed means. Standard error of the mean is represented by the light-coloured areas around the mean line. Number labels indicate the number of colonies used to calculate the mean. Points that do not have a number label had the same number of observations as the previous point. Dashed line shows the 1 million spores/bee nominal threshold. Drop lines indicate dates when samples were taken. Asterisks indicate significant differences ($P < 0.05$, Slice) between locations within dates.

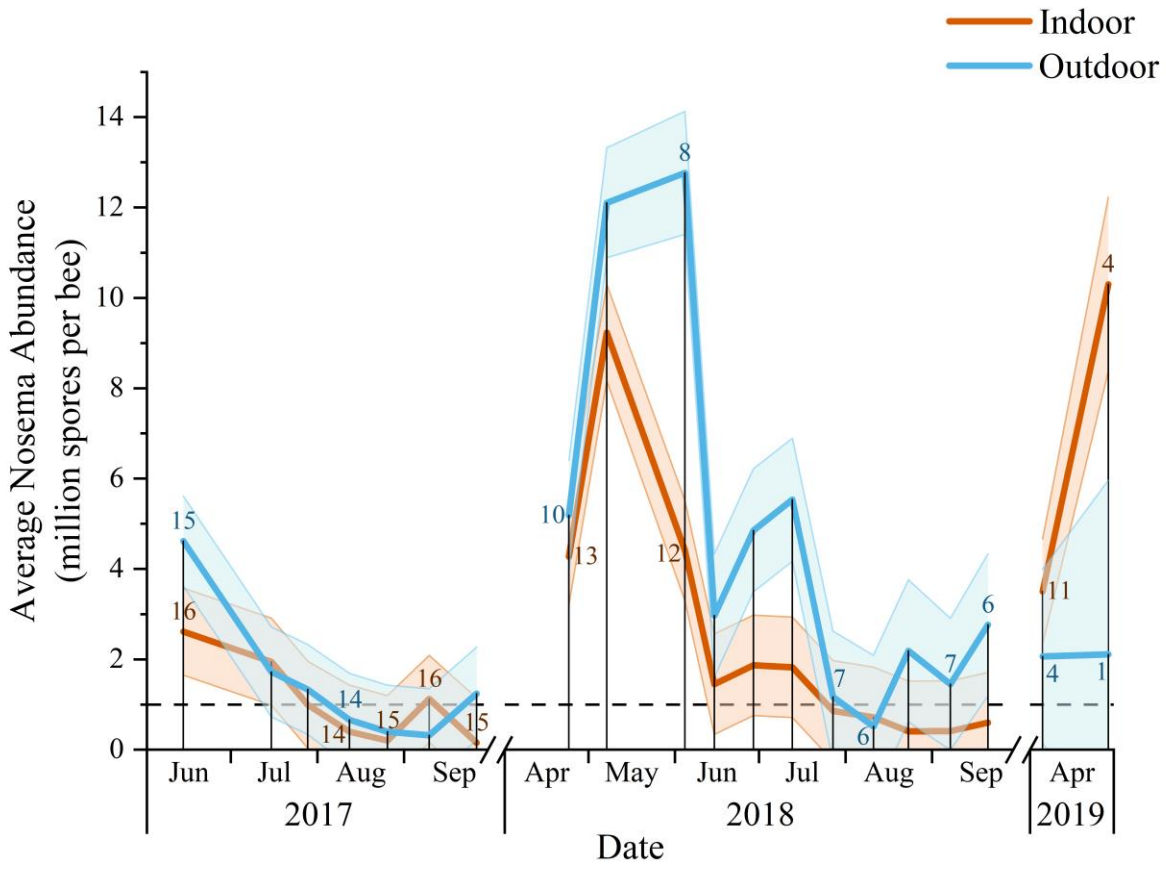

Fig. 2.3 – Influence of wintering method on average *Nosema* abundance over 23 months. Indoor represents colonies moved to an indoor wintering building, and outdoor represents colonies kept outdoors in insulating wraps. Data are plotted as untransformed means. Standard error of the mean is represented by the light-coloured areas around the mean line. Number labels indicate the number of observations used for the mean. Points that do not have a number label had the same number of observations as the previous point. Dashed line shows the historical 1 million spores/bee nominal threshold. Drop lines indicate dates when samples were taken.

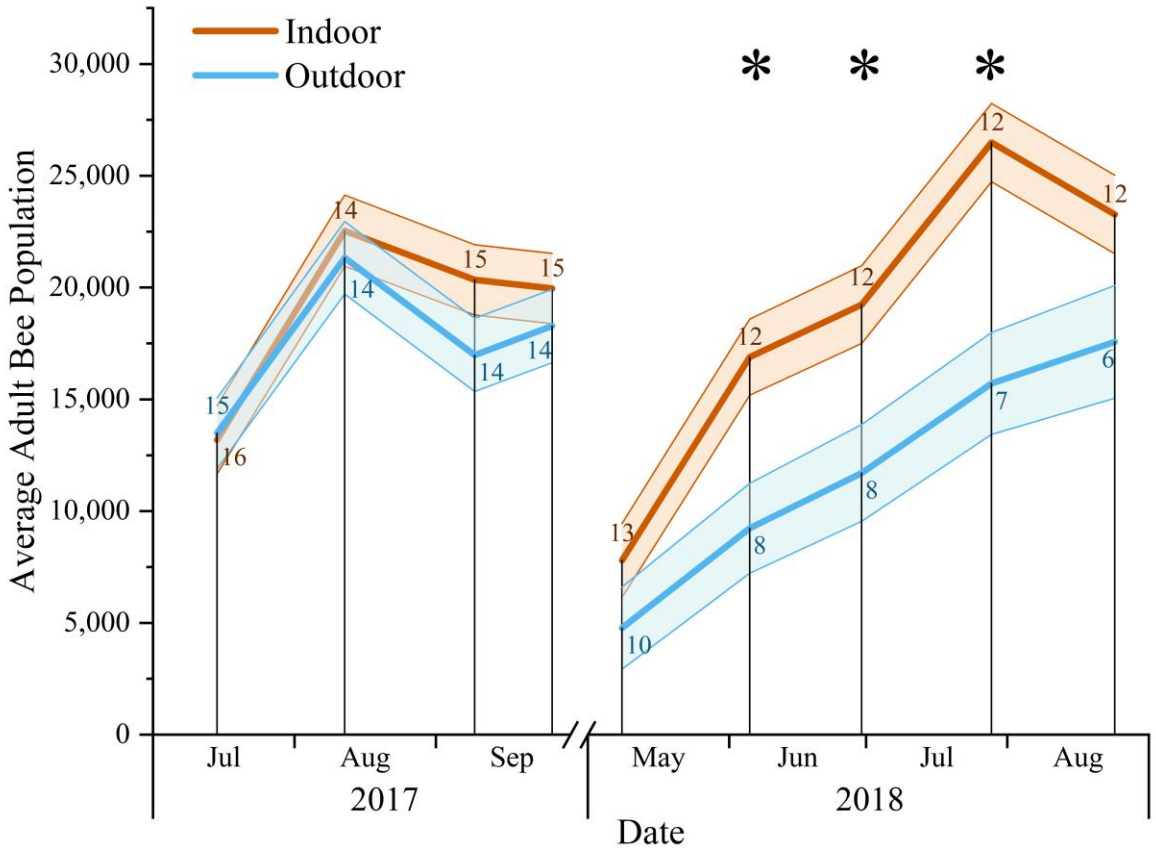

Fig. 2.4 – Influence of wintering method on average adult bee population (live colonies) over 23 months. Indoor represents colonies moved to an indoor wintering building, and outdoor represents colonies kept outdoors in insulating wraps. Standard error of the mean is represented by the light-coloured areas around the mean line. Number labels indicate the number of colonies used to calculate the mean. Drop lines indicate the date when samples were taken. Asterisks indicate significant differences between wintering methods.

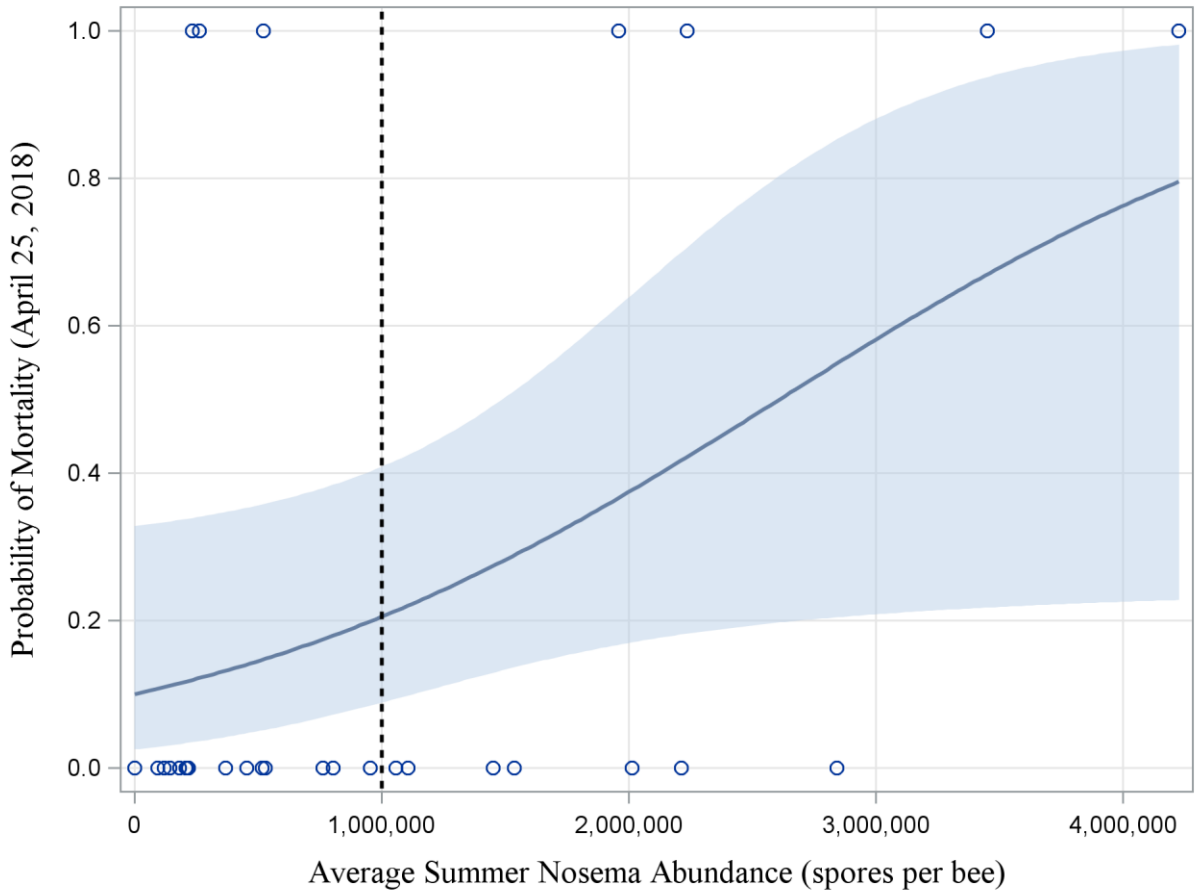

Fig. 2.5 – The effect of average *Nosema* abundance in the previous summer (July-August, 2017) on the predicted probability of having dead colonies in spring 2018 (cumulative colony mortality on April 25, 2018). Shaded area is the 95% confidence limit. Dashed line shows the 1 million spores/bee nominal threshold.

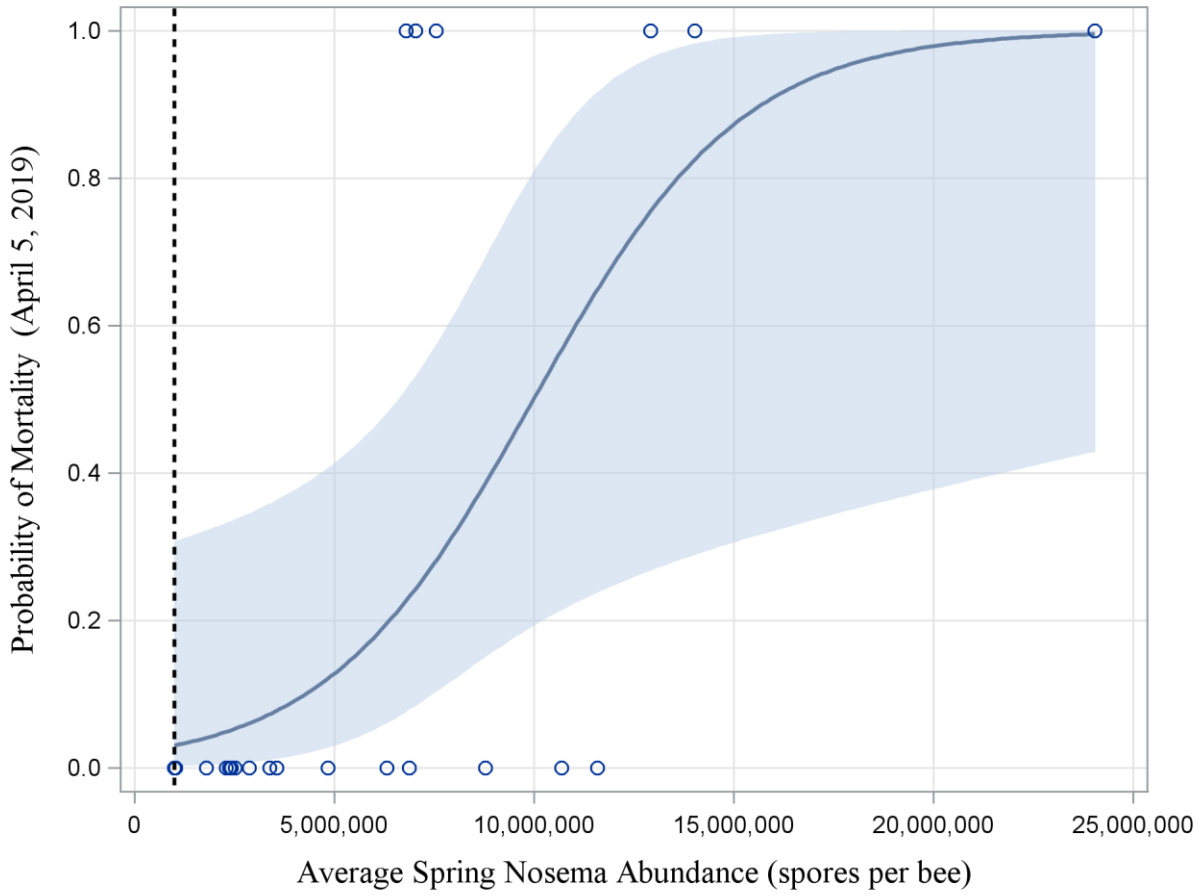

Fig. 2.6 – The effect of average *Nosema* abundance in the previous spring (April - June, 2018) on the predicted probability of having dead colonies in spring 2019 (cumulative colony mortality on April 5, 2019). Shaded area is the 95% confidence limit. Dashed line shows the 1 million spores/bee nominal threshold.

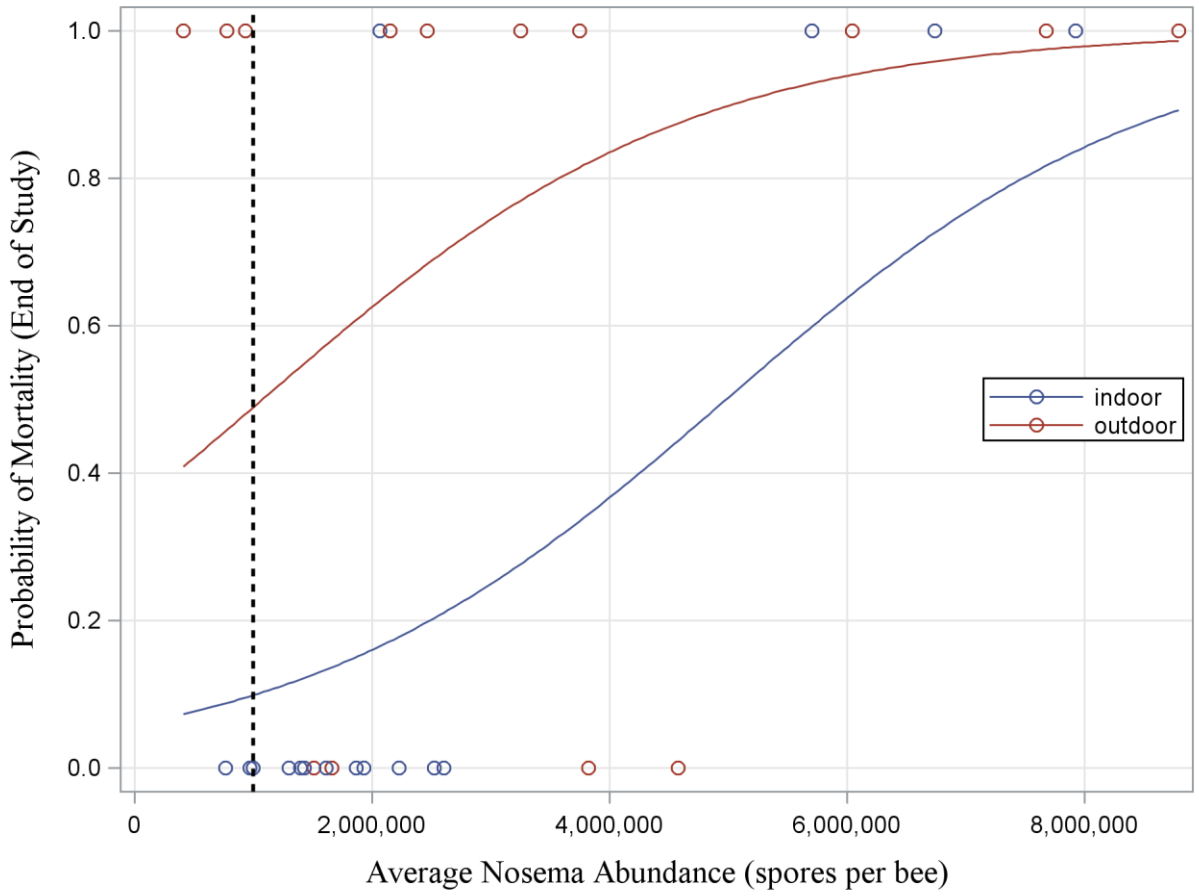

Fig. 2.7 – The effect of wintering method on the predicted probability of observing dead colonies at the end of 23 months (cumulative mortality at the end of the study) as influenced by the average *Nosema* abundance over the study (June 2017-September 2018). Indoor represents colonies moved to an indoor wintering building, and outdoor represents colonies kept outdoors in insulating wraps. Dashed line shows the 1 million spores/bee nominal threshold. Samples of dead bees taken from the bottom board (due to the unavailability of live bees in the colony) are included in the average.

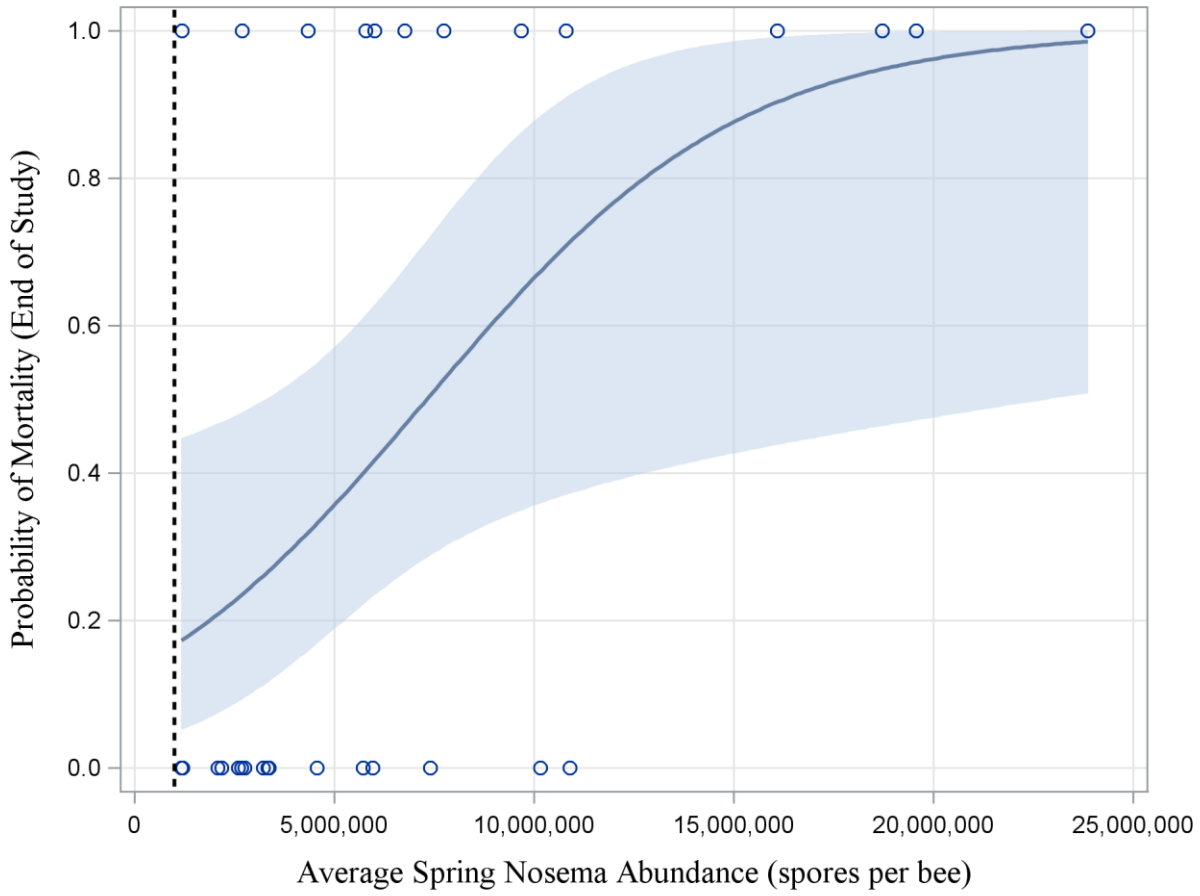

Fig. 2.8 – The effect of two-year average spring *Nosema* abundance over the study (June 2017, April-June 2018) on the predicted probability of observing dead colonies at the end of 23 months (cumulative mortality at the end of the study). Shaded area is the 95% confidence limit. Dashed line shows the 1 million spores/bee nominal threshold. Samples of dead bees taken from the bottom board (due to the unavailability of live bees in the colony) are included in the average.

Chapter 3: Effect of Fumagilin-B® treatment timing on *Nosema* spp. and honey bee colony strength and survival under indoor and outdoor winter management in the Canadian Prairies

Abstract

Nosema infection has been shown to negatively affect honey bee colony productivity and survival. Fumagilin-B® is the only product registered in Canada to combat this parasite; however, it is unclear whether fumagillin treatment consistently reduces the abundance of *Nosema ceranae* spores and improves honey bee colony strength and survival under Canadian management and climatic conditions. This study assessed the effects of combinations of spring and fall fumagillin treatment on *Nosema* abundance and colony strength and mortality in different beekeeping regions within Alberta using indoor or outdoor wintering management practices. Colonies were assigned to one of four treatments: Spring-only, Fall-only, Spring-and-Fall, and Control (no treatment). The spring treatment was administered using the drenching method at a dose of ~68 mg/colony, whereas the fall treatment was given in bulk at a dose of 120 mg or 48 mg/colony, depending on the year. In this study, the colonies were infected predominately with *N. ceranae*, with *Nosema apis* present only in mixed infections. The prevalence and composition of mixed infections varied over time and location, with mixed infections being more prevalent in the North following winter in one year. Although fumagillin treatment in either the spring or fall did reduce *Nosema* abundance in the short term, it did not eliminate the infection at the doses used in this study. Therefore, continued monitoring is necessary. Colony strength and productivity were improved by spring treatment in some locations but not consistently, possibly due to the treatment being applied late in the spring

below the label recommended dose. Spring treatment combined with fall treatment increased colony survival over winter in one of two years, but there was no interaction between treatment and wintering method. Wintering method also did not interact with fumagillin treatment to affect *Nosema* spore abundance in the spring. There did not appear to be a significant residual effect of fumagillin over winter, as none of the three fumagillin treatments reduced spring *Nosema* abundance or increased colony population. Therefore, spring treatment should be considered to reduce spring *N. ceranae* abundance rather than relying on residual efficacy from treatments the previous fall.

Introduction

Nosema is an economically important disease in honey bees (*Apis mellifera* Linnaeus) caused by two species, *Nosema apis* Zander and *Nosema ceranae* Fries et al. Of these two species, *N. ceranae* has become the dominant form in most beekeeping regions (Klee et al. 2007; Paxton et al. 2007; Chen et al. 2008; Martín-Hernández et al. 2012). With the emergence of *N. ceranae* in Canada, research is needed to determine the effect of *N. ceranae* on honey bee colonies.

Nosema can be controlled using fumagillin, a product registered in Canada under the trade name, Fumagilin-B. Since its discovery, fumagillin has been an effective treatment against *N. apis* (Bailey 1953a; Webster 1994), with fall treatment causing reduced *N. apis* abundance in the following spring (Furgala 1962; Furgala and Boch 1970; Furgala et al. 1973). *Nosema ceranae* infections are also suppressed by fumagillin treatment (Pernal et al. 2009; Giacobino et al. 2016; McCallum et al. 2020), but the infection returns in the following months (Williams et al. 2008a; Higes et al. 2011; Mendoza et al. 2017). Furthermore, treatment for *N. ceranae* may

not improve colony strength or productivity (Williams et al. 2011; Giacobino et al. 2016; Mendoza et al. 2017). Alberta's current control recommendations are to apply fumagillin in the spring and fall when spore abundance is above one million spores per bee (Alberta Agriculture and Forestry 2020a). However, this nominal threshold used throughout North America was established for *N. apis* infections and has not been appropriately validated for either *N. apis* or *N. ceranae* under different beekeeping winter management and climatic conditions.

Lengthy Canadian Prairie winters are a significant challenge for honey bee colony survival. Cold winter temperatures prevent bees from performing defecation flights, causing bees to hold their feces within their rectum for long periods, increasing *Nosema* spore abundance in the gut. Eventually, bees may be forced to defecate within the hive, which could increase the spread of infection (Retschnig et al. 2017). In Canada, colonies can be wintered outdoors in insulated winter wraps that allow the bees to enter or exit the hive on warmer days. Alternatively, colonies can be wintered indoors in buildings maintained between 4-5°C with air circulation and ventilation systems, with colonies kept in the complete darkness or red light only to prevent bee flight (Currie et al. 2015). Although outdoor wintering may allow for defecation flights during milder weather, thus reducing their *Nosema* spore load, indoor wintering may reduce stress, a factor that often exacerbates nosema infection, by providing an environment with warmer ambient temperatures and avoiding extreme temperature fluctuations. Wintering management methods must be considered when exploring issues related to honey bee diseases in Canada.

The objectives of this study were to: (1) assess the effects of spring and/or fall fumagillin treatment on *Nosema* abundance, adult bee population, total brood area, and honey production in two geographic regions within Alberta using different wintering management methods (indoors

versus outdoors) and (2) characterize the effect of treatment combinations in these environments on colony mortality.

Methods

Experimental design

Experimental honey bee colonies borrowed from local beekeepers were located in two apiaries near Edmonton, Alberta (53°38'49.5"N 113°21'25.5"W and 53°39'32.2"N 112°38'38.2"W), and two apiaries near Rainier, Alberta (50°22'33.6"N 112°05'19.3"W and 50°23'50.0"N 112°06'41.6"W) (see Chapter 2 for details). For simplicity, the Edmonton apiaries will be referred to as "North" (as it is North of Rainier) and the Rainier apiaries as "South." In each apiary, 32 colonies of approximately 40 colonies were randomly selected for this study. Colonies were placed on pallets in groups of four, with two colonies facing the same direction on either side.

This study was comprised of two experiments with the same treatments but minor differences in experimental design. The first experiment of this study was carried out from May 2017-May 2018. Before the study, the adult bee and brood population were equalized for all colonies in each apiary, as described in Chapter 2. For winter 2017-2018, all of the colonies from each apiary were either wintered outdoors or indoors at each of the two geographic regions. In the late autumn, the outdoor-wintered colonies were wrapped with a western insulation wrap with a top pillow, usually in groups of four as per standard practice (Currie et al. 1998). Indoor-wintered colonies were transported to a wintering building in each location and stored along with non-experimental colonies from the beekeeping operation. The wintering building was maintained at 4-5℃ with a ventilation rate of 0.25 L/s per colony (in the wintering building) and was kept dark to prevent bee flight (Currie et al. 2015).

The surviving colonies from the first experiment were used in the second experiment of the study, which was carried out from June 2018-April 2019. Additional colonies were added to make up for any colony mortality in the previous experiment. Again, the colonies were equalized on May 23 and 25 in the North and May 29-30 in the South as previously described. However, for winter 2018-2019, half of the colonies from each treatment group in each apiary were randomly assigned to be moved to an indoor wintering facility. The other half remained in the apiary to be wintered outdoors. Additionally, if a colony became queenless during the study, this was noted, and it was given a mated replacement queen (Kona Queen Hawaii, USA).

Treatments

Colonies were blocked by pre-experiment *Nosema* abundance and then randomly assigned to one of the following four treatments: (1) Spring Only treatment, (2) Fall Only treatment, (3) Spring and Fall treatment, and (4) Control (no treatment). Each treatment group had eight replicate colonies per apiary site for a total of 32 colonies per treatment. The formulated product Fumagilin-B® (Medivet Pharmaceuticals, High River, Alberta) was used for both spring and fall treatments. To ensure a homogenous mixture, Fumagilin-B was mixed with enough water to dissolve it before adding it to sugar syrup, as per the label instructions. This study intended to administer 120 mg of fumagillin per colony for each treatment so that the spring and fall could be directly compared. However, errors were made when converting from milligrams of active ingredient to grams of Fumagilin-B powder. The doses given are described below.

The spring treatment used the drenching method to encourage the bees to consume the fumagillin quickly. The treatment consists of 250 mL of treated 1:1 sugar syrup that was poured

onto the bees using a ladle four times, once every 4th-day. Each colony received 1 L of syrup for a cumulative dose of 68.25 mg of fumagillin over 13 days. Colonies that were not treated in the spring (Fall only and Control) were given non-medicated sugar syrup in the same manner. The spring treatments were given in the North apiaries from June 30-July 12, 2017, and June 13-25, 2018. The spring treatments were given in the South apiaries from July 6-18, 2017, and June 15-27, 2018. It should be noted that the treatment application occurred late in the spring as time and appropriate temperatures were needed to equalize colony populations and assign treatments.

The fall treatment used the bulk feeding method where 3.7 L of 2:1 sugar syrup containing fumagillin was provided in frame feeders. Colonies received a dose of 120 mg fumagillin per colony in 2017 and 48.46 mg per colony in 2018. Colonies that were not treated in the fall (Spring only and Control) were given non-medicated sugar syrup in the same manner. The fall treatments were given in the North apiaries on September 8, 2017, and August 31, 2018. The fall treatments were given in the South apiaries on September 6, 2017, and August 29, 2018.

Varroa *monitoring and control*

Varroa populations were monitored throughout both experiments. *Varroa* infestation level (recorded as mites per 100 bees) was estimated using an alcohol wash, as described in Chapter 2. Samples were collected from the interior brood frames every two weeks from spring until colonies were prepared for winter. At the beginning of September 2017 and 2018, all colonies were treated with Apivar® (500 mg Amitraz/strip) as some colonies had infestation levels above the fall threshold (Nasr and Muirhead 2017).

Nosema abundance

Bee samples were collected from either the outer honey frames in the brood chamber or from honey supers every two weeks from spring until colonies were wintered (Fries et al. 2013). *Nosema* samples were analyzed in the same manner as described in Chapter 2 and recorded as spores/bee.

Determining Nosema species

To determine which species were infecting the colonies, a subset of samples was selected from three time periods each year: before spring treatment, before fall treatment, and after wintering. For each year, two colonies from each treatment group (Spring, Fall, Spring-Fall, and Control) were randomly selected from each apiary. For the 2017-2018 field season, the sample dates were June 12-16, 2017, August 23-29, 2017, and April 24-26, 2018. For the 2018-2019 field season, the sample dates were June 4-7, 2018, August 20-29, 2018, and April 3-8, 2019.

Nosema samples of 30 bees were frozen using liquid nitrogen and crushed in a mortar using a pestle. DNA was extracted from ~100 µL of homogenized bee using the DNeasy Blood and Tissue Kit (Cat. No. 69504; Qiagen, Mississauga, ON, Canada) with the QIAcube (Qiagen) automatic DNA extraction instrument as described in Chapter 2. DNA samples were quantified for *N. apis* and *N. ceranae* using the methods described in Chapter 2.

Evaluating colony strength

Colony strength assessments based on measures of the adult bee population, total brood area, and stored honey were performed approximately once per month until the colonies were

wintered. Depending on weather conditions, one of two methods described in Chapter 2 was used to estimate bee population. A cluster size estimate was used in September 2018 when cold temperatures prevented a more detailed assessment. The two methods used different units with adult bee population being expressed as number of bees and cluster size using number of frames covered with bees.

Frames containing brood were photographed and analyzed using ImageJ (National Institutes of Health, USA) to calculate the square centimetres of capped brood per colony. This was done by inputting the height (top bar to bottom bar) of the frame and tracing the brood area. The amount of stored honey was estimated by recording the approximate percentage (to the nearest quarter) of capped honey on each side of every frame in the hive and converted to frames of honey per colony. Brood area was not evaluated after August 2018 due to inclement weather, which would harm the brood. Queen presence, supersedure, and acceptance were recorded when observed throughout the study.

Post-winter evaluation

Colonies were sampled for *Nosema* and *Varroa* twice. First, when indoor colonies were moved out of the wintering building, and outdoor colonies were unwrapped in the spring of the following year. The second sample was taken two weeks later. When temperatures were below 10℃, only bee population was estimated using cluster size as described in Chapter 2, which occurred in April 2018 and 2019. A colony was considered dead when no live queen or bees were left in the hive.

Statistical Analysis

The fumagillin treatments were split into two periods (spring and fall) and analyzed separately. The analyses of the spring treatment considered the groups Spring only and Spring-Fall as treated and Fall only and Control as untreated. The spring treatment analysis was restricted to June 14-August 12, 2017, and June 5-August 12, 2018. The analyses of the fall treatment included only the Fall only and Control treatments and were restricted to August 26-September 27, 2017, and August 24-September 22, 2018. All treatments (Spring only, Fall only, Spring-Fall, and Control) were considered separately for the *Nosema* abundance over winter and colony mortality analysis. One colony was removed from the 2017-2018 experiment as the accepted mated queen never laid eggs. Two colonies were removed from the 2018-2019 experiment as they were kept in a single brood chamber rather than a double chamber and had fewer frames to rear brood.

The effects of spring treatment, sampling date, and location on *Nosema* abundance, adult bee population, brood area, and honey were analyzed with PROC MIXED (SAS v.9.4) using a repeated measures design with hives nested within apiary as the subject and sampling date as the repeated measure using the REML statement (restricted maximum likelihood). *Nosema* abundance was logarithmically transformed to meet the assumption of normality. The Kenward-Roger Degrees of Freedom Approximation was used to improve the homogeneity of variance. When significant interactions occurred, an analysis of simple effects was used to test for differences between means within spring treatment by sampling date and location (Slice option, LSMEANS statement). Additionally, contrasts were used to test the treatment*time interaction for the change in *Nosema* abundance from before and after treatment application in the treatment

and control. Analyses were performed on the transformed data but are presented using the untransformed means in tables and figures.

The effects of fall treatment, date, and location on *Nosema* abundance, adult bee population, and brood area were analyzed with PROC MIXED (SAS v.9.4) using the same methods as above. Repeated measures were not used for adult bee population in 2018 as the units were different for the two sample dates (number of bees and number of frames). For 2018, brood area was not analyzed as there was only one sample date, which occurred before the fall treatment. When significant interactions occurred, an analysis of simple effects was used to test for differences between means within fall treatment by sampling date and location (Slice option, LSMEANS statement). Additionally, contrasts were used to test the treatment*time interaction to assess the change in *Nosema* abundance from before and after treatment application in the treatment and control groups.

The effect of fumagillin treatment, sampling date, location, and wintering method on *Nosema* abundance, adult bee population, and brood area following winter was analyzed with PROC MIXED (SAS v.9.4) using the same methods as above. When significant interactions occurred, post-hoc analyses were performed using a Bonferroni adjusted alpha value. This analysis was restricted to September 27, 2017-May 8, 2018, and September 22, 2018-April 5, 2019.

The proportion of colonies infected with either *N. ceranae*, *N. apis*, or both species in each location was compared within sampling month (June, August, or April) with Fisher's exact test. The effect of fumagillin treatment on colony mortality was analyzed using survivorship curve analysis (PROC LIFETEST, SAS v.9.4). This analysis was done separately for both years

(2017-2018 and 2018-2019). Pairwise comparisons between treatments and the control were performed using a log-rank chi-squared test.

Results

The first experiment (2017-2018) began with 127 colonies; however, by autumn, only 117 colonies remained alive (Fig. 3.1A). Over winter 2017-2018, 24 colonies (20.5%) died, leaving 93 live colonies the following spring. The second experiment (2018-2019) started with 126 colonies, with three colonies dying before winter (Fig. 3.1B). Over winter, 28 colonies (22.8%) died, leaving 95 live colonies.

Nosema species

In the 2017-2018 field season, colonies were infected only with *N. ceranae* as no *N. apis* was detected in the samples. *Nosema ceranae* was seen in all tested colonies at all three time periods (Table 3.1). In the 2018-2019 field season, colonies were infected with either only *N. ceranae* or both *N. ceranae* and *N. apis* (Table 3.1). In June 2018, *N. apis* was not detected in any of the tested colonies. Infection type prevalence was significantly different between the North and South apiaries in August 2018 ($P=0.014$, Fisher's exact test) and April 2019 ($P<0.0001$, Fisher's exact test). Nearly all colonies after winter (April) in the North were infected with both species, whereas just over half of colonies were infected with both species in August in the South (Fig. 3.2). Additionally, *N. ceranae* was dominant in mixed infections in the North, but *N. apis* was slightly more dominant in mixed infections in the South.

Spring treatment

In the spring of 2017, the main effect of fumagillin treatment and its interactions with sampling date and location on *Nosema* abundance were not significant (P>0.05; Table 3.2). However, the interaction between treatment and sampling date showed a tendency effect (P=0.081) that merited further exploration. Following spring treatment, treated colonies (Spring only and Spring-Fall) had marginally lower *Nosema* abundance than untreated colonies (Fall only and Control) on July 15, 2017 (F=3.5, df=1, 123, P=0.064; Fig. 3.3). Contrasts showed *Nosema* abundance decreased more sharply after treatment (July 15) for treated colonies than untreated colonies (F=3.93, df=1, 126, P=0.0497, Contrast; Fig. 3.3). However, no significant differences in the change in *Nosema* abundance between treatments were found for subsequent sampling dates (July 28: P=0.32; August 12: P=0.19, Contrast). The three-way interaction between spring treatment, location, and sampling date was significant for the effect on the adult bee population (F=5.09, df=1, 119, P=0.026) (Fig. 3.4). In the North, treated colonies had larger bee populations than untreated colonies, whereas in the South, bee population was similar among treated and untreated colonies (Table 3.3). Brood area was not affected by spring treatment alone or in combination with sampling date and location and sampling date (Table 3.2). There was a significant interaction of spring treatment and sampling date on the number of honey frames produced (F=3.68, df= 2, 117, P=0.028), but when partitioned by date, no significant differences were found (P>0.05, Slice).

In 2018, *Nosema* abundance was affected by an interaction between spring fumagillin treatment and sampling date (F=3.10, df=5, 288, P=0.0096) (Fig. 3.5). After spring treatment, treated colonies had lower *Nosema* abundance than untreated colonies on June 30, 2018 (F=15.46, df=1, 122, P=0.0001, Slice). However, treatment differences did not remain in

subsequent sample dates (July 14: P=0.16; July 28: P=0.78; August 12: P=0.12, Slice). In 2018, spring fumagillin treatment, sampling date, and location did not affect adult bee population, brood area, or honey production (Table 3.2).

Fall treatment

In the North apiaries in 2017, colonies treated with fumagillin in the fall had significantly lower *Nosema* abundance than untreated colonies (F=6.63, df=1, 112, P=0.011; Fig. 3.6). The change in *Nosema* abundance from before to after treatment application was not significantly different between treated and untreated colonies (F=1.45, df=1, 170, P=0.23, Contrast). For the adult bee population, the interaction between treatment, location, and sampling date showed marginal effects (P=0.067), but there were no significant differences between treated and untreated when partitioned by sampling date and location (P>0.05, Slice). Fall fumagillin treatment did not affect brood area before winter (Table 3.2).

In 2018, fall fumagillin treatment and its interactions with sampling date and location had no significant effect on *Nosema* abundance (Table 3.2). The change in *Nosema* abundance from before to after treatment application was not significantly different between treated and untreated colonies (F=0.06, df=1, 175, P=0.80, Contrast). Fall treatment also had no effect on adult bee population (F=0.005, df=1, 59, P=0.94) and cluster size (F=1.64, df=1, 57, P=0.21).

Post-winter effects

In 2017-2018, *Nosema* abundance was affected by an interaction between fumagillin treatment and sampling date (F=4.47, df=3, 176, P=0.0047; Fig. 3.7). Before winter, only Fall-

treated colonies had significantly lower *Nosema* abundance than control colonies (t=2.91, df=176, P=0.0040; Table 3.4). After winter, *Nosema* abundance was similar between all treated and control colonies. Fumagillin treatment and its interactions with sampling date, location, and wintering method had no significant effect on either adult bee population or brood area (Table 3.5).

In 2018-2019, fumagillin treatment did not affect *Nosema* abundance post-winter, nor were there interactions between treatment and sampling date, location, or wintering method (Table 3.5). There was an interaction between the effects of fumagillin treatment, sampling date, and location on cluster size (F=3.79, df=3, 77.9, P=0.014), but there were no significant differences between treatments and control within sampling date and location (Bonferroni-adjusted pairwise comparisons, P>0.0043).

Colony survival

In the first year (May 2017-2018), colony survival curves were not significantly different between the treatments and control (P>0.05; Fig. 3.8). In contrast, colony survival curves were affected by fumagillin treatment in the second year (June 2018-April 2019; Fig. 3.9). Colonies treated in the spring and fall had a greater probability of survival than control colonies (χ^2=4.76, df=1, P=0.029).

Discussion

This study evaluated the effects of fumagillin treatment on *Nosema* abundance and colony strength and mortality within Alberta using different winter management practices. The

colonies were naturally infected with predominately *N. ceranae*, although mixed infections with *N. apis* occurred in the second year. More mixed infections occurred in the Northern operation than in the Southern operation following winter. Since the northern sites in this study had colder temperatures during the study period, these results do not refute the theory that climatic differences may affect the regional prevalence of *N. ceranae*. Our fumagillin treatment in either the spring or fall reduced *Nosema* abundance but did not entirely eradicate *N. ceranae,* suggesting monitoring following treatment is necessary. Colony strength was improved in some locations following spring applications but not consistently, possibly due to our treatments being applied slightly after the spring *Nosema* peak below the label recommended dosage. Following winter, none of the three fumagillin treatments impacted spring *Nosema* abundance or colony strength of surviving colonies compared to the control. However, the data showed spring treatment in combination with fall treatment increased colony survival over winter in one of two years. Based on these results, previous fall fumagillin treatment did not appear to have enough residual activity to reduce post-winter *Nosema* abundance.

Nosema ceranae was found to be the predominant species in Alberta, with *N. apis* being present in one year of this study and always in combination with *N. ceranae*. In 2018-2019, the proportion of single and mixed infections varied over time and by location. Overall, more mixed infections occurred in the Northern operation than in the Southern operation following winter. Additionally, relative species composition in mixed infections tended to vary with location, with *N. ceranae* being more dominant in the North in April and *N. apis* being slightly more dominant in the South in August. The sudden appearance of *N. apis* was not likely due to the addition of imported colonies as *N. apis* was not present at the beginning of the experiment. More likely, *N. apis* was present, and some unknown factors created conditions suitable for its proliferation. This

study's results agree with previous research in Alberta that found that most colonies were infected with either only *N. ceranae* or both species (Currie et al. 2010; Emsen et al. 2016). Furthermore, several other Canadian studies have also found *N. ceranae* to be the predominant species in other provinces (Williams et al. 2008a, 2010; Currie et al. 2010; Copley et al. 2012; Emsen et al. 2016; McCallum et al. 2020). It has been suggested that regional *N. ceranae* prevalence is affected by climate as *N. apis* was believed to be better suited than *N. ceranae* to cold temperatures (Gisder et al. 2010; Fries 2010). These results do not refute this theory as the colder northern sites had more mixed infections with *N. apis* than the southern sites. However, *N. ceranae* was still more predominant in mixed infections suggesting cold climate may not provide much of an advantage to *N. apis* as previously thought. Further research is needed to ascertain what factors are affecting the success of *N. ceranae*.

Spring fumagillin treatment reduced *N. ceranae* abundance, supporting previous Canadian studies (Pernal et al. 2009; McCallum et al. 2020). Importantly, however, this reduction in *Nosema* abundance did not appear to have a consistent effect on colony strength. In the first year, treated colonies had larger adult bee populations than untreated colonies in the North following spring treatment. However, brood production was similar between treated and untreated colonies. Honey production did not increase from spring treatment in either year, possibly because the benefits in population size resulting from the spring treatment caused colonies to peak too late to exploit the nectar flow.

The lack of consistent colony-level benefits from spring fumagillin treatment observed in this study could be due to the timing of the treatment and the applied dosage. In both years, the spring treatment was applied in the late spring/early summer (June-July) due to time constraints in setting up the experimental colonies. The *N. ceranae* peak occurs in early spring, around May

(Traver et al. 2012; McCallum et al. 2020). By the time colonies were treated in this study, they had likely experienced the full effects of the spring *Nosema* peak. Additionally, the dosage used in this study (68.25 mg/colony) is slightly below (68-91%) the label recommended dosage of 75-100 mg for a two-chamber colony in the spring. However, the dosage used in this study was high enough to suppress *N. ceranae* abundance rather than increase spore proliferation, as seen in Huang et al. (2013) at doses less than 50 μg/L. Perhaps if the spring fumagillin treatment were applied before the peak at the higher label dosage, the reduced *Nosema* levels would have had a more significant impact on the colony's population and productivity. Further research is needed to determine the spring economic threshold of *N. ceranae* in Canada. Despite this, spring fumagillin treatment did appear to reduce *Nosema* in this study. To the best of my knowledge, this is the first study to investigate the effect of spring fumagillin treatment on colony strength metrics in colonies infected with *N. ceranae* in Canada. Additional research that tests the timing of spring treatment before and following the spring *Nosema* peak at a range of doses is needed to determine whether spring fumagillin treatment could be used to consistently increase bee population and honey production.

The effectiveness of fall fumagillin treatment differed between experiment years, possibly due to the different dosages used. In the second year, the fumagillin dosage was significantly lower than the first year (48 mg vs 120 mg) and did not reduce *Nosema* abundance. At the higher dosage, fall fumagillin treatment did reduce *Nosema* abundance before winter, supporting past studies from South America (Giacobino et al. 2016; Mendoza et al. 2017). However, a recent study from Nova Scotia found fall fumagillin treatment at an even higher dosage (200 mg/colony) did not reduce *Nosema* abundance (McCallum et al. 2020). It should be noted that their colonies were also treated in the spring, which may have reduced fall *Nosema*

abundance, making fall treatment less necessary. In contrast, this study evaluated the effectiveness of fall treatment by comparing colonies treated only in the fall to control colonies. Fall-only fumagillin treatment did not affect adult bee population or brood production before winter in either year. This was probably due to the natural reduction in population size that occurs in the fall which tends to obscure treatment effects. Additionally, fall treatment is usually applied with the intent of reducing the impact of *Nosema* during the winter and subsequent spring.

Following winter, this study found that previously treated colonies (Spring-only, Fall-only, or Spring-Fall) were not significantly different from control colonies in terms of spring *Nosema* abundance in both years. Also, fumagillin treatment did not improve colony strength, as seen in previous studies (Williams et al. 2008a, 2011; Mendoza et al. 2017). This suggests that the effects of neither the spring nor the fall treatment on *Nosema* abundance lasted until the following spring. Furthermore, the presence of *Nosema* in treated colonies after winter suggests that fumagillin does not completely eradicate *N. ceranae* and must be regularly monitored. In contrast, studies from Nova Scotia found that fall treatment reduced spring *Nosema* abundance, though a higher dosage of 190 mg/colony was used in those studies (Williams et al. 2008a, 2011). In Manitoba, fall fumagillin treatment also suppressed spring *Nosema*, but only for colonies wintered indoors (Desai and Currie 2016). Past studies have shown a residual effect of fall fumagillin treatment on *N. apis* (Furgala 1962; Furgala and Boch 1970; Furgala et al. 1973). In this study, it is unclear whether *N. ceranae* was unaffected by residual levels of fumagillin or that long-lasting residual levels were not achieved due to lower doses.

However, colony survival increased for colonies treated with fumagillin in both spring and fall in the second year, but not the first. The difference between years could be due to

various factors, from the presence of *N. apis* to winter conditions. For example, higher mortality in the first year due to the long winter could have negated any improvements in survival from fumagillin treatment. Previous studies have found that fumagillin treatment did not improve colony winter survival, though this could be due to only treating in the fall rather than both the spring and fall (Traver et al. 2012; Mendoza et al. 2017). Based on this study's results, fall fumagillin treatment did not consistently reduce the abundance of *Nosema* post-winter. There appeared to be a benefit of spring treatment, suggesting that if colonies were treated with fumagillin in the spring before the infection peak, greater benefits might be observed. However, this study did show that late spring treatment combined with fall treatment did increase colony survival in one of the two years.

It was thought that wintering method might impact how fumagillin treatment affects *Nosema*. Desai and Currie (2016) found that fumagillin-treated colonies had significantly lower *Nosema* than untreated colonies when wintered indoors, but the reduction in *Nosema* was not significant for outdoor-wintered colonies. However, this study found that wintering method did not interact with fumagillin treatment in affecting spore abundance. This does not necessarily mean that wintering method does not impact disease. Other studies have found that colonies infected with high levels of parasites and/or pathogens were more likely to survive when wintered indoors than when wintered outdoors (Williams et al. 2010; Bahreini and Currie 2015; Desai and Currie 2016). Therefore, wintering management should be considered when investigating honey bee diseases in Canada, as effects may be context-specific.

In conclusion, *N. ceranae* has become the predominant species in Alberta. Further research is needed to determine the factors affecting the success of *N. ceranae* relative to *N. apis* as the latter species is still present. Although the fumagillin treatments did reduce *Nosema*

abundance, they did not eliminate the infection. Therefore, this research reinforces the need for continuous monitoring of this disease in the spring and fall. Additionally, fumagillin treatment in both the spring and fall increased colony survival in one of two years and may be worth implementing as a strategy to reduce the risk of winter loss. The late, low-dose spring treatment did not increase brood and honey production. Although it did increase bee population, but it did not do so consistently between sites and years. Additional research is needed to determine if treating with a range of doses before the spring *Nosema* peak results in improved colony strength and productivity. In this study, wintering method did not interact with fumagillin treatment to influence any impact of *Nosema* on colony health and survival. There also appeared to be little residual effects of treatment as following winter, previous spring and/or fall fumagillin treatment did not reduce spring *Nosema* abundance. Therefore, another spring treatment should be considered to reduce spring *N. ceranae* abundance.

Tables and Figures

Table 3.1 – Prevalence (percent of colonies) of *Nosema* species in tested colonies (*n*) during two experimental years by location (North or South) in Alberta, Canada. Tested samples were taken before spring treatment (June), before fall treatment (August), and after wintering (April) for both years.

		2017-2018 Prevalence (%)			**2018-2019 Prevalence (%)**			
Location	**Month**	*n*	*N. ceranae*	*N. apis*	*n*	*N. ceranae*	*N. apis*	*N. ceranae* + *N. apis*
North	June	13	100	0	16	100	0	0
	August	14	100	0	14	92.9	0	7.1
	April	15	100	0	15	6.7	0	93.3
South	June	16	100	0	16	100	0	0
	August	14	100	0	15	46.7	0	53.3
	April	13	100	0	14	92.9	0	7.1

Table 3.2 – Effect of fumagillin treatment (Spring or Fall), sampling date, and location on *Nosema* abundance and colony measurements in 2017 and 2018. Significant effects are in boldface. *F* = *F* value, df = degrees of freedom, *P* = *P*-value.

			Treatment			**Treatment*Date**			**Treatment*Date* Location**		
Year	**Treatment**	**Parameter**	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
2017	Spring	*Nosema* abundance	0.06	1, 157	0.81	2.28	3, 162	0.081	0.08	3, 162	0.97
		Adult bee population	**7.50**	**1, 120**	**0.0071**	1.43	1, 119	0.23	**5.09**	**1, 119**	**0.026**
		Brood area	0.70	1, 114	0.40	0.20	1, 116	0.66	0.58	1, 116	0.45
		Honey	1.10	1, 121	0.30	**3.68**	**2, 117**	**0.028**	1.45	2, 117	0.24
	Fall	*Nosema* abundance	**6.63**	**1, 112**	**0.011**	0.45	1, 112	0.50	3.28	1, 112	0.073
		Adult bee population	0.02	1, 54.7	0.90	0.02	1, 52.6	0.90	3.49	1, 52.6	0.067
		Brood area	0.20	1, 52.2	0.66	0.06	1, 50.5	0.81	1.00	1, 50.5	0.32
2018	Spring	*Nosema* abundance	**5.19**	**1, 218**	**0.024**	**3.10**	**5, 288**	**0.0096**	0.31	5, 288	0.90
		Adult bee population	0.37	1, 122	0.54	0.01	1, 122	0.90	0.06	1, 122	0.80
		Brood area	0.28	1, 122	0.60	0.12	1, 122	0.73	0.74	1, 122	0.39
		Honey	0.22	1, 121	0.64	0.31	2, 120	0.74	0.09	2, 120	0.92
	Fall	*Nosema* abundance	2.64	1, 116	0.11	0.09	1, 116	0.77	0.38	1, 116	0.54

Table 3.3 – Partitioning of the interaction between 2017 spring fumagillin treatment × sampling date × location sliced by location (North or South) and sampling date (July 15 or August 12) for adult bee population. Significant effects are in boldface. *F* = *F* value, df = degrees of freedom, *P* = *P*-value.

Parameter	**Location**	**Sampling Date**	***F***	**df**	***P***
Adult bee population	North	July 15, 2017	**4.73**	**1,123**	**0.032**
		August 12, 2017	**9.74**	**1,118**	**0.0023**
	South	July 15, 2017	2.51	1,123	0.12
		August 12, 2017	0.11	1,120	0.74

Table 3.4 – Post-hoc analysis of the interaction between time × fumagillin treatment split by winter period (before or after) for *Nosema* abundance. Significant effects are in boldface (Bonferroni adjusted $\alpha = 0.0085$). *t* = *t* value, df = degrees of freedom, *P* = *P*-value).

Parameter	**Winter (2017-2018)**	**Treatment vs Control**	***t***	**df**	**P**
Nosema abundance	Before	Spring	2.25	176	0.026
		Fall	**2.91**	**176**	**0.004**
		Spring-Fall	2.09	176	0.038
	After	Spring	-1.00	176	0.32
		Fall	-1.92	176	0.057
		Spring-Fall	-2.05	176	0.042

Table 3.5 – Effect of fumagillin treatment, sampling date, location, and wintering method on *Nosema* abundance and colony measurements after winter in 2017-2018 and 2018-2019. Significant effects are in boldface. *F* = *F* value, df = degrees of freedom, *P* = *P*-value.

		Treatment*Date			**Treatment*Date* Location**			**Treatment*Date* Wintering method**			**Treatment*Date* Location* Wintering method**		
Winter	**Parameter**	***F***	**df**	***P***	***F***	**df**	***P***	***F***	**df**	***P***	***F***	**df**	***P***
2017-2018	*Nosema* abundance	**4.47**	**3, 176**	**0.005**	0.88	3, 176	0.45	1.95	3, 176	0.12	1.57	3, 176	0.20
	Adult bee population	1.96	3, 74.8	0.13	1.97	3, 74.8	0.13	0.23	3, 74.8	0.88	0.26	3, 74.8	0.85
	Brood area	0.44	3, 78.7	0.73	0.05	3, 78.7	0.99	0.17	3, 78.7	0.91	0.46	3, 78.7	0.71
2018-2019	*Nosema* abundance	0.96	3, 192	0.41	1.52	3, 192	0.21	1.55	3, 192	0.20	0.25	3, 192	0.86
	Adult bee population	1.61	3, 77.9	0.19	**3.85**	**3, 77.9**	**0.013**	0.62	3, 77.9	0.60	1.30	3, 77.9	0.28

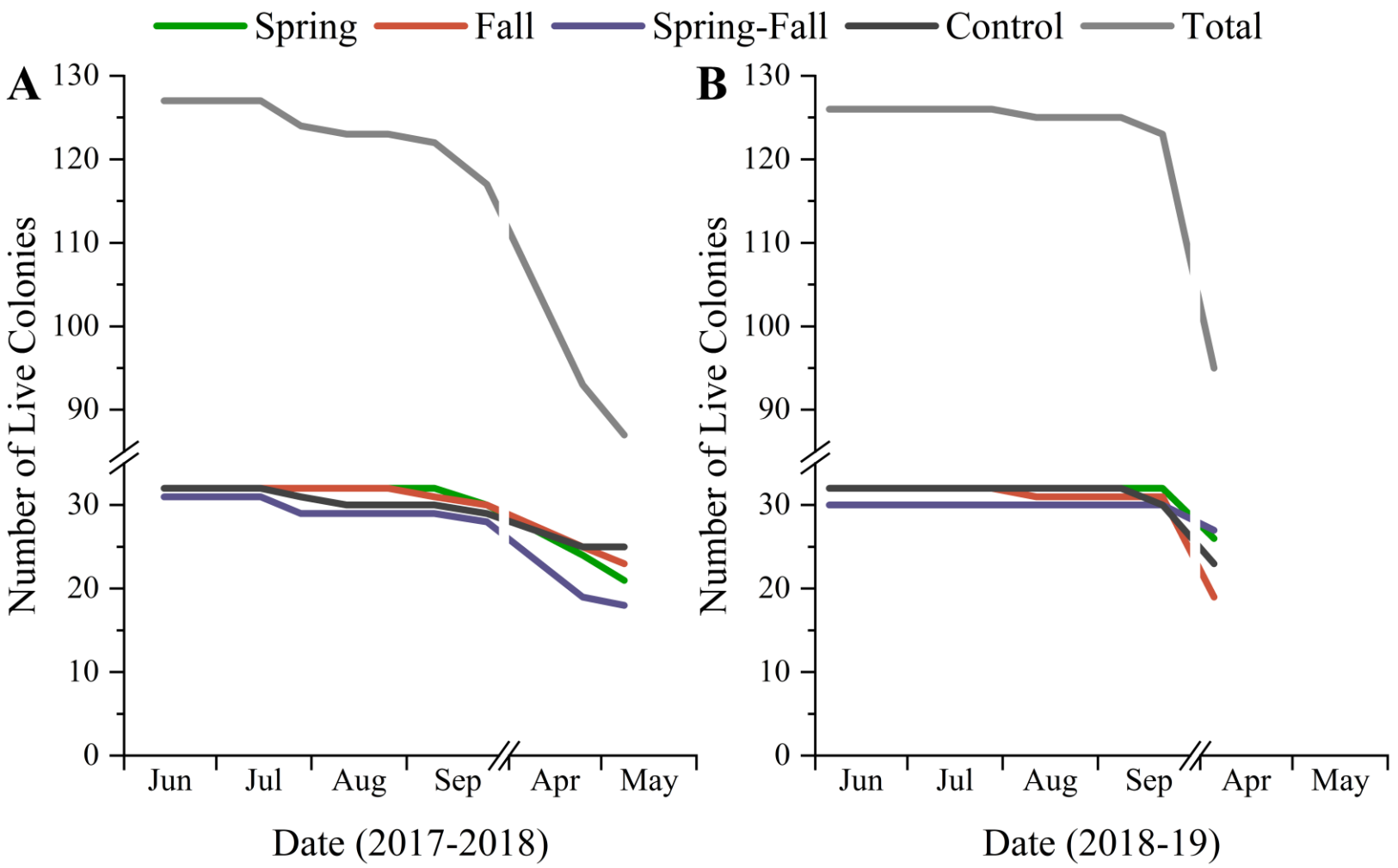

Fig. 3.1 – Number of live colonies in total and by treatment over 11 months during the first experiment (A) and second experiment (B) with x-axis breaks indicating winter.

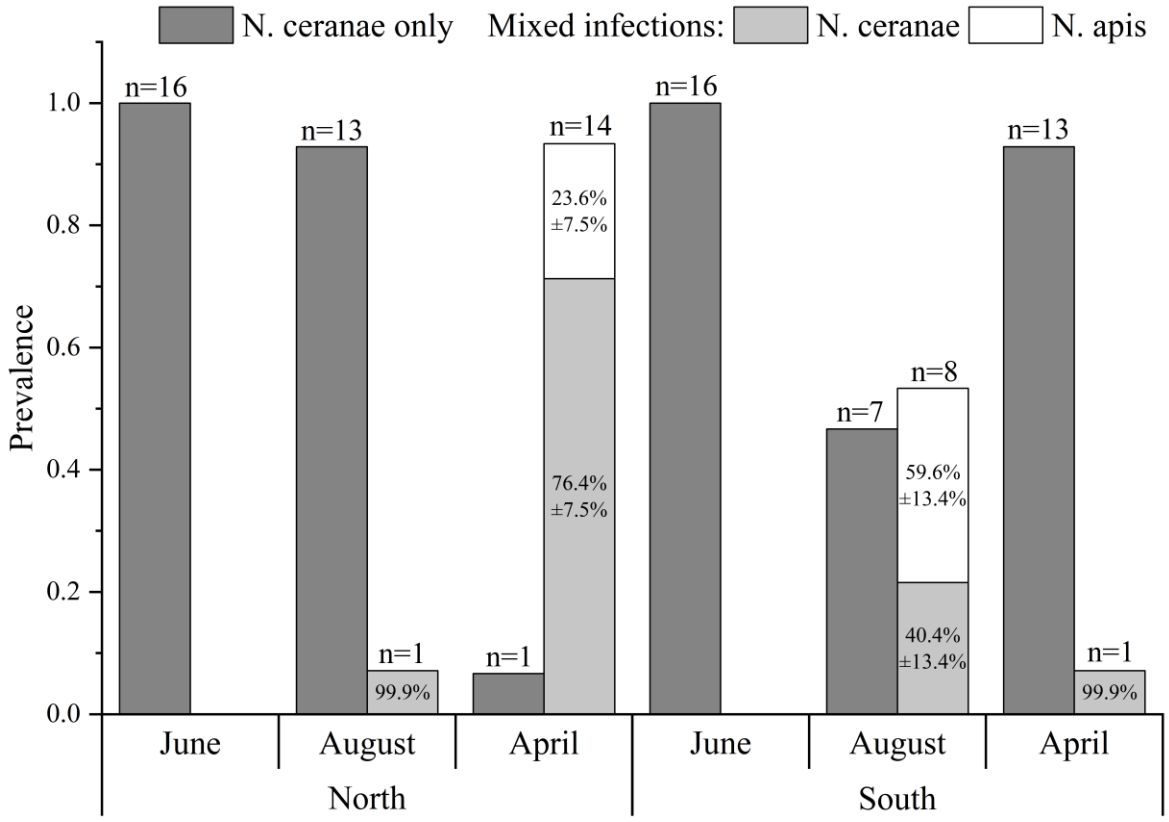

Fig. 3.2 – Prevalence (proportion of colonies) of infection types (single versus mixed) and average species composition (% ± SE) in mixed infections in the subset of colonies tested during 2018-2019 by location (North or South) in Alberta, Canada. Samples were taken before spring treatment (June), before fall treatment (August), and after wintering (April). n is the number of colonies with that infection type.

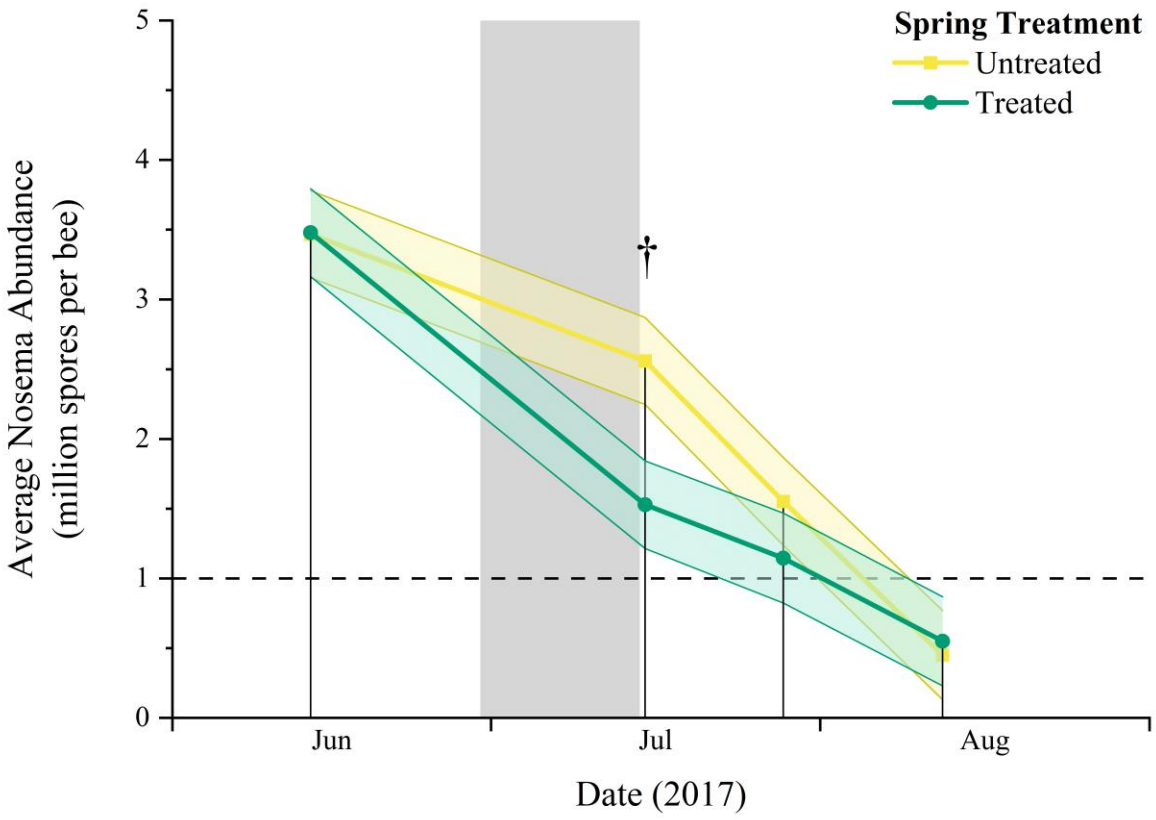

Fig. 3.3 – The effect of 2017 spring fumagillin treatment on average *Nosema* abundance by sampling date. Data are plotted as untransformed means. One standard error is represented by the light-coloured areas around the mean line. Spring fumagillin treatment (grey area) was applied from June 30-July 12, 2017 for the North apiaries and from July 6-18, 2017 for the South apiaries. Dashed line shows the 1 million spores/bee threshold. The dagger (†) indicates a marginally significant difference between treated and untreated colonies.

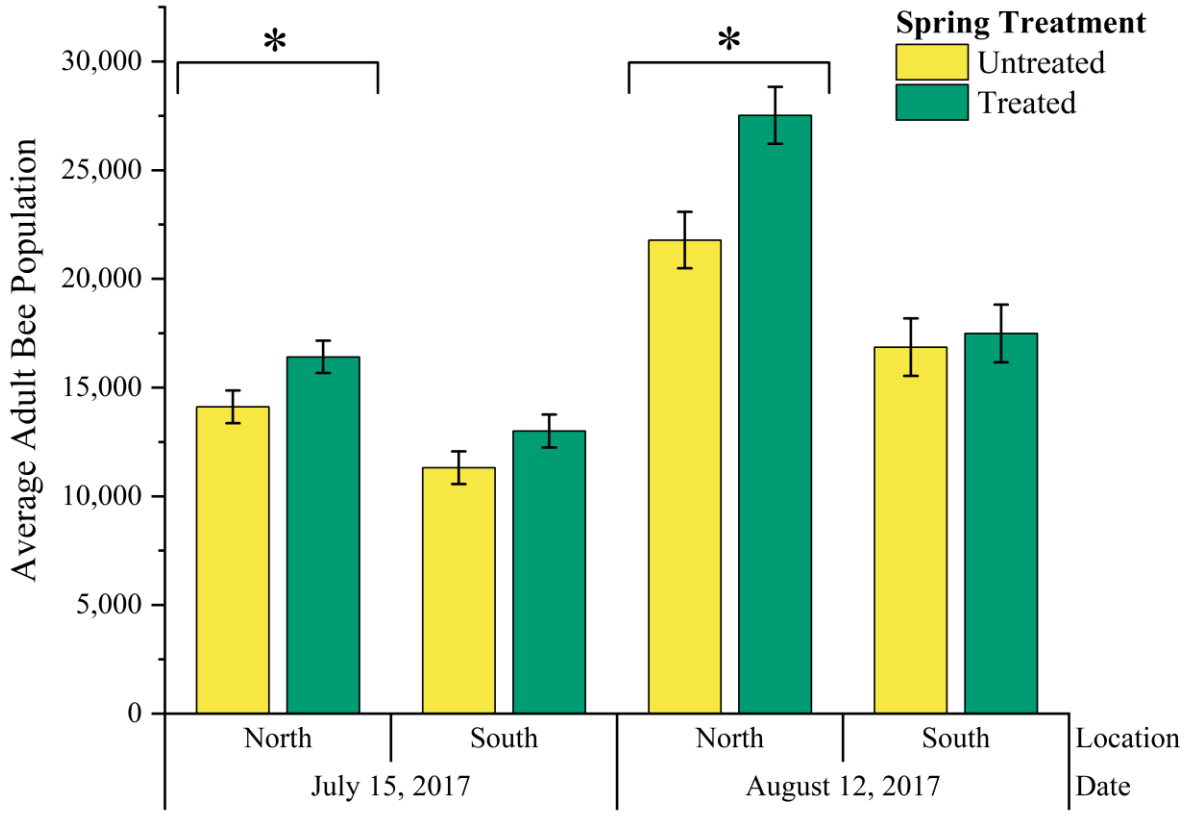

Fig. 3.4 – The post-treatment effect of 2017 spring fumagillin treatment on colony size (average adult bee population) by location and sampling date showing one standard error of the mean. Spring fumagillin treatment was applied from June 30-July 12, 2017 for the North apiaries and from July 6-18, 2017 for the South apiaries. Asterisks indicate significant differences between treated and untreated.

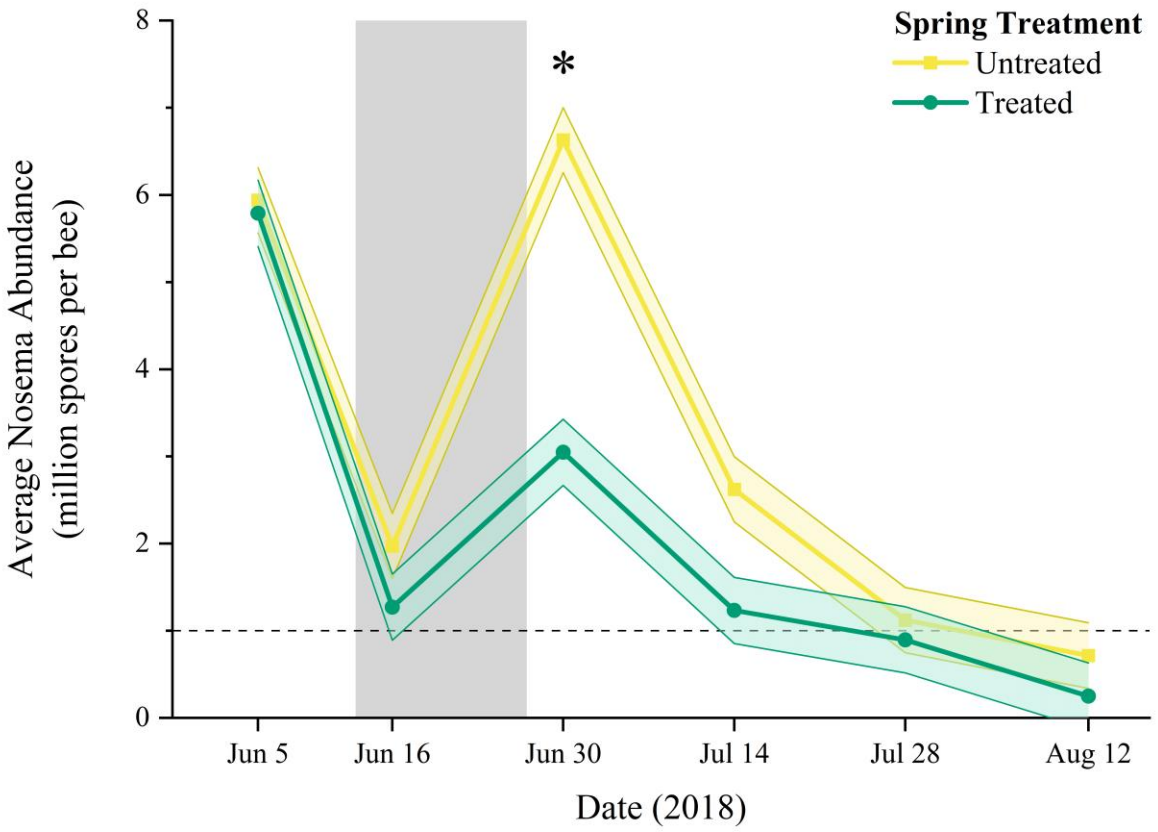

Fig. 3.5 – The effect of 2018 spring fumagillin treatment on average *Nosema* abundance by date. Data are plotted as untransformed means. One standard error is represented by the light-coloured areas around the mean line. Spring fumagillin treatment (grey area) was applied from June 13-25, 2018 for the North apiaries and from June 15-27, 2018 for the South apiaries. Dashed line shows the 1 million spores/bee threshold. The asterisk indicates a significant difference between treated and untreated on June 30.

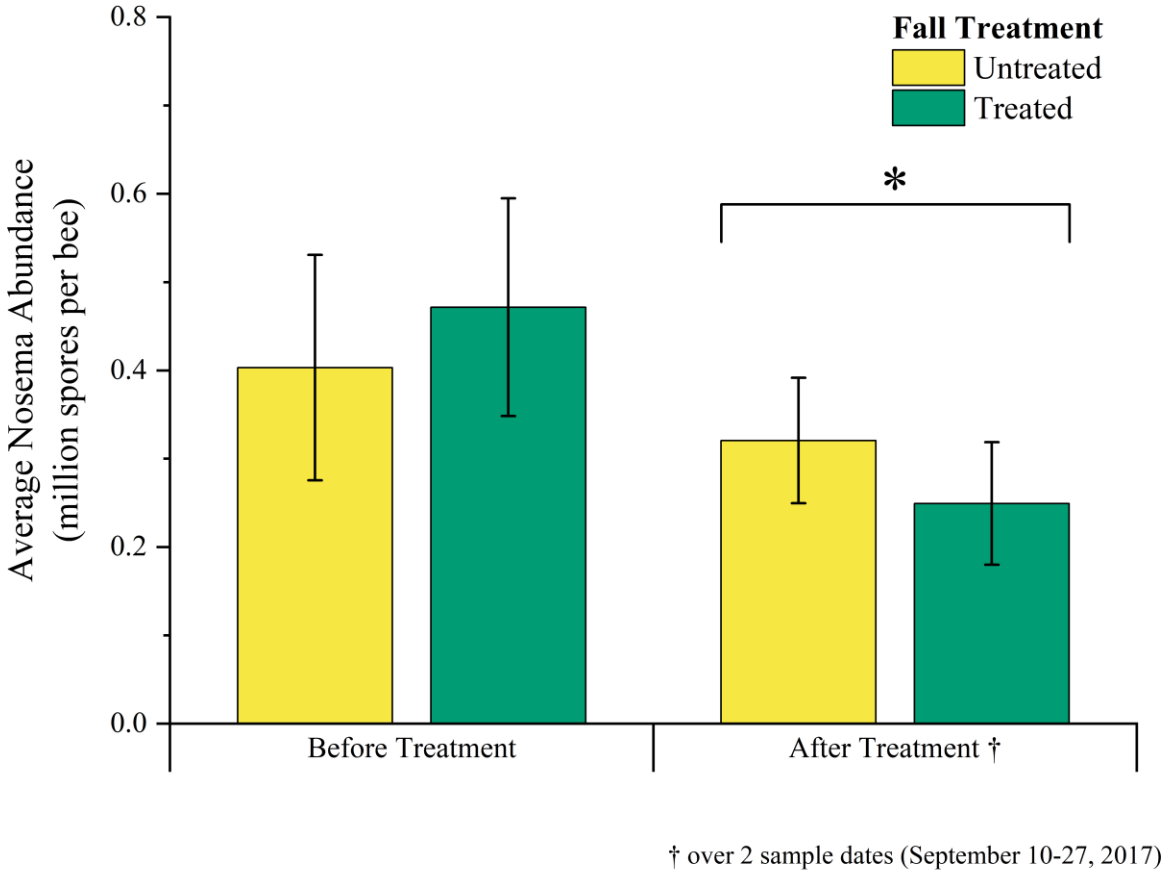

Fig. 3.6 – The effect of 2017 fall fumagillin treatment (120 mg/colony) on average *Nosema* abundance before and after treatment application. Data are plotted as untransformed means with standard error bars. Treatment was applied on September 6 and 8, 2017. The asterisk indicates a significant difference between treated and untreated colonies.

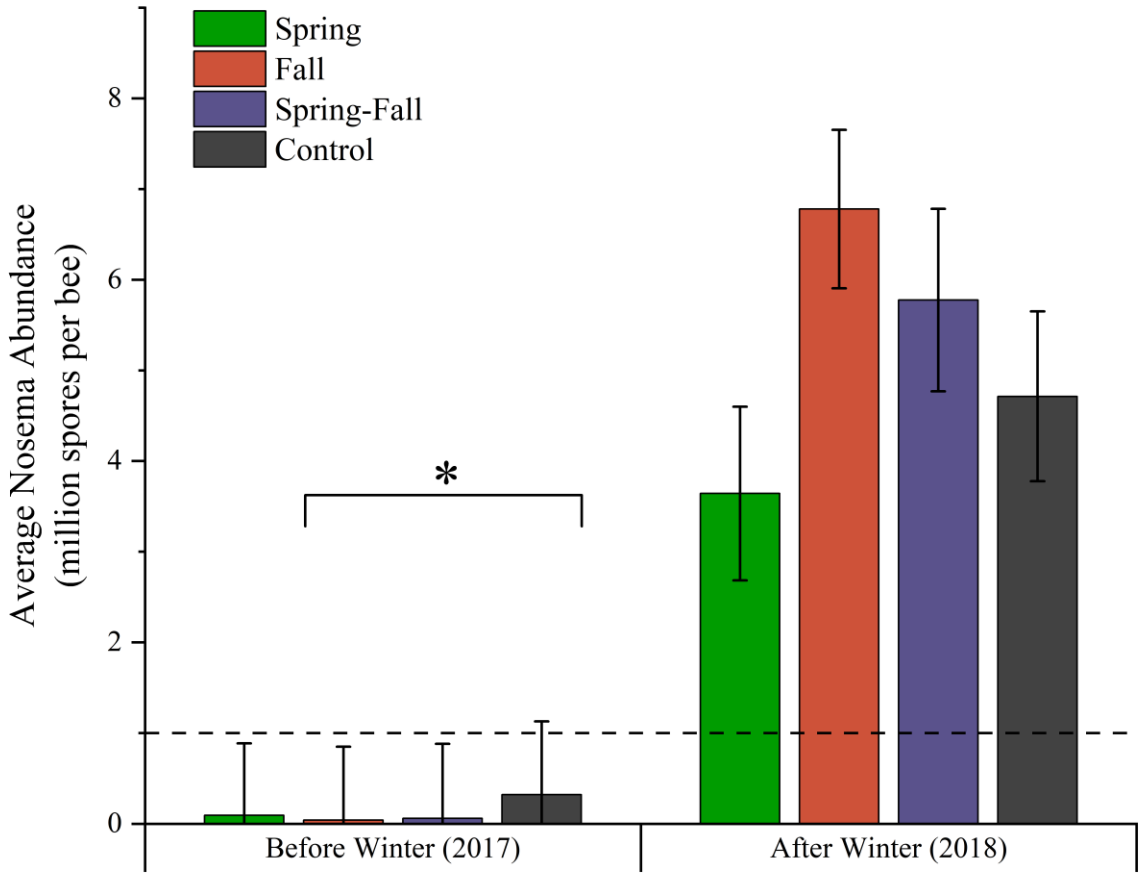

Fig. 3.7 – The effect of 2017 fumagillin treatment on average *Nosema* abundance before and after winter. Error bars show one standard error of the mean. Dashed line shows the 1 million spores/bee threshold. The asterisk indicates a significant difference between Fall-only treatment and control. Total fumagillin treatment dose per colony: Spring – 68 mg, Fall – 120 mg, Spring-Fall – 188 mg, Control – 0 mg.

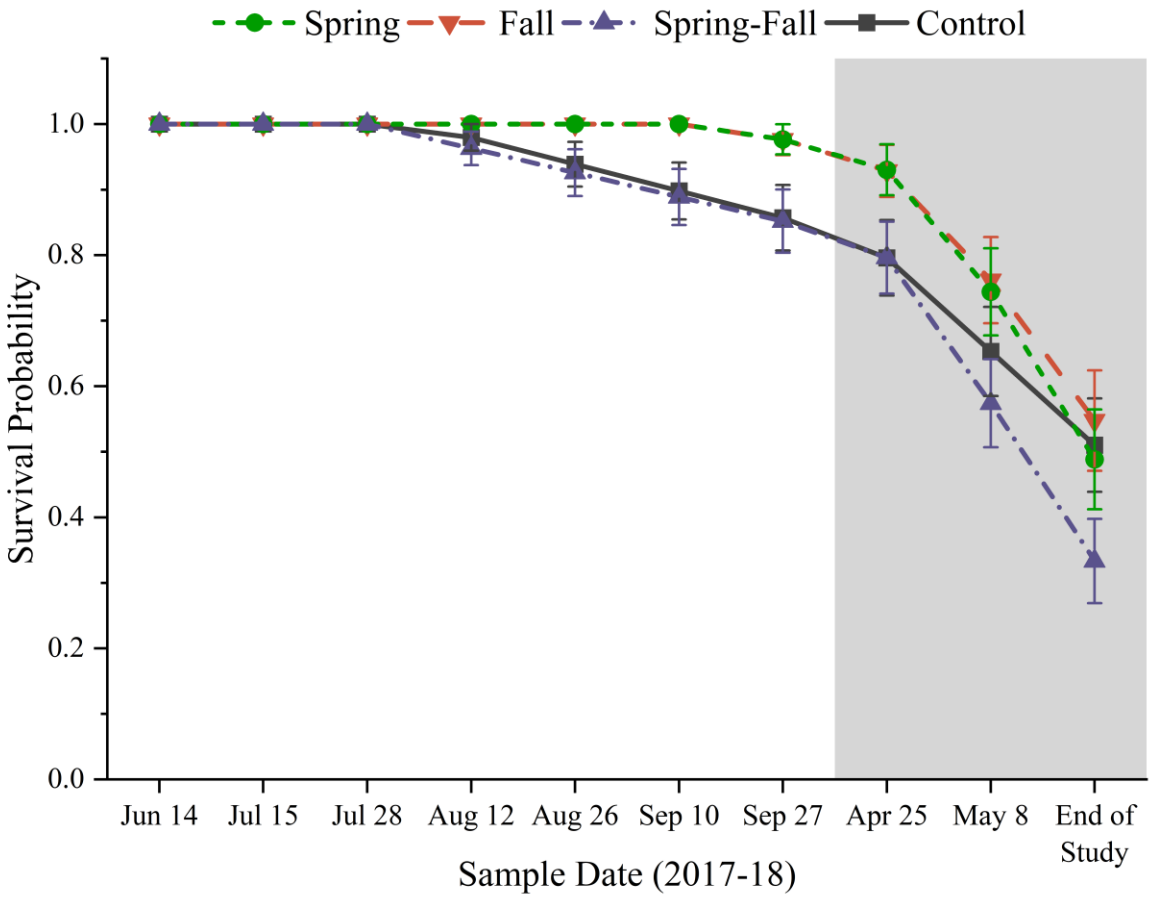

Fig. 3.8 – The effect of 2017 fumagillin treatment on the survival probability of colonies over the first experiment (2017-2018) with standard error bars. Shaded area indicates after winter. Total fumagillin treatment dose per colony: Spring – 68 mg, Fall – 120 mg, Spring-Fall – 188 mg, Control – 0 mg.

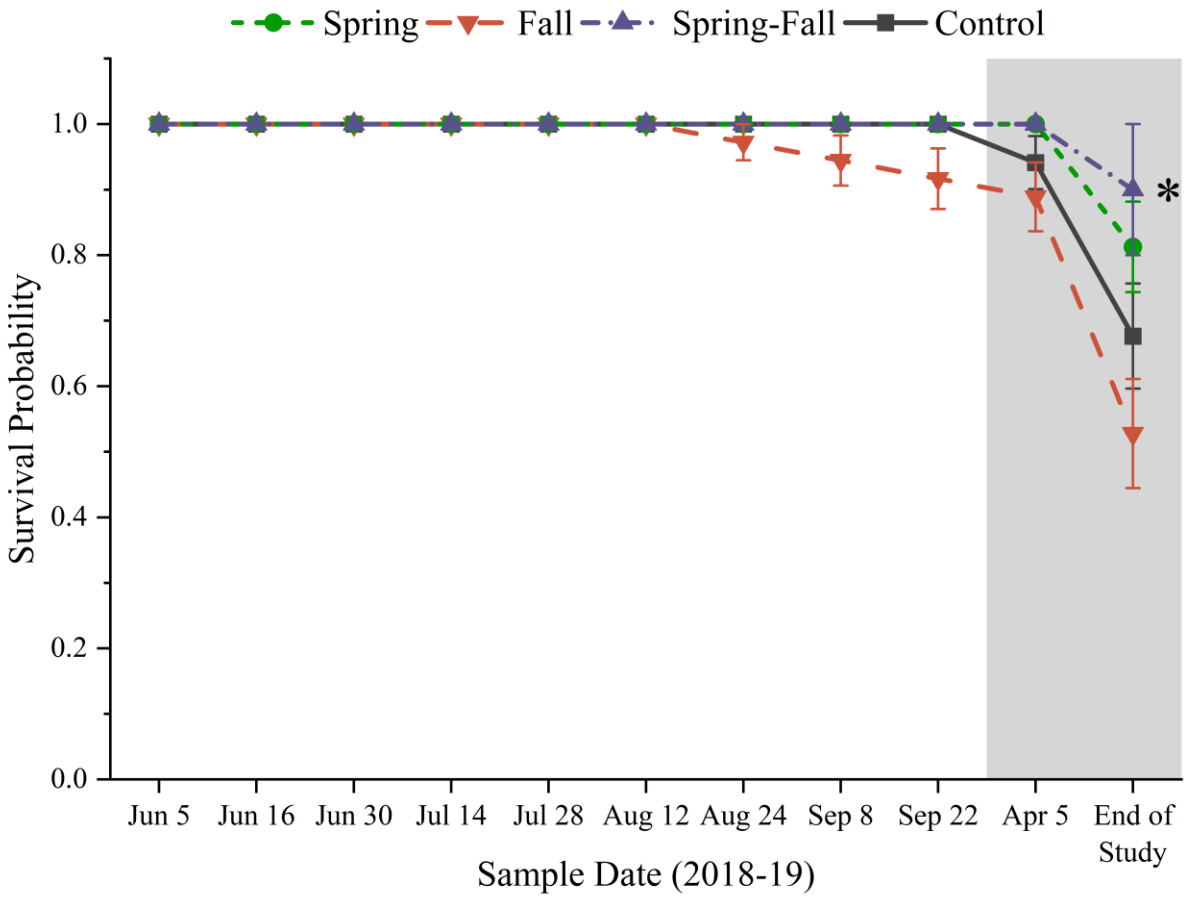

Fig. 3.9 – The effect of 2018 fumagillin treatment on the survival probability of colonies over the second experiment (2018-2019) with standard error bars. Shaded area indicates after winter. Asterisk (right side) indicates significant differences between spring-fall treatment and control. Total fumagillin treatment dose per colony: Spring – 68 mg, Fall – 48 mg, Spring-Fall – 116 mg, Control – 0 mg.

Chapter 4: General Discussion

Beekeepers need to keep honey bee colonies healthy, with low levels of pests, parasites, diseases, and pathogens for the colonies to survive and be productive. Implementing an effective pest management strategy relies on having accurate information on pests and their treatments. *Nosema apis* is a long-time parasite of *Apis mellifera,* with fumagillin being an effective treatment (Bailey 1953a; Furgala et al. 1973; Webster 1994). With the emergence of *Nosema ceranae*, information is needed on how this species differs from *N. apis* and whether the same treatment is effective. *Nosema ceranae* appears to be more successful than *N. apis* as it has become the predominant species in many regions (Klee et al. 2007).

The impact that *N. ceranae* has on honey bee colonies varies from none (Gisder et al. 2010; Williams et al. 2011; Desai and Currie 2016) to severe (Higes et al. 2008, 2009b; Botías et al. 2013a). Additionally, fumagillin, the only registered antifungal to treat *Nosema* in Canada, has variable efficacy against *N. ceranae* (Williams et al. 2008a; Higes et al. 2011; Huang et al. 2013; Mendoza et al. 2017). Furthermore, the epidemiology of *Nosema* may be affected by winter conditions as cold temperatures and different wintering methods (indoor and outdoor) provide varying levels of temperature stress and defecation flight opportunities. Across the Canadian Prairies, the length and severity of winter vary in different geographic locations. With winter being a challenging period for honey bee colonies, factors affecting winter environment need to be considered when investigating pest management issues. In this thesis, the seasonal variation of *Nosema ceranae* abundance in honey bee colonies was characterized in different climatic zones within Alberta when managed using two different wintering methods. Also, the impact of *Nosema* in these environments on honey bee colony population, survival, and

economic viability was described. The effects of fumagillin treatment on *Nosema* abundance and colony strength under these experimental conditions was also assessed. Finally, the effect of treatment combinations in these environments on colony mortality was characterized.

The dynamics of how *N. apis* and *N. ceranae* interact are poorly understood; therefore, the species of *Nosema* infecting the experimental colonies was determined. Both studies (Chapter 2 and 3) showed that colonies were infected with either only *N. ceranae* or both *N. apis* and *N. ceranae*, agreeing with previous findings from Alberta (Currie et al. 2010; Emsen et al. 2016). Pure *N. apis* infections were not observed. Furthermore, in colonies with mixed infections, *N. ceranae* was generally predominant over *N. apis* (Chapter 3), even in the spring when *N. apis* infection traditionally peaks. The higher prevalence of *N. ceranae* in this study and previous Canadian studies (Williams et al. 2008a, 2010; Currie et al. 2010; Copley et al. 2012; Emsen et al. 2016; McCallum et al. 2020) indicates that *N. ceranae* can be successful in cold environments despite being less cold tolerant than *N. apis* (Gisder et al. 2010; Fries 2010).

Nosema Epidemiology in Alberta

For treatments to be effective, awareness of the seasonal trends of parasites is needed to inform the timing of treatments to prevent outbreaks. Our two-year study (Chapter 2) showed *N. ceranae* has a consistent spring peak and low summer and fall levels, supporting previous North American studies (Pernal et al. 2010, Traver et al. 2012, Emsen et al. 2020, McCallum et al. 2020). The seasonal pattern for *N. ceranae* seen in this study has some similarities to the established pattern for *N. apis* but lacks the typically fall peak (Fries 1993, Guzmán-Novoa et al. 2010, Copley et al. 2012). It is possible that *N. ceranae* may have had a fall peak in October, as seen in Nova Scotia (McCallum et al. 2020), which was not captured as this study halted

sampling in September. However, if such a peak did occur, it would not affect judgements about treatment thresholds as October is too late to start nosema treatment in Alberta. The difference in fall *Nosema* abundance for these two species further demonstrates that sampling timing needs to be reassessed in order to make sound control decisions and prevent economic losses.

Winter is particularly challenging for honey bees as they are confined to the hive and often unable to perform defecation flights. During long periods of cold weather, bees may be forced to defecate within the hive, which could increase the spread of *Nosema* infection when the comb is cleaned in the spring (Retschnig et al. 2017). It was predicted that *Nosema* abundance would be lower in southern Alberta ("South") than in central Alberta ("North") due to colonies in the South often having warm periods in winter that allow for defecation flights. However, this study found no consistent pattern for differences in *Nosema* abundance by location within Alberta (Chapter 2), possibly because the small number of sites used in this study did not adequately represent Alberta's full climatic variation.

The impact of temperature stress and cleansing flight opportunities on *Nosema* abundance was investigated by comparing indoor and outdoor winter management. Indoor-wintered colonies experience lower temperature stress and no cleansing flights, whereas outdoor-wintered colonies have greater temperature stress with cleansing flight opportunities. In this study, the effects of wintering method on *Nosema* abundance in untreated colonies sampled after winter were not consistent (Chapter 2). There was no difference between wintering methods during the first winter, whereas *Nosema* abundance only increased over the second winter for indoor-wintered colonies. Previous Canadian studies have had varying results and are not directly comparable to this study as some of the participating beekeeper's colonies were treated with fumagillin (Williams et al. 2010; Desai and Currie 2016).

Wintering method significantly affected the impact of *Nosema* infection on colony performance. Indoor-wintered colonies were less likely to die from *Nosema* infections and had larger bee populations in the following summer than outdoor-wintered colonies at equivalent infection levels (Chapter 2). The better survival in the presence of nosema during indoor wintering in this study supports other Canadian studies that demonstrate colonies infected with high levels of parasites and/or pathogens are more likely to survive when wintered indoors than when wintered outdoors (Williams et al. 2010; Bahreini and Currie 2015; Desai and Currie 2016). The impact of wintering method on survival when nosema is present suggests that mitigation of temperature stress during indoor wintering, where flight does not occur, affected the impact of *Nosema* infections on colonies more than any benefits associated with the availability of late winter cleansing flights. This may be due in part to cleansing flight opportunities being unpredictable and short-lived, while reduced temperature stress can occur all winter. Furthermore, these results quantified the impact of different spore loads on colony health and suggested the damage threshold for *Nosema* infection should be lowered for outdoor-wintered colonies, which suffer more damage than indoor-wintered colonies under equivalent *Nosema* abundance. Indoor wintering is an attractive option for beekeepers looking to reduce mortality due to *Nosema* and increase summer colony population size.

Nosema abundance was found to be a significant predictor of colony mortality. Spring and summer *Nosema* abundance was a better predictor of colony mortality than fall abundance in untreated colonies (Chapter 2). While previous Canadian studies have found that *N. ceranae* is not correlated with mortality (Guzmán-Novoa et al. 2010; Williams et al. 2010, 2011; Desai and Currie 2016; Emsen et al. 2020), these studies restricted sampling to a period from early fall through to the spring as it was assumed that fall *Nosema* abundance would be most likely to

predict winter mortality. Perhaps these studies would have found associations between *Nosema* and mortality if sampling had been carried out in the spring to early summer.

The Effectiveness of Fumagillin Treatment

Fumagillin is known to effectively treat *N. apis* infections, with long-lasting effects (Furgala 1962; Furgala and Boch 1970; Furgala et al. 1973). This study found that spring fumagillin treatment reduced *Nosema* abundance in colonies infected predominantly with *N. ceranae* (Chapter 3), supporting previous Canadian studies (Pernal et al. 2009; McCallum et al. 2020). This reduction in *Nosema* abundance increased aspects of colony performance in some sites but did not consistently increase colony population. Averaged over all sites, brood and honey production did not increase from spring treatment in either year. The lack of consistent colony-level benefits from spring fumagillin treatment observed in this study could be due to late treatment application using a lower dosage than the label recommended. The *N. ceranae* peak occurs in early spring, around May (Traver et al. 2012; McCallum et al. 2020). If the spring fumagillin treatment were applied before the peak at the correct dosage, the reduced *Nosema* levels could have had a more significant impact on the colony's population and productivity. This was not possible using the experimental design utilized as time was required to equalize colony populations and the spring period in Alberta is quite short. Additional research is needed to determine whether spring fumagillin treatment increases bee population and honey production when applied earlier at the label recommended dosage.

This study found that immediately after overwintering all previously treated colonies (Spring only, Fall, only, Spring and Fall) had similar *Nosema* abundance compared to control colonies in both years (Chapter 3). Also, fumagillin treatment did not improve spring colony

strength, as seen in previous studies (Williams et al. 2008a, 2011; Mendoza et al. 2017). The presence of *Nosema* in treated colonies post-winter suggests that fumagillin does not completely eradicate *N. ceranae* and must be continuously monitored. This supports several studies where treated colonies had higher spore abundance again (Williams et al. 2008a; Higes et al. 2011; Mendoza et al. 2017).

Usually, fall fumagillin treatment is applied with the intent of reducing the impact of *Nosema* during the winter and subsequent spring. Fall fumagillin treatment at the higher dosage (120 mg/colony) reduced *Nosema* abundance before winter, supporting past studies from South America (Giacobino et al. 2016; Mendoza et al. 2017). However, this difference was gone by the spring, suggesting little residual effect of fumagillin. In contrast, Nova Scotia studies found that fall treatment reduces spring *Nosema* abundance, though this could be due to the higher dosage of 190 mg/colony used in that study (Williams et al. 2008a, 2011). In Manitoba, fall treatment also suppressed spring *Nosema*, but only for colonies wintered indoors (Desai and Currie 2016).

Colony survival increased for colonies treated with fumagillin in both spring and fall, but not in both years. Previous studies found that fumagillin treatment does not affect colony winter mortality, though this could be due to only treating in the fall rather than both the spring and fall (Traver et al. 2012; Mendoza et al. 2017). Based on these results, fall fumagillin treatment appears not to consistently reduce spring *Nosema* post-winter. Instead, colonies should be treated with fumagillin in the early spring before the infection peak, and in combination with fall treatment, this may increase colony survival in some years. Given the high cost of colony replacement at over $230 CAD per colony, fumagillin treatment may be economically viable as a strategy to reduce risk even if it does not pay off each year, especially if economic thresholds can be utilized in decision-making.

Summary

In conclusion, this thesis demonstrates the presence of a seasonal pattern of *N. ceranae* abundance with a spring peak in the Canadian Prairies. There was no consistent evidence that *Nosema* was a greater threat to colony health in the colder 'North' region of Alberta than the warmer South. There was no consistent effect of wintering method on *Nosema* abundance; however, indoor wintering reduced colony mortality due to *Nosema* infection and increased summer colony population size relative to outdoor-wintered colonies. This study found clear evidence that spring *N. ceranae* abundance impacts the long-term health of the colony, as the probability of survival post-winter decreased with increasing *Nosema* loads above 1 million spores/bee.

Although fumagillin treatment did reduce *Nosema* abundance, it did not eliminate the infection, making monitoring is necessary in the spring and fall. Furthermore, fall fumagillin treatment did not reduce *Nosema* abundance in the following spring at the comparatively low doses applied in this study. Additional research is needed to determine if treating before the spring *Nosema* peak results in improved colony strength and productivity. Wintering method did not influence the effect of fumagillin treatment on *Nosema* and colony health and survival. The results suggest that in order to reduce spring *Nosema* abundance, colonies should be treated in the spring before the *Nosema* peak, rather than in the previous fall.

Appendix

Appendix 1: Monthly climate normals data from weather stations near apiary sites

Data provided by Environment and Climate Change Canada https://climate.weather.gc.ca (retrieved on June 2, 2020).

North Apiaries

Station name: Elk Island National Park

Climate ID: 3012275

Location: 53°40'58.000" N 112°52'05.000" W

Elevation: 716.2 m

South Apiaries

Station name: Vauxhall North

Climate ID: 3036690

Location: 50°10'57.080" N 112°07'19.040" W

Elevation: 760.0 m

	Daily Average (°C)		Daily Maximum (°C)		Daily Minimum (°C)		Days with Maximum Temperature above 10°C	
Apiary	North*	South**	North*	South**	North*	South**	North†	South‡
January	-12.0	-9.2	-6.2	-2.9	-17.8	-15.4	0.4	1.9
February	-9.3	-6.1	-2.5	0.5	-15.9	-12.7	0.8	4.1
March	-5.1	-0.9	1.2	6.1	-11.4	-7.8	3.3	12.0
April	4.2	6.0	10.8	13.7	-2.4	-1.6	17.5	21.4
May	10.6	11.4	17.0	19.0	4.1	3.8	27.3	28.1
June	14.6	15.4	20.8	22.3	8.4	8.6	29.7	29.7
July	17.0	17.7	23.2	25.5	10.8	10.0	31.0	30.9
August	15.7	17.4	22.1	25.8	9.2	8.9	30.7	31.0
September	10.1	12.4	16.1	20.7	4.0	4.1	24.7	28.2
October	4.3	5.7	9.9	13.4	-1.3	-1.9	15.9	21.1
November	-5.2	-1.9	-0.3	4.5	-10	-8.2	2.1	7.4
December	-9.5	-6.8	-4.1	-0.6	-15.1	-12.9	0.6	3.0
Year	3.0	5.1	9.0	12.3	-3.1	-2.1	183.8	218.8

*calculated using data from 1981-2007 (total 20 years, 8 years missing; ~98% of possible observations)

**calculated using data from 1987-2007 (total 21 years, 1 year missing; ~95% of possible observations)

†calculated using data from 1981-2007 (total 20 years, 8 years missing; 92.3% of possible observations)

‡calculated using data from 1988-2007 (total 20 years, 3 years missing; 87.4% of possible observations)

Appendix 2: Monthly climate data from weather stations near apiary sites for 2017-2019

Data provided by Alberta Agriculture and Forestry, Alberta Climate Information Service (ACIS) https://acis.alberta.ca (retrieved June 3, 2020).

North Apiaries

Station name: Elk Island National Park

Climate ID: 3012275

Location: 53°40'58.000" N 112°52'05.000" W

Elevation: 716.2 m

South Apiaries

Station name: Vauxhall CDA CS

Climate ID: 3036682

Location: 50°03'00.000" N 112°08'00.000" W

Elevation: 779.00 m

		Average Air Temperature (°C)		Minimum Air Temperature (°C)		Maximum Air Temperature (°C)		Days with Maximum Temperature above 10°C	
Year	**Apiary**	North	South	North	South	North	South	North	South
2017	May	12.5	14.1	-0.5	0.5	29.9	31.8	29	31
	June	15.2	17.0	3.9	4.9	28.1	33.0	30	30
	July	17.4	21.2	5.6	4.6	29.7	36.9	31	31
	August	15.8	18.7	4.7	4.9	28.4	35.1	31	31
	September	10.9	13.3	-0.6	-3.8	30.1	35.4	25	28
	October	4.1	6.2	-5.9	-7.8	20.2	24.1	15	20
	November	-8.1	-3.9	-21.6	-24.9	6.3	16.5	0	4
	December	-8.6	-7.5	-34.6	-35.2	9.2	14.9	0	5
2018	January	-10.8	-7.9	-35.4	-39.8	9.0	8.4	0	0
	February	-13.4	-14.6	-32.0	-38.5	7.2	4.8	0	0
	March	-6.3	-7.0	-26.0	-29.2	10.7	6.4	1	0
	April	0.3	1.9	-26.6	-20.0	27.5	28.4	12	16
	May	14.3	15.3	-1.0	0.0	29.3	31.1	30	31
	June	15.9	16.9	3.9	4.4	29.8	31.5	30	30

Continued…

		Average Air Temperature (°C)		Minimum Air Temperature (°C)		Maximum Air Temperature (°C)		Days with Maximum Temperature above 10°C	
Year	**Apiary**	North	South	North	South	North	South	North	South
2018	July	17.2	19.1	5.4	6.1	31.4	35.3	31	31
	August	15.4	17.7	1.8	2.4	34.6	39.9	31	31
	September	5.7	9.3	-3.1	-3.6	25.7	31.7	15	23
	October	2.9	4.9	-6.4	-7.1	24.3	23.5	14	22
	November	-3.9	-0.7	-19.3	-19.9	11.4	14.2	1	10
	December	-7.9	-3.2	-28.4	-21.7	7.2	11.5	0	3
2019	January	-9.2	-4.5	-31.1	-25.8	8.8	13.7	0	2
	February	-22.3	-20.3	-43.9	-35.0	-1.5	1.3	0	0
	March	-5.3	-4.2	-39.8	-40.2	18.2	18.2	2	11
	April	4.0	6.9	-6.6	-6.3	20.6	22.6	15	26
	May	10.7	10.7	-4.3	-5.0	29.1	30.1	25	28

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