

Spatiotemporal Surveillance of *Varroa destructor* in Ontario, Canada

by

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ABSTRACT

SPATIOTEMPORAL SURVEILLANCE OF *VARROA DESTRUCTOR* IN ONTARIO, CANADA

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There has been continued concern regarding the sustained levels of overwinter colony losses experienced by beekeepers in Canada. Although not entirely attributable to one single cause, these losses have been repeatedly linked to the parasitic mite, *Varroa destructor*, and thus a concerted effort towards their control has been ongoing. Many jurisdictions in North America have adopted an integrated pest management (IPM) approach to *Varroa* control, with the primary aim of maintaining mite levels below critical thresholds, above which there is a decreased likelihood for colony survival. Developing and optimizing IPM strategies is reliant on a comprehensive understanding of the biology, risk-factors, and distribution of the pest across the population to efficiently allocate resources and target interventions. Until now, the distribution of *Varroa* in Ontario has not been presented in the literature. The purpose of this thesis is to enhance the current state of *Varroa* surveillance across the province of Ontario by providing missing population-level context of the distribution of honey bee colonies and mites across space and time. Through geospatial interpolation of registered honey bee colonies, a province-wide population distribution map is presented, essential for standardizing future epidemiological studies, and permitting further insight into the implications of population density on colony health. Using spatial scan statistics and geostatistical modelling, a continuous depiction of the

distribution of *Varroa* mite infestation intensities is developed and several regions of substantially increased risk are highlighted. Through time series analyses, a repeating seasonal pattern of provincial *Varroa* mite levels is described and evidence is presented to suggest an association between mite counts and weather variables at a 7-week lag interval. As a culminating project for this thesis, a practical information dashboard is developed for all members of the beekeeping community, to disseminate the key findings of these presented works, and enable, potentially, the future collection and analysis of improved quantities of data through citizen science. The results presented in this thesis offer valuable information for guiding future research and policy decisions regarding *V. destructor* in Ontario, while simultaneously demonstrating the methods available for the assessment of diseases in honey bees or other animal species.

DEDICATION

To my parents.

For their continuous encouragement and support through all my endeavours.

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STATEMENT OF WORK

Kurtis Sobkowich, under the primary guidance of Dr. Olaf Berke, was responsible for the design and implementation of all of the studies included in this thesis. The data used in the presented work was collected by the following organizations: Ontario Ministry of Agriculture, Food and Rural Affairs, the Ontario Beekeepers' Association and the Ontario Animal Health Network. All necessary data cleaning and management required specifically for use in this thesis was conducted by Kurtis Sobkowich. The statistical analysis, and the development of the associated *R* script was performed by Kurtis Sobkowich, with guidance from Dr. Olaf Berke and reference to sample code presented and written by Dr. Berke. Kurtis Sobkowich is responsible for the design and development of the information dashboard presented in this thesis with feedback from Dr. Olaf Berke, Dr. Theresa Bernardo, Dr. David Pearl and Paul Kozak. Kurtis Sobkowich is responsible for the writing of this thesis in whole, with critical feedback and suggestions from Dr. Olaf Berke, Dr. Theresa Bernardo, Dr. David Pearl and Paul Kozak.

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LIST OF ABBREVIATIONS

ACF	Autocorrelation Function
AIC	Akaike Information Criterium
API	Application Programming Interface
AR	Autoregressive Model
ARIMA	Autoregressive Integrated Moving Average Model
ARMA	Autoregressive Moving Average Model
BYM	Besag, York, & Mollie Model
CAD	Canadian Dollars
CAPA	Canadian Association of Professional Apiculturists
CCD	Colony Collapse Disorder
CCS	Census Consolidated Subdivisions
CI	Confidence Interval
COVID-19	2019 Novel Coronavirus (SARS-CoV-2)
FPE	Final Prediction Error
GLARMA	Generalized Linear Autoregressive Moving Average Model
GLM	Generalized Linear Model
IPM	Integrated Pest Management
KT	Knowledge Translation and Transfer
LEA	Lambert Azimuthal Equal-Area Projection
LOESS	Locally Weighted Least Squares Regression
MA	Moving Average Model
MAE	Mean Absolute Error
MAUP	Modifiable Areal Unit Problem
OAHN	Ontario Animal Health Network
OBA	Ontario Beekeepers' Association
OIE	World Organisation for Animal Health
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
PACF	Partial Autocorrelation Function
PIT	Probability Integral Transform
SARIMA	Seasonal Autoregressive Integrated Moving Average Model
SMR	Standardized Morbidity Ratio
STL	Seasonal-Trend Decomposition using LOESS
USA	United States of America
VAR	Vector Autoregressive Model
VSH	<i>Varroa</i> Sensitive Hygiene
WLSE	Weighted Least Squares Estimator

Throughout this thesis the term “**colony**” is used to refer to the collection of honey bees living together in a single social unit. The term “**hive**” is used to refer to the physical structure housing the colony. The term “**yard**” (apiary) is used to refer to a collection of colonies in a common location under common beekeeper management. The term “**beekeeping operation**” is used to refer to all of the colonies under common management, regardless of geographic location.

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1 CHAPTER ONE: INTRODUCTION

1.1 Status of the Canadian Beekeeping Industry

Beekeeping is an integral part of the Canadian agriculture system and contributes an estimated \$5.5 billion per year to the national economy (Agriculture and Agri-Food Canada, 2020), although it is difficult to attribute a concrete value because of the complex relationships between bees and their environment. Managed honey bees in Canada, *Apis mellifera*, provide economic value through both the pollination of food crops as well as the production of honey and by-products (i.e., wax and propolis). It is estimated that roughly 80% of the commercial crops produced for human and animal feed are reliant on insect pollination (including pollination by honey bees) either fully or partially (Chauzat *et al.*, 2013) or are benefited from insect pollination through improved quality and yield (Klatt *et al.*, 2014).

The province of Ontario constitutes 12 – 15 % of the national total of managed honey bee colonies (five-year average of 99,133) and 24 – 31% of the nation’s beekeepers (five-year average of 2,923) (Agriculture and Agri-Food Canada, 2020). Beekeepers in Ontario fall into one of two broad groups, hobbyists and commercialists, as defined by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) as managing less than 50 colonies or managing 50 colonies or greater, respectively (OMAFRA, 2021b). This categorization of beekeepers is not inherently indicative of the level of experience possessed by the beekeeper. Commercial operations can then be further categorized based on their main production outcome, be it pollination and honey harvesting or breeding for the sale of queens and nucleus colonies. In comparison to other provinces, Ontario beekeepers on average operate a relatively small number of colonies (five-year average of roughly 34 colonies per beekeeper), whereas beekeepers in Alberta, Prince Edward Island, and Manitoba operate on average over 100 colonies per

beekeeper (Agriculture and Agri-Food Canada, 2020). No matter the scale or the production goal, all beekeepers in Ontario are required under the Ontario Bees Act to register their colonies with the provincial government through OMAFRA (Bees Act, 2019).

In response to early reports about higher than normal levels of bee mortality, including “Colony Collapse Disorder”, from the United States of America in 2006 (VanEngelsdorp, Cox-Foster, Frazier, Ostiguy, & Hayes, 2006), the Canadian Association of Professional Apiculturists (CAPA) began to publish annual statements on overwinter colony losses in Canadian apiaries. Since the onset of these reports, Ontario beekeepers have regularly reported losses above the accepted 15% and have reported losses as high as 58% following the 2013/2014 overwintering period (Canadian Association of Professional Apiculturalists, n.d.). Contrary to this high annual loss of colonies, there has been a net increase in the number of Ontario colonies over the same 15 year span, which increased by approximately 33% (Canadian Association of Professional Apiculturalists, n.d.), a testament to the success of Ontario honey bee breeding and the ability to replace lost stocks through methods such as hive splitting (Currie, Pernal, & Guzmán-Novoa, 2015). However, there is a clear need to address the causes of overwinter colony loss to prevent disruptions in the Canadian agri-food sector and economic losses experienced by beekeepers. In a survey of Ontario beekeepers conducted in 2020, infestation of colonies by the parasitic *Varroa destructor* mite was perceived to be the greatest contributor to overwinter colony losses (Claing *et al.*, 2020).

Beekeeping is a unique form of animal husbandry in which animals from different operations have the opportunity to interact with one another, presenting regional competition for food sources, and the ability to transmit disease not only between units (colonies) of one bee yard (containing one or more colonies) but among yards within the same vicinity. In addition to

the regional mixing of bees is the demand for mobile and out-of-province pollination services in which regionally diverse colonies are brought together in high concentrations for a relatively short period of time to pollinate high-demand crops before being relocated back to their home regions, presenting further opportunity for disease spread across a large scale. In 2017 (the most recent report on the subject), approximately 23,000 colonies were moved from Ontario to Eastern Canada for blueberry pollination, decreasing from 38,000 in 2016 (Kozak, 2017). Beekeepers will also often rent out their hives for pollination within the province and therefore the locations of hives may change multiple times over the course of a season.

1.2 The Western Honey Bee (*Apis mellifera*)

1.2.1 Life Cycle of the Honey Bee

The life cycle of honey bees is centered around the single fertilized queen bee within the colony and occurs in the following series: (1) mating, (2) oviposition, (3) and development of the brood. The queen bee will only undergo the mating process once in her life, roughly one to two weeks after emerging from her brood cell. Mating of the queen occurs over several mating flights, in each of which a single male drone bee will transfer spermatozoa into the spermatheca and subsequently perish (Philippe, 2007; Winston, 1987). Over the course of these mating flights, the queen will accumulate enough spermatozoa from multiple drones to be reproductively productive for up to 5 years (although commercial queens are more commonly replaced after only one or two seasons), after which she will be replaced by a new queen, either by the colony or the beekeeper (Page & Peng, 2001; Remolina, Hafez, Robinson, & Hughes, 2007; Rueppell, Bachelier, Fondrk, & Page, 2007).

Roughly seven days following mating, the queen will begin laying eggs in the cells of the wax comb structure of the hive, in the process of ovipositioning (Woyke *et al.*, 2008). Wax comb

containing eggs, larvae, or pupae is collectively referred to as brood. The queen will utilize the stored spermatozoa to fertilize eggs to become female workers or will lay unfertilized eggs to develop into male drones (Winston, 1987). The time from laying to emergence is dependent on the sex of the bee and ranges from 21 days for workers to 24 days for drones. Eggs that are destined to develop into new queens are laid in larger oval shaped cells called queen cups and emerge more quickly in only 16 days (Vidal-Naquet, 2018). Queen eggs are fertilized like workers, but the queen larvae are fed a continuous diet of royal jelly, triggering a cascade of hormonal changes leading to the development of a queen (Wirtz & Beetsma, 1972).

Development of all castes consist of an egg, larva and pupa stage lasting three, six, and twelve days respectively on average (Vidal-Naquet, 2018). Capping occurs roughly 9 days after laying in all castes and involves the cell being covered in a layer of wax and propolis by workers. The reproductive stages of the honey bee play a considerable role in reproduction of the parasitic mite, *Varroa destructor* (elaborated further in subsequent sections).

In climates with a winter season temperature of less than 10°C, an additional “overwintering” component is introduced into the bee’s life cycle. During the winter, the queen will lay fewer eggs, or pause laying completely, and the colony will engage in hive temperature regulation by means of clustering and shivering to maintain an internal temperature of between 27°C and 34°C (Stabentheiner, Pressl, Papst, Hrassnigg, & Crailsheim, 2003).

1.2.2 The Colony

Honey bees are eusocial animals and exist within a large community referred to as the colony. The term colony is not synonymous with the hive which is instead in reference to the physical structure that contains the colony. A honey bee colony is a highly organized social structure consisting of three castes: drones, workers, and the queen. Drones are male bees and are

only reared in the spring for the purpose of mating and are markedly less abundant than workers (Rangel & Fisher, 2019). Female workers comprise the majority of the colony population (10,000 to 60,000 depending on the time of year (Winston, 1987)) and occupy all roles related to honey production, cleaning and feeding the queen and larvae. The primary role of the sole queen within the colony is to lay eggs, and she will do so at a rate of over 1,000 per day (Cale & Gowen, 1956; Harbo, 1986). The queen will also act to unite the colony through pheromones that influence behaviours such as swarming and colony cohesion (Butler, Callow, & Johnston, 1962). Communication between bees is primarily achieved through the sharing of pheromones, but several physical signals can also be used such as the famed “waggle dance” to indicate the location of food sources (von Frisch, 1967).

1.2.3 The Hive

The industry standard hive structure for beekeeping is the Langstroth hive, invented in 1851. This hive structure utilizes a simple wooden box with removable frames separated by no less than 6mm of space and no greater than 9mm, as discovered by the Reverend Lorenzo Lorraine Langstroth as the optimal distance in which enough space remains for the bees to move, but not such a large gap that they attempt to build comb or close the gap with propolis (Langstroth, 1847, 1853). The design of the Langstroth hive offers beekeepers the ability to isolate boxes of brood frames from boxes of honey frames for easy harvest, through the use of honey supers and queen excluders. A secondary benefit of this hive design is the ability to remove frames for general colony health inspection, such as in the sampling for *V. destructor*. The regular box shape of the hive also allows for ease of transport for mobile pollination operations. Several hives placed in close proximity comprise a single yard, and multiple yards operated under the same management make up a single operation.

1.2.4 Notable Honey Bee Behaviours

1.2.4.1 Robbing

Robbing is a behavior exhibited by honey bees when nearby nectar sources are scarce, or when an opportunity arises for an easy source of honey. Often, stronger colonies will initiate the attack on weaker colonies, and attempt to steal their honey stores. This behavior can lead to the loss of large numbers of bees, and colonies that have been robbed will have a low chance of surviving the winter (Hamdan, 2010). Robbing can also serve as a transmission mechanism for communicable diseases, where the strong, healthy colony, robs from the weakened, diseased colony, inadvertently picking up pathogens that are then brought home (Hamdan, 2010).

1.2.4.2 Swarming and Absconding

Swarming is a behaviour naturally used by honey bees to advance the species and can be thought of as a means of reproducing at the colony level (Simpson, 1958). Swarming involves the interplay of several factors and is not fully understood from a mechanistic standpoint (Caron & Connor, 2013; Simpson, 1958). Swarming in its simplest, is the splitting of the colony into a parent swarm and a new colony. Days prior to swarming, the parent colony and queen will begin the process of rearing new queens. Shortly before the emergence of these new virgin queens, the original queen and roughly 60% of the colony will leave the hive for a new nesting site (Caron & Connor, 2013). Swarming inevitably causes the new colony to endure a lull in offspring production while the new wax comb is built. This is referred to as a brood-less period. The most common reasons for swarming appear to be overpopulation of the hive, and excess brood production without sufficient hive into which to expand (Winston, 1987). In feral bees, swarming is necessary to build and maintain populations, however, in modern beekeeping, swarming can lead to untimely weakening of a colony and loss in production. Swarming is primarily observed

in the late spring but can occur throughout the productive season. This behaviour can be leveraged or induced by beekeepers in an attempt to grow the number of colonies within the apiary by supplying empty hives for colonies to swarm to and artificially triggering a swarm (Vidal-Naquet, 2018).

Honey bees have also been observed to demonstrate a complete abandonment of a hive, in a behaviour termed absconding. The process of absconding is similar to swarming, in that scout bees will search for a suitable nesting location before the mass of bees mobilizes. However, instead of only 60% of the bees leaving, the entire colony will abandon their hive. Absconding similarly results in a brief brood-free period while a wax comb is constructed in the new location. This abandonment behaviour is primarily believed to be a stress response from a lack of food, environmental nuisances, or over-involvement by the beekeeper (Caron & Connor, 2013).

1.2.5 Nutrition

Honey bees require carbohydrates (honey and honeydew), proteins (pollen), fats (pollen), and water at differing ratios depending on caste and life stage (Vidal-Naquet, 2018). Water is the only component of the bee's diet that can not be stored within the hive and therefore bees require constant access to it to maintain colony health (Vidal-Naquet, 2018). Honey bees will utilize honey stores as their primary food source throughout the season, but can be supplemented with high-sugar supplements to sustain them following honey harvest, throughout the overwintering period, and prior to sufficient nectar flow in the spring (OMAFRA, 2021a; Vidal-Naquet, 2018).

1.3 Factors Impacting Honey Bee Health

1.3.1 “Colony Collapse Disorder”

During the winter and spring of 2006-2007, North American apiarists saw a dramatic decline in their honey bee colonies, with some beekeepers reporting losses upwards of 80% and others experiencing a compete loss of their bee stocks (Kluser & Peduzzi, 2007). This sudden phenomenon puzzled entomologists, ecologists and beekeepers alike, and was given the unequivocal name “Colony Collapse Disorder” (CCD) (VanEngelsdorp *et al.*, 2006). Colony collapse disorder at this time was defined as the unexplained desertion of the colony by the worker bees and was not synonymous with overall colony death. When a colony falls victim to CCD, there are not visible piles of deceased bees littering the surrounding area of the hive. CCD differs from absconding, in that the worker bees leave behind food, capped brood, and the queen. The cause of CCD is hypothesized to be a multifactorial combination of pathogens, pests, environmental influences, and human-mediated influences (VanEngelsdorp *et al.*, 2010; Vidal-Naquet, 2018). In the foreground of the numerous hypotheses of CCD causes, is infestation by *Varroa destructor*, and intoxication by neonicotinoid pesticides (Watson & Stallins, 2016). No study has been able to identify a single cause for CCD, and therefore it is thought that various CCD outbreaks can be defined as having similar symptoms but with differing or multifactorial causes (Vidal-Naquet, 2018). It is because of this lack of clear cause that the term CCD has begun to become interchangeable with any colony loss (VanEngelsdorp & Pettis, 2014).

1.3.2 Environment

1.3.2.1 Food Availability and Climate

Because honey bees are foraging animals, the diversity and abundance of their diet is directly tied to the landscape surrounding them and the weather impacting said landscapes.

Honey bees will travel great distances if necessary to acquire the nectar and pollen required for survival and honey production, and have been reported to forage as far as 14.4 km from their hive in times of extreme scarcity (Beekman & Ratnieks, 2000). However, bees more commonly forage within 3 km of their colony. Because of the connection with their surroundings, honey bees have been considered as primary health indicators of the local environment, and bee products can be used as sentinel gauges of environmental toxins, such as lead (Lambert *et al.*, 2012). Lambert *et al.* (2012) demonstrated that honey bees kept near an urban environment appear more contaminated than those kept near cultivated farmland. Meteorological factors can play an indirect role in colony health as determinants of forageable plant health and diversity and modifiers of honey bee behaviours (Vidal-Naquet, 2018). Storm conditions can lead to prolonged confinement of honey bees in their hive leading to reduced foraging and ultimately a weakening of the colony (VanEngelsdorp & Meixner, 2010), whereas drought conditions can lead to a reduction of available nectar sources, increased robbing, and abandonment of the hive if persistent (Le Conte & Navajas, 2008; Winston, 1987). A balance of sufficient but not excessive precipitation in combination with warm temperatures has been shown to improve colony productivity indirectly through an increase in surrounding nectar availability (Shuel, 1992). Forager bees appear to be busier and take fewer breaks on sunny days and in contrast spend less time outside and are less productive on dark days or in poor weather (Riessberger & Crailsheim, 1997). Inside the hive, nurse bees also appear to be affected by poor weather, and in as little as a single day of rain they reduce their work by about half (Riessberger & Crailsheim, 1997). Climate factors can also predispose the colony to disease and worse health outcomes in miscellaneous ways, such as increasing the relative humidity in the hive leading to fungal

diseases, or through physical hazards such as high winds or flooding (in extreme scenarios) causing the hive to no longer be inhabitable (Vidal-Naquet, 2018).

1.3.2.2 Intoxication

With the industrialization of agriculture and the demand for greater yields in less space, with reduced crop loss, there has become a greater reliance on agrochemicals, many of which have negative health implications for honey bees (Cullen *et al.*, 2019; Mullin *et al.*, 2010; Siviter *et al.*, 2021). Even agrochemicals deemed to be “bee safe” have been shown to be detrimental to honey bee development when applied onto crops in combinations (Ricke, Lin, & Johnson, 2021). The residue of these chemicals can be detected in pollen, wax and honey stored within the hive, indicating that the effects of these chemicals may continue beyond their use in the crop season (Mullin *et al.*, 2010). Agrochemicals are considered to be another key factor in the increase in colony losses experienced in modern times (Henry *et al.*, 2012; Lu, Warchol, & Callahan, 2014; Mullin *et al.*, 2010; VanEngelsdorp & Pettis, 2014; VanEngelsdorp *et al.*, 2010). The location of a colony and agricultural use of the land surrounding the colony can therefore be reasonably hypothesized to be an indirect risk factor impacting colony health.

Agrochemicals developed specifically for use within the apiary such as miticides (to control levels of *Varroa destructor* mites), can likewise lead to adverse health events in honey bees if administered irresponsibly or for prolonged periods (Giovenazzo & Dubreuil, 2011; Mondet, Goodwin, & Mercer, 2011; Vidal-Naquet, 2018). Experts in the field have made comments about the difficulty of finding suitable chemical treatments in beekeeping to treat for parasitic pests as it is equivalent to “killing an insect on the back of another insect, that you do not want to harm, inside of a box containing food to be consumed by humans” (Kozak, 2021).

1.3.2.3 Overwintering

In general, honey bees in temperate climates are involved in a biphasic behavioural pattern, involving a productive season and a winter season. The productive season involves the months in which honey production is actively being pursued through foraging. The productive season is broadly stated as occurring between early spring and late autumn in the Canadian climate. However, more specifically, the productive season begins when viable nectar and pollen sources are available, typically between last and first frost. Outside of the productive season, honey bees endure a period of overwintering. During this time, brood production comes to a near or complete stop, as well as nectar foraging and thus honey production (Caron & Connor, 2013). The halt of foraging means that honey bees become entirely reliant on the honey they have produced and stored over the productive season.

Overwintering is a stressful time for honey bees and involves a variety of behavioural and physiological changes (Döke, Frazier, & Grozinger, 2015). The most observable change in behavior is the clustering of the colony around the central frames of the hive (Caron & Connor, 2013). The cluster serves the function of maintaining climatic homeostasis within the hive while simultaneously conserving the limited food stores. This cluster of bees will initially congregate in the lower sections of the hive, and progressively move upwards on the comb as the honey stores run dry. Nearing the end of the winter, as the days begin to lengthen, the bees will resume brood production in preparation for the spring nectar flow (Caron & Connor, 2013).

The stressors of the winter season cause the hive to become more fragile and is the most common time for a colony to die. There are several reasons for a colony to die during winter including management practices of removing too much of the honey stores without supplemental feeding, and the presence of unmanaged disease and pests in the months leading up to wintering

(Dainat *et al.*, 2012). In particular, the presence of *Varroa destructor* has been identified as the strongest predictor for an overwinter colony loss (Dainat *et al.*, 2012; Guzmán-Novoa *et al.*, 2010; Van Der Zee *et al.*, 2015). In Ontario, beekeepers have self-reported that *V. destructor* load in the fall is among the top reasons for an overwinter colony loss (Ferland *et al.*, 2018). The vulnerability of honey bees during the winter months emphasizes the need for strong colonies entering the winter season, free from disease, and in possession of adequate food stores (Ferland *et al.*, 2019).

1.3.3 Management Factors

The World Organisation for Animal Health (OIE) recognizes that the process of animal husbandry and farming possesses numerous points of risk in food safety and animal health (OIE: World Organisation for Animal Health, 2010). The threat to honey bee health due to mismanagement manifests in three forms: biohazards, chemical hazards, and physical hazards (Vidal-Naquet, 2018). Inappropriate sourcing and distribution of bees and biological material such as sperm can also be a biosecurity issue for honeybee health. The trade of honey bee biomatter, such as queens, poses the main risk of global disease spread and introduction of new disease, so appropriate sourcing and shipping practices must be maintained (Mutinelli, 2011). Beekeeping management practices and experience are major influencers of the overwinter survival of a colony (Giacobino *et al.*, 2016; Jacques *et al.*, 2017). Specific management factors found to be associated with higher overwinter mortality include: not attending beekeeping courses within the past three years, not being a member of a beekeeping organization, lack of qualifications or licensing, and being a hobbyist level beekeeper (Jacques *et al.*, 2017). Management factors have been demonstrated to have a stronger association with colony loss than environmental stressors, after controlling for access to food (Giacobino *et al.*, 2017).

1.3.4 Viruses, Bacteria, Fungi, & Parasites

Playing a considerable role in world-wide honey bee colony health are colony diseases associated with various viral, bacterial, fungal, and parasitic pathogens. These infectious diseases can lead to weakening of the colony, modified behaviour, loss of production, and colony loss. Honey bee colonies worldwide are susceptible to 22 known viruses with six being of greatest concern due to their virulence: deformed wing virus, black queen cell virus, sacbrood virus, Kashmir virus, acute bee paralysis virus, and chronic bee paralysis virus (Chen & Siede, 2007; Tantillo *et al.*, 2015). These viruses may present a notable risk to colony survival, and are considered by some as a key contributor to the elevated levels of colony loss, especially if the colony is coinfecte

d with the parasitic microsporidium *Nosema sp.* (Tantillo *et al.*, 2015). A common vector of viruses is the *Varroa destructor* mite, responsible most notably for transmitting deformed wing virus and associated with substantial colony weakening (Dainat *et al.*, 2012a; Gisder *et al.*, 2009; Le Conte *et al.*, 2010; Posada-Florez *et al.*, 2020; Rosenkranz, Aumeier, & Ziegelmann, 2010). The mite, *Tropilaelaps clareae* is another emerging parasitic vector of disease in the global beekeeping landscape (not yet present in Canada) that is expected to further contribute to substantial colony losses (Chantawannakul *et al.*, 2018; de Guzman *et al.*, 2017; Khongphinitbunjong *et al.*, 2016). Bacterial diseases such as the American and European foulbrood (*Paenibacillus larvae* and *Melissococcus plutonius*) are the most detrimental bacterial diseases known to affect honey bee colonies and also contribute to large levels of colony loss (Forsgren, Locke, Sircoulomb, & Schäfer, 2018; Hansen & Brødsgaard, 1999; Stephan, de Miranda, & Forsgren, 2020). Of all of the pests and diseases, the *Varroa destructor* mite is still widely regarded as the greatest threat to beekeeping and honey bee health (Guzmán-Novoa,

Eccles, Calvete, McGowan, *et al.*, 2010; Ramsey *et al.*, 2019; Rosenkranz, Aumeier, & Ziegelmann, 2010; Traynor *et al.*, 2020).

1.4 The *Varroa* Mite (*Varroa destructor*)

The parasitic mite, *Varroa destructor*, is a pest of major concern in the global and Canadian beekeeping industry. To date, nearly 20,000 peer-reviewed articles have been published concerning the “*Varroa* mite”, with approximately 12,000 publications in the past decade (Google Scholar, 2022). At the 2019 International Apicultural Congress (Apimondia), several keynote presentations were devoted to the topic of mite control, breeding for mite resistance, and the status of mite research, further illustrating the global concern and interest in *V. destructor* and the timeliness of the subject (*Apimondia 2019 Abstract Book*, 2019).

Repeatedly, researchers investigating the causes of elevated colony losses discover the *Varroa* mite to be the greatest contributor, despite beekeepers believing the losses to be caused by a synergistic effect of multiple factors (Guzmán-Novoa, Eccles, Calvete, McGowan, *et al.*, 2010; Le Conte *et al.*, 2010; Staveley, Law, Fairbrother, & Menzie, 2014).

1.4.1 History

Originating in Asia, the native host for *Varroa destructor* is the Asian honey bee, *Apis cerana*, which unlike *A. mellifera* has co-evolved with *Varroa* mites where the mites are not known to cause any substantial harm to infested colonies (Rosenkranz *et al.*, 2010). The *Varroa* mite is hypothesized to have been introduced to South America, through the illegal trade of queens, before being first identified in the United States of America in 1987, and rapidly distributed across North America through the movement of hives and nucleus colony sales, arriving in Canada shortly afterwards in 1989 (Connor, 2015; Melhim, Weersink, Daly, & Bennett, 2010; Peck, 2021). Today, *Varroa* mites can be found world-wide (select island

locations are still considered to be *Varroa*-free at this time including; Australia, the Isle of Man, and New Zealand's Chatham Islands), and in Canada can be found in every province with the exception of Newfoundland and Labrador (Peck, 2021).

1.4.2 Reproductive Cycle

The *Varroa* reproductive cycle is highly intertwined with that of the honey bee and can not reproduce outside of the honey bee brood. Mites exist in one of two main stages in their life cycle, the phoretic and the reproductive stages. A mite in the phoretic stage is a mature female mite that has latched onto an adult bee to feed upon the bee's fats stores (a recent discovery made by Ramsey *et al.* (2019) contrary to the long-standing theory that mites feed only on the hemolymph of adult bees similar to a tick feeding on blood (Rosenkranz *et al.*, 2010)). Mites that have latched will do so in a way that allows them to be protected from innate honey bee grooming behaviours that would otherwise dislodge them, and do so by nestling between the plates of the abdomen (Delfinado-Baker, Rath, & Boecking, 1992; Ramsey *et al.*, 2019). Mites will feed for an average of 12-14 days and will conveniently use the movements of their host bee to travel to new sites for the reproductive phase of their life cycle to begin.

The reproductive cycle of the *Varroa* mite is a multistep process involving precise timing and synchronization with the reproductive cycle of the honey bee. The reproductive site of the mite is within a cell of honey bee brood, from which the mother and offspring mites can feed upon the developing bee out of sight of nurse bees. A mature female mite looking for a cell to lay her eggs is guided by a naturally secreted pheromone by the honey bee larva signalling the nearing of the prepupae stage (Le Conte *et al.*, 1989). The mother mite will enter the brood cell just before the cell is capped in wax and orient herself beneath the larva and into the larval jelly (the food that sustains the bee larva during development) as a mechanism of hiding from

hygienic worker bees (Rosenkranz *et al.*, 2010). The mother mite will then proceed to pierce through the outer integument of the larva and begin feeding for approximately 70 hours before the first egg is laid. The first egg laid is unfertilized and will develop into a male for the purpose of fertilizing subsequent female mites. Multiple mother mites may enter a common brood cell to facilitate mating (Martin, 1995), but in the absence of a second mother mite, inbreeding of the male and female offspring mites will occur. Subsequent eggs are then laid approximately every 30 hours (1-6 eggs in total), all of which are females (Rosenkranz *et al.*, 2010). The male mite(s) waits for the female mites to achieve sexual maturity then proceeds with mating. Once the wax capping of the brood cell has been breached, only the fertilized females will emerge and enter the phoretic stage or find a new cell for reproduction. When honey bee brood is abundant, approximately 70% of *Varroa* mites in the colony will exist in the capped brood (Boot, Schoenmaker, Calis, & Beetsma, 1995).

Of important note is the affinity of *Varroa* mites for honey bee drone brood during reproduction. *Varroa* mites will preferentially select drone brood over worker brood for three key reasons: a larger brood cell size compared to worker brood; a longer development time for drones allowing for more eggs to be laid by the mite in a single reproductive cycle; and a greater attention to drone larva by nurse bees, providing more food to the larva and therefore to the mites (Vidal-Naquet, 2018). This attraction to drone brood can be exploited by beekeepers as a control measure for *Varroa* mites by setting “trap brood” in which frames of drone brood are removed by the beekeeper before coming to maturity, effectively removing the *Varroa* mites trapped inside the cells (Ontario Beekeepers’ Association, 2014).

1.4.3 Population Dynamics

The population growth rate of *Varroa* is largely dependent on the population growth rate of bees and the amount of brood available, and therefore their population dynamics are highly correlated across the beekeeping season, albeit shifted in time. The population of *Varroa* mites within a colony throughout the season is dictated by the initial level of mites existing in the spring, a factor determined by the mite infestation level prior to overwintering, as mature female *Varroa* mites are able to survive in the cluster and resume egg laying in the spring brood. As more brood is produced during the summer months, there is a greater availability for mites to lay eggs and the population of mites can grow rapidly, with a doubling time of approximately 20 days (Fries, Camazine, & Sneyd, 1994; OIE: World Organisation for Animal Health, 2021). Although the population of honey bees in a colony peaks between the months of July and August, *Varroa* mite populations typically remain relatively low until the late summer and then will dramatically increase in population size by October (OIE: World Organisation for Animal Health, 2021). However, mite population growth is dictated by several factors both inside and outside of the colony, including: presence of drone brood, swarming behaviour, genetic predisposition to *Varroa* sensitive hygiene behaviour, ambient climate, nectar flow, and management and treatment for *Varroa* mites (Guzmán-Novoa *et al.*, 2010; Rosenkranz *et al.*, 2010).

1.4.4 Varroosis

The term varroosis is often used to describe the clinical state of infestation of a colony by *Varroa destructor*. Varroosis has effects at both the colony and the individual level. At the colony level, clinical signs of varroosis include a reduction in honey production, reduced and scattered brood cells (rather than the typically observed solid brood patterns and near-full

frames), a reduction in overall bee population, and a general weakening of the colony described by less than normal levels of activity (Vidal-Naquet, 2018). Colonies experiencing a high level of *Varroa* are also more predisposed to viral and bacterial pathogens. At the individual level, varroosis can involve numerous adverse health outcomes, namely a reduction in bee weight, a shortened life span, physical deformities (characteristic of a bee infected with deformed wing virus transmitted by *Varroa*), and immunosuppression (Vidal-Naquet, 2018). Recently, researchers at the University of Guelph Honey Bee Research Centre have discovered that varroosis may also lead to impaired memory, and behavioural changes, building off of previous findings that suggest varroosis leads to a reduced ability to navigate on foraging trips (Kralj, Brockmann, Fuchs, & Tautz, 2007; Kralj & Fuchs, 2006; Morfin, Goodwin, & Guzman-Novoa, 2020; Rosenkranz *et al.*, 2010).

1.4.5 Transmission and Risk-Factors

Varroa mites primarily transfer to a new host via close contact between the infected bee and the new host bee. Within the same colony, opportunities for mites to transfer from one bee to the next are ample as bees are continuously in close proximity to each other. Mite transfer between colonies can occur in several ways and is still primarily dependent on physical contact between bees. It has been hypothesized, however, that a transfer of mites can also occur indirectly at foraging sites, being left behind by one bee and picked up by another, but this transmission route has not been fully proven and is not expected to be overly common even if viable (Peck, Smith, & Seeley, 2016). The primary means of inter-colony *Varroa* mite transfer are generally believed to be robbing, drifting, wandering drones (drone bees tend to be less particular to which colony they return), absconding, and swarming (Peck & Seeley, 2019; Vidal-Naquet, 2018). The transfer of mites by all these routes occurs similarly where a *Varroa*-carrying

bee from one colony comes in contact with a *Varroa*-free bee from another. This can occur when either of the bees (*Varroa*-carrying or *Varroa*-free) enters a colony other than that from which it originated. The two primary means of mite transfer between colonies appear to be by either drifting or robbing (Peck, 2021). Drifting occurs as a chance contact between neighbouring colonies in which a returning bee enters the wrong hive by mistake and either acts as the mite donor or recipient. Drifting behaviours can be minimized by making hive entrances distinctive, typically done through the use of colours, physical landmarks, and hive entrance orientation (Jay, 1971).

Recently, beekeepers have noted that when a colony fails because of *Varroa*, there is a subsequent surge of *Varroa* counts in nearby colonies. Two theories have been proposed to explain this phenomenon: “mite bombs” and “robber lures”. The “mite bomb” theory suggests that the failing colony will experience a greater than normal level of bees drifting into new colonies, supposedly due to the navigational and behavioural effects of *Varroa* infestations, and thus an emigration of bees carrying mites to healthy colonies (Kralj & Fuchs, 2006; Oliver, 2018). In contrast to the “mite bomb” theory, the “robber lure” theory, involves bees from healthy colonies seeing the weakened colony as an easy robbing target and inadvertently bring mites back to their own colony (Peck & Seeley, 2019). Regardless of the mechanism, there is a clear regional effect at play and neighbouring apiaries can influence each other’s risk for *Varroa*. This regional effect has received more attention in recent years as beekeepers in Ontario have begun to cite “*Varroa* from nearby beekeepers” as a key factor responsible for overwinter colony loss (Claing *et al.*, 2020). In New Zealand, researchers provided further evidence for this as they reported a 17% decrease in the odds of *Varroa* infestation for every kilometer increase in distance from a currently infested hive (Stevenson, Benard, Bolger, & Morris, 2005). Therefore,

the density and spatial relationship of colonies in a given region are thought to be potential risk factors for *Varroa* infestations but this has yet to be adequately studied.

Previous research in Argentina utilizing mixed-effects logistic regression to identify pertinent risk-factors for high-level *Varroa* infestations has suggested that failing to appropriately monitor for mites is among the strongest management-based risk factors (Giacobino *et al.*, 2014). Likewise, the same research noted replacing queens, and adequate supplemental feeding of pollen and carbohydrates appeared to offer a level of protection against high-level *Varroa* infestations (Giacobino *et al.*, 2014), although these results could be confounded by the general level of beekeeper involvement and experience. Further management factors found to influence the risk for high-level *Varroa* infestation included: level of beekeeping experience, treatment of mites before overwintering, and spring sampling of mite levels (Giacobino *et al.*, 2016). When compared to management factors, however, environmental variables (i.e., surrounding land-use, temperature and humidity) appear to be the dominant driver of mite infestations when the study area is large enough to capture a great enough contrast between regional features, but in small-scale studies, lacking regional diversity, management factors appear to be dominant (Giacobino *et al.*, 2017).

1.4.6 Sampling for *V. destructor*

In Ontario, three *Varroa* sampling methods are approved and promoted by OMAFRA: alcohol wash, ether roll, and sticky board (OMAFRA, 2021c). Both the alcohol wash and ether roll methods involve removing a sample of approximately 300 adult bees (a $\frac{1}{2}$ cup scoop) from the hive and dislodging mites through means of killing both the bees and the mites in alcohol. Then the number of mites that were released are counted and divided by 3 to estimate a *Varroa* count per 100 bees. This form of sampling provides an indication of the number of live mites in

the phoretic stage, but it does not capture the number of mites that have died (i.e., dropped to the bottom of the hive) or are currently present in the brood. Sampling for mites using this method should be done in such a way to ensure that the queen is not sampled and that the sample is taken from young healthy bees (preferably nurse bees) on a brood frame, where mites are more likely to be present (Lee *et al.*, 2010). Alternatively, the sticky board method involves placing a sticky sheet beneath the frames of the hive and counting the number of dropped mites over a specific time frame, be it 24, 48 or 72 hours to estimate a daily drop rate or the “natural mite fall”. The main disadvantage of the sticky board method is the lack of representation for the number of mites still present either on the bees or in the brood, and therefore this method is most effective when used immediately following mite treatment, known as the “total mite drop” (Coffey & Breen, 2013; Pernal & Clay, 2013). Both the live mite (alcohol wash) and dropped mite (sticky board) sampling methods provide accurate estimations of the mite pressure being faced by the colony but both have disadvantages and must be interpreted differently (Vidal-Naquet, 2018).

OMAFRA recommends that sampling should occur at least twice a year (in the spring and fall) and that an approved treatment strategy be implemented if sampling reveals mite counts greater than the ministry threshold values (OMAFRA, 2021c). Critical mite threshold values in Ontario vary based on time of year and sampling method, but as an example, sampling by the alcohol wash method (most common amongst inspectors and beekeepers in Ontario) would use a threshold of 2 mites per 100 bees in spring and 3 mites per 100 bees in late summer to determine if treatment is required (OMAFRA, 2021c). Many beekeepers will experience an increase in the number of mites present in a sample during the fall months. As the honey bees enter their broodless period, mite reproduction must also stop and mites within the colony will be more likely to exist outside the comb and are therefore available for sampling (Peck, 2021).

1.4.7 Control and Treatments

Without proper control and treatment measures, *Varroa* mites will decimate a colony within only a few seasons (Rosenkranz *et al.*, 2010). Control and treatment measures can include beekeeping management practices as well as the application of synthetic or natural chemical substances to the colony to kill the mites present. In Ontario, three synthetic and three organic treatment chemicals are approved for use by OMAFRA. Approved synthetic treatments include: amitraz (trade name: Apivar®), tau-fluvalinate (trade name: Apistan®), flumethrin (trade name: Bayvarol®) and coumaphos (trade name: CheckMite+™). Approved organic chemical treatments include: formic acid strips (trade name: Mite Away Quick Strips®), oxalic acid dehydrate, and thymol (trade names: Apiguard® and Thymovar®) (Kozak *et al.*, 2021).

Misuse and prolonged use of these treatment chemicals can lead (and has led) to mite resistance, adverse health outcomes for the bees, and residues in bee products that could pose human health risks (Vidal-Naquet, 2018). It is therefore recognized that chemical treatment strategies, although necessary, should be used as a supplement to management/prevention strategies and natural forms of *Varroa* defence by the bees. Because *Varroa* mites are fully dependent on honey bee brood for reproduction, many non-chemical management practices for mite control involve the disruption of the colony's brood production to interrupt mite population growth. Non-chemical control measures can include: removal of drone brood, queen trapping to temporarily prevent egg laying, drone brood traps, removal of brood frames, and artificial swarming (Vidal-Naquet, 2018).

A long-term goal for controlling *Varroa* has become breeding bees to be more equipped to resist mites and suppress mite populations naturally within the hive. Breeding bees with greater *Varroa* sensitive hygiene (VSH) tendencies is an ongoing venture in research and

beekeeping, in which worker bees are able to detect *Varroa* mites capped in the brood, and extract and dispose of the infested bee before the mites reach maturity (Rinderer, Harris, Hunt, & de Guzman, 2010). Other *Varroa* resistant traits include: general hygienic behaviour, suppression of mite reproduction, grooming, uncapping/recapping behaviour, reduced development time, and “survivor” bees (Peck, 2021). Since 1984, over 153 studies have been conducted on natural and selective breeding towards *Varroa* resistant stocks of bees, showing promise for the future (Mondet *et al.*, 2020). However, at present, there is no commercially available stock of bees that show signs of enough *Varroa* resistance to eliminate the need for treatment (Peck, 2021).

1.4.7.1 Integrated Pest Management

Integrated pest management (IPM) approaches have become a key component of *Varroa* mite defense in many jurisdictions because no single control measure is 100% effective and the eradication of *Varroa* mites is considered impossible (Vidal-Naquet, 2018). IPM strategies focus on maintaining mite levels below a critical threshold, recognizing that a certain level of the pest is both inevitable and tolerable (Jack & Ellis, 2021), while simultaneously reducing the dependence on chemical treatments (van Alten, Tam, & Bryans, 2013). Maintaining mites at hypoendemic levels is done through a combination of chemical treatments, management strategies, enhanced monitoring, and education outreach. The steps of an effective IPM strategy involve the development of a critical pest threshold, continuous monitoring of the pest population, implementation of prevention techniques, and application of treatments whenever critical thresholds are reached (Jack & Ellis, 2021). IPM approaches favour control measures with the least risk first and progress into more invasive methods as needed, following a general order of: (1) cultural controls, (2) mechanical controls, (3) biological control, and (4) acaricides (Jack & Ellis, 2021; Roth, 2020). Cultural control methods involve modifying the environment to

be less suitable for mites without large impacts on the bees themselves (i.e., using mite resistant stocks, occasionally disrupting egg laying, and sanitary beekeeping practices). Mechanical control measures are slightly more invasive and can involve either physically removing mites from the colony or mechanical segregation of mites from the bees (i.e., removing drone brood, and installing screened bottom boards). In the context of honey bees and *Varroa* mites, biological controls do not have a large practical role, but in theory would involve the introduction of a natural enemy of *Varroa* mites into the colony. Research into entomopathogenic fungi is ongoing and may offer some level of biological control in the future (Chandler, Sunderland, Ball, & Davidson, 2001). IPM is not synonymous with treatment-free beekeeping, and does not eliminate the need for acaricides, but rather reserves chemical treatments for times of greater need to prevent the development of resistance or contamination of the environment or honey bees. For this reason, chemical control measures are at the top of the IPM pyramid and are to be used only when the lower risk control measures cannot keep mite levels below the threshold. However, in practice, beekeepers continue to rely heavily on chemical control measures, leading to increased concern for acaricide resistant mites and toxicity (Jack & Ellis, 2021). Chemical treatments continue to be the most effective control measures for *Varroa* mites, and although they can lead to unwanted side-effects, the complete abandonment of their use will almost certainly result in a colony becoming overwhelmed with mites. It is therefore important to continue to use chemical treatments but to do so judiciously.

IPM strategies are fluid and should adapt to changing circumstances. Knowledge of the transmission routes and risk factors, as well as continuous monitoring of *Varroa* mite levels in the population, are important components in addressing the effectiveness of an IPM strategy and

informing regional adaptations as needed throughout the seasons. Surveillance of mites is key to recognizing if current approaches are effective in controlling mites at a population level.

1.5 Geographic Epidemiology

Geographic (often referred to as spatial or geospatial) epidemiology is a subfield of epidemiology focussed on the description, quantification, and analysis of spatial patterns of health status and the known or presumed determinants associated within a population (Berke & Waller, 2010). Methods and applications of geographic epidemiology for use in public health have been reviewed thoroughly, and provide unique information for disease surveillance (Waller & Gotway, 2004). Geographic epidemiology is based on available spatial data containing information regarding a position in space. Spatial data can exist at various levels of specificity and can be representative of a single observation or an aggregation of multiple observations existing within a common location or region. However, the aggregation of point data into regional data can itself impose a level of bias, as the choice of boundary may affect the perceived rate of disease as described by Openshaw (1984) as the modifiable areal unit problem (MAUP). Special consideration must be given to spatial data due to the inherent existence of autocorrelation as stated in Tobler's first law of geography: "everything is related to everything else, but near things are more related than distant things." (Tobler, 1970). Applying standard epidemiologic methods to spatial data can be an erroneous endeavour as these methods assume independent observations, which is not the case for communicable diseases in relatively close proximity.

Geographic epidemiologic methods classically fall into one of three broad categories based on objective: (1) descriptive mapping to visualize distributions and patterns of disease; (2) disease cluster detection to identify areas of elevated (or reduced) disease risk; and (3)

geographic correlation studies investigating the association of a disease and its risk factors. All of these methods can be particularly beneficial in disease surveillance, where knowledge of the distributions of a disease over space can inform intervention strategies and provide broad geographical context.

A classic example of the application of spatial data to explain the transmission of disease is John Snow's investigation into the London cholera outbreak of 1854, in which hand drawn disease maps led to the realization of the origin of the disease and subsequently to the end of the outbreak (Snow, 1855). Although Louis Pasteur would not present his renowned "Germ Theory" for another several years, Snow (through geographic epidemiology) was able to determine the route of disease transmission and curb the spread, despite not fully understanding the proximate cause (i.e., *Vibrio cholerae*). Likewise, today, geographic epidemiology methodologies can be applied to public health problems to identify and explain patterns of disease transmission without the need for prior knowledge of the proximate cause.

1.5.1 Disease Mapping

Descriptive disease mapping is an exploratory and often initial step in geographic epidemiological studies in which a disease metric is displayed on a map of the study area, corresponding to the location it is representing. Depending on the type of spatial data available, disease maps can take on various forms. Spatial data exist as one of three categories: (1) spatial point data, (2) regional data, and (3) geostatistical data. Spatial point data are comprised of a collection of locations each with their own corresponding information regarding the disease status, or metric of interest. Therefore, point data naturally lend themselves to be depicted with a dot map. A dot map shows each data point as a discrete mark on the map, stylized through colour and/or shape to illustrate the disease status. Regional data are a collection of observations across

a study area that have been aggregated by some form of regional structure dividing the study area into non-overlapping subsections. These subsections can be regular or irregular in shape and/or size, and thus should be carefully selected from a predefined regional structure with cultural or biological relevance, as to not impose bias through the MAUP. Regional data are most readily displayed through choropleth maps where the study area, broken up into the defined regions, is coloured to depict the metric of interest. Data aggregated by political or administrative regions are the most common format for population health data. Geostatistical data are typically represented by so called isopleth maps. Geostatistical data entails a quantitative metric (i.e., *Varroa* infestation level or risk) in relation to a geographic location without subdividing the study area into regions. Common examples of maps depicting geostatistical data include weather maps and topographic maps, but maps of similar visual characteristics can be developed for epidemiologic values as well. A common application of geostatistical data and isopleth maps in disease surveillance is the development of a continuous risk map for a given disease (Berke, 2004).

1.5.2 Kriging

Kriging is a geostatistical method with origins in geology and gold mining developed by the French mathematician Georges Matheron building on work by Danie Krige to interpolate continuous measurements across a study area using only a limited number of samples (Matheron, 1960). These methods can also be applied to health data to produce continuous spatial predictions of disease status across a population from data with a finite number of observation locations, or from data aggregated to a regional level (Berke, 2004; Carrat & Valleron, 1992). Producing geostatistical predictions can allow for routine disease surveillance efforts to be transformed into risk maps representative of areas with limited or no data. Kriging methods

borrow information from neighbouring observations to supplement the information obtained at and between sampling locations. Observations closer together in space are expected to provide more information (Tobler's first law of geography) and are therefore given more weight compared to more distant observations. The amount of weight given to neighbouring observations is determined through a spatial dependence structure described by a variogram function, proposed by Georges Matheron (1963). The variogram model typically contains several key features: the nugget, sill and range. The nugget parameter represents the measurement error variance. The sill of the variogram represents the overall variance in the spatial process. And the range denotes the Euclidean distance between locations where observations can still influence each other; Observations from locations further apart than the range are deemed independent.

1.5.3 Disease Cluster Detection

In addition to disease mapping, some epidemiologic studies aim to identify disease clusters, which are aggregations of cases occurring within a common area, whose intensity differs from the expected amount (greater or less than expected). The identification of disease clusters can be achieved through scan test statistics, namely the spatial scan test and the flexible scan test (Kulldorff, 1997; Tango & Takahashi, 2005). Scan tests utilize scanning windows that move across the study areas visiting each observation location, increasing in radius until a maximum percentage of the population is covered. For each window the likelihood of the observed cases is estimated. For the window maximizing the likelihood function, a likelihood ratio test is performed to compare the likelihood of observations within and external to the window. A disease cluster is identified if the observed number of cases within the window differs substantially from what would be expected (Kulldorff, 1997; Kulldorff & Nagarwalla, 1995). The interpretation of a disease cluster is that the risk of disease within the cluster is

meaningfully higher (or lower) than in the remaining study population. These clusters can then be used to guide future research and inform intervention efforts.

1.5.4 Geostatistical Modelling

Standard regression models assume independence of their residuals. This assumption is likely violated when modeling of geostatistical/spatial data is the focus. Neglecting the independence assumption when modeling spatial data may lead to overdispersion. In the case of overdispersion all forms of model diagnostics are biased due to a biased estimate of the residual variance. Attempting to fit a standard regression model to spatial data can result in overdispersion, due to spatial clustering, and outliers, due to spatial clusters. Geostatistical models solve the problem of overdispersion with the addition of a spatially correlated random effect to account for spatial clustering and better provide estimates of the true correlation between an outcome and a presumed risk-factor (Liang & Zeger, 1986; Waller & Gotway, 2004). Julian Besag, Jeremy York, and Annie Mollié (1991) extended this further with the proposal of the Besag-York-Mollié (BYM) model in which a second, spatially uncorrelated, random effect is included to account for the outliers, or spatial clusters. Alternatively, the inclusion of a nugget parameter in a variogram can also be used to model a second random effect (i.e., clusters) in a Gaussian model. Additionally, universal kriging provides the ability to combine the benefits of spatial regression modelling with kriging to achieve a continuous risk interpolation across a study area (Matheron, 1969). The risk at a location can be predicted as the sum of a deterministic (generalized linear model) and a stochastic process (kriging predicted residuals).

1.5.5 Past Applications of Geostatistical Epidemiology in Veterinary Science

Although geostatistical epidemiology is not well represented in the literature of honey bee research, there have been some exemplar applications. In 2005, Stevenson *et al.* described

the spatial distribution of *Varroa destructor* in apiaries across the North Island of New Zealand, and demonstrated how the degree of autocorrelation decayed per kilometer away from the originating site (Stevenson *et al.*, 2005). This appears to have been the first large-scale study to investigate the spatial pattern of *Varroa* infested colonies, and to quantify the rate of local spread based on the found level of autocorrelation. More specifically, Stevenson *et al.* (2005), through disease mapping were able to identify that the odds of a colony being infested by *Varroa* were greatest in the area immediately surrounding the Auckland International Airport, due to the temporary storage of imported honey bees arriving with existing mite infestations. Identification of the site of incursion, and the rate at which mite infestations can spread, allowed for government stakeholders and beekeepers to make changes to how they survey the regions in close proximity to better contain potential outbreaks.

A recent application of cluster detection methods to *A. mellifera* and *V. destructor* outbreaks in North-Central Argentina identified several clusters of infestations of both high and low-risk (Molineri *et al.*, 2018). This study detected clusters ranging in size from 4.73 km up to 117.23 km with relative risks as high as 3.55 and as low as 0.083. The implication of these findings is evidence towards the existence of unknown environmental factors serving as risk-modifiers for *Varroa* infestations at a regional scale. By identifying the location of these aggregated cases, Molineri *et al.* facilitate further comparative studies to be initiated in attempts to determine the key differences between the low and high risk regions and thus identify potential causes for high levels of *Varroa*. Combining the results of this spatial analysis with results from a beekeeper management survey and information on the surrounding vegetation, Molineri *et al.* (2018) further indicate that the risk for *Varroa* is apparently highest in areas with

a poor surrounding landscape, despite optimum management practices. Suggesting that environmental factors may be more important to bee health than management factors.

Further examples of geostatistical modelling in application to *A. mellifera* are limited but have been successful in explaining some of the spatial variation of varroosis and other diseases through climate and weather variables in the United Kingdom (Rowland *et al.*, 2021). Despite the rare application of geostatistical modelling to varroosis in the literature, the need for such methodologies has been recognized, as open-access data sources designed for spatiotemporal modelling of *Varroa* mites have become available (Rubinigg *et al.*, 2021). Moreover, examples of geostatistical modelling in areas of veterinary public health and epidemiology outside of honey bee research are abundant and can further attest to their value and application to disease control. Specific examples of spatial modelling being applied to veterinary public health problems include predictive risk mapping of rabies in Morocco (Khayli *et al.*, 2021), *E. multilocularis* in red foxes of Germany (Berke, Romig, & von Keyserlingk, 2008), and bovine tuberculosis in Mexico (Martinez, 2007). Additionally, spatial scan statistics have been used frequently in veterinary science to investigate a variety of diseases and species (Costa & Kulldorff, 2009; Nwosu, Berke, Pearl, & Trotz-Williams, 2019; Thomas-Bachli, Pearl, Parmley, & Berke, 2020).

1.6 Time Series Analysis

When observations are recorded on the same object or population repeatedly over time, they are called time series data. As with spatial data, special consideration must be given in time series data analysis to account for the autocorrelation fundamentally present. Tobler's first law of geography (1970) can be conveniently adapted to time series data in that when repeat observations are made over time, those that are made in the recent past are expected to be more

similar to the current observation, than those made in the more distant past. Time series data are a natural product of continuous disease surveillance efforts and are therefore common in epidemiological studies. Time series analyses can be used for descriptive statistics or applied for the purpose of explanation or prediction (i.e., risk factor assessment and forecasting). Furthermore, continuous monitoring of time series data can be useful in early outbreak detection.

1.6.1 Descriptive Time Series Analysis

Descriptive time series analysis starts with a time series plot where time is on the x-axis and the health variable of interest on the y-axis. From this point, initial patterns can be identified, and the process of decomposing the data into its component parts can begin. Time series data are made up of two primary component parts: the signal component and the residual noise (Tukey, 1977). The signal component of time series data is predictable/explainable, in theory, and can be further divided into trend, season and autocorrelation components. Extracting these components from a dataset can be accomplished through seasonal-trend decomposition using locally estimated scatterplot smoothing (LOESS) (STL) (Cleveland, Cleveland, McRae, & Terpenning, 1990).

1.6.2 Time Series Modelling

Because of the intrinsic autocorrelation existing in time series data, standard regression modelling methods are not suitable, and specific time series modelling approaches must be used instead. The two most basic time series models, the autoregressive (AR) and the moving average (MA) models, were popularized by George Box and Gwilym Jenkins (1976) building from the Ph.D. work by Peter Whittle (1951). In the AR model, observations are modelled using lagged values of past observations. Contrarily, in the MA model, observations are modelled based on the errors of the previous forecasts. However, both AR and MA component models can be

combined into an ARMA model, which will take into account both the past observations and the error of previous predictions to estimate the present value (Box & Jenkins, 1976; Whittle, 1951). Both the ARMA model and the isolated AR and MA models assume stationarity and thus can only be applied to data free from long term trend and seasonal effects. Alternative models, the autoregressive integrated moving average (ARIMA) and seasonal autoregressive integrated moving average model (SARIMA) adjust for trend, and for season and trend components, respectively (Box & Jenkins, 1976).

Time series modelling approaches can further be used in conjunction with linear regression models to assess the influence of presumed risk-factors on the data while simultaneously accounting for autocorrelation. In a generalized linear autoregressive moving average model (GLARMA), count data (common in instances of disease incidence investigations) are defined by a general linear model component with a Poisson or negative binomial distribution in addition to an ARMA error component (Davis, Dunsmuir, & Wang, 1999). GLARMA models are fit iteratively, adding AR and MA terms until the residual error components are void of any remaining autocorrelation. Time series models have previously been applied to veterinary public health research, demonstrating a high degree of sensitivity and prediction ability for applications of disease surveillance in swine (Petukhova, Ojkic, McEwen, Deardon, & Poljak, 2018). Time series modelling approaches have also been demonstrated as a suitable methodology for use in automated forecasting of public health data and surveillance systems (Berke, Trotz-Williams, & de Montigny, 2020).

1.6.3 Past Applications of Time Series Analysis in Veterinary Science

With many production animals, routine data are collected consistently for the purpose of disease surveillance or monitoring of growth and production progress, resulting in time series

data sets being commonplace in veterinary science applications. However, like geographical epidemiology, the application of descriptive time series analysis or time series modelling has been largely absent from the literature regarding *V. destructor* and *A. mellifera*. Previous studies have used some basic time series principles to aid in the development of mathematical models of *V. destructor* population dynamics, but offer only a limited description of the observed data and use it primarily for the purpose of comparison and validation of a theoretical model (DeGrandi-Hoffman & Curry, 2004; Messan, Rodriguez Messan, Chen, DeGrandi-Hoffman, & Kang, 2021).

Time series analysis has, however, been applied in a multitude of scenarios across veterinary science across communicable and non-communicable diseases. A review of the literature regarding time series analysis in veterinary medicine highlighted the need for more comprehensive time series analysis when presenting time series data, and concluded that these methods are currently underutilized given their value in veterinary applications (Ward, Iglesias, & Brookes, 2020). This review found that in 37 articles published from 1990 to 2019, with a focus on time series analysis, all but three used data from routine surveillance efforts with data occurring either at the day or month scale and a median study period of 10 years. However, despite these available data, only about two thirds of the studies went beyond describing the time series data and appropriately applied times series analysis methods. The authors state that the application of time series analysis enables greater insight into the patterns and distributions of disease over time and can facilitate more effective disease surveillance and control through prediction of occurrence and early detection systems (Ward *et al.*, 2020). Further advocating for the effective use of time series analysis in veterinary science applications, Ward *et al.* (2020) provide an exemplary demonstration of how these methods can be applied from descriptive

statistics to multivariate ARIMA modelling and prediction using surveillance data of canine parvovirus as the event of interest.

1.7 Information Dashboards for Population Health Data

Knowledge translation and transfer (KTT) is a term encompassing a broad variety of activities aimed at making scientific research findings more readily accessible to those who need to know and those who can apply them in practice. According to the Canadian Institutes of Health Research: “Knowledge Translation is defined as a dynamic and iterative process that includes synthesis, dissemination, exchange and ethically-sound application of knowledge to improve the health of Canadians, provide more effective health services and products and strengthen the health care system” (Canadian Institutes of Health Research, 2016). KTT efforts can include, but are not limited to clear language reports, infographics, social media posts, educational events, and interactive online dashboards. With the omnipresence of the internet, online information dashboards emerged as a popular tool for presenting data in a digestible format in combination with relevant information addressing a broad audience of various educational backgrounds. In a public health context, dashboards present primary descriptive statistics and plots (time series plots, disease maps, and summary statistics) as a means of monitoring the progression of a disease throughout a population over time and space. In the early stages of the novel coronavirus (COVID-19) pandemic, hundreds of dashboards were developed across multiple countries to provide an automated and consistent stream of unbiased information, enabling the rapid translation of information to decision-making (Ivković *et al.*, 2021). Many of these dashboards required minimal human effort once launched (with the exception of housekeeping and reprogramming as government data sources altered how information was published (Gillis, Sobkowich, & Bernardo, 2020)) and therefore allowed researchers to focus

their efforts on more comprehensive and intensive analyses while basic statistics were still automatically being reported. The automation of disease surveillance information provides the added benefit of real-time updates, as new data can be analyzed and published shortly after collection, and repeatedly over a single day if needed. In contrast to traditional static reporting methods, many online dashboards allow for interactive exploration of the data by permitting users to disaggregate by region, time frame or both, such that basic patterns over time and space can be visualized for a specific region of interest. Although not yet extensively adopted by veterinary public health, online information dashboards have demonstrated their value for human disease applications throughout the COVID-19 pandemic, and have been recognized as a valuable tool by the Global Burden of Animal Disease project (Global Burden of Animal Disease, 2022). Consequently, the development and application of dashboards are therefore expected to be useful in the surveillance of *V. destructor*, and the broader field of honey bee disease surveillance.

1.8 Disease Surveillance

Disease surveillance is a branch of epidemiology formally defined by the World Health Organization as “the continuous, systematic collection, analysis, and interpretation of health-related data needed for the planning, implementation, and evaluation of public health practice” (World Health Organization, 1968). John Graunt’s analysis of the London “Bills of Mortality” in the 1600’s is an early example of surveillance data being applied to public health and is arguably a key turning point for the development of modern epidemiology (Berke, Sobkowich, & Bernardo, 2020). Through the simple monitoring of publicly disclosed death records, Graunt was able to determine that the frequency of communicable diseases varied by place and time, whereas non-communicable diseases remained stable. Furthermore, Graunt provided evidence

towards simple risk-factors for overall health (living in the country-side versus living in the city) drawn from basic surveillance data. Since then, disease surveillance efforts have evolved from manual record keeping towards more automated reporting of diseases, however the basic principles have remained the same, and surveillance has become a cornerstone of public health. The efficiency and effectiveness of disease surveillance is largely related to the timeliness of data collection and analysis (Webb, Bain, & Page, 2017).

The key outcome of disease surveillance is the collection of data on a disease(s) of interest to be analyzed for the purpose of informing decisions about public health interventions, estimating the burden of disease in a population, and earlier identification of outbreaks. Surveillance allows for insight into the changing nature of a disease and monitoring of how it is progressing at a population level. At the broadest level, disease surveillance exists in two forms: event-based surveillance and indicator surveillance. Event-based surveillance has a primary focus of monitoring outbreak events in a population, while indicator-based surveillance relies on the routine reporting of individual disease cases which can then later be analyzed as a group (Webb *et al.*, 2017). More specifically, disease surveillance efforts can exist in a variety of forms and can be active or passive in nature, where the latter involves waiting for notifications of a disease and the former attempts to seek out cases within a population. Depending on the nature of the disease of interest, a surveillance program may aim to provide an early warning of potential threats, contain an already existing outbreak, monitor an endemic disease to prevent an outbreak, or evaluate the current burden of a disease in a population. The identification of clusters over both space and time is a critical component of a sophisticated disease surveillance system, but requires information on the location and time of identified cases.

With the modern ubiquity of the internet, disease surveillance systems have benefited from access to an online stream of health data while simultaneously requiring less labour-intensive data collection methods (i.e., “shoe-leather epidemiology”). Reporting systems for notifiable and reportable diseases have become commonplace in veterinary medicine and act as a continuous source of surveillance data. “Rumor surveillance” involves the monitoring of unofficial sources to identify potential cases and outbreaks, such as screening of social media (e.g., Twitter – individuals tweeting about symptoms they are experiencing), review sites (e.g., Yelp – individuals leaving reviews of a restaurant from which they contracted a food-borne disease) and email chains (e.g., Promed – disease reports submitted by subscribers) (Webb *et al.*, 2017). As of late, rumor surveillance has become more widespread and has been successful in early outbreak detection, as evidenced by the success of projects such as BlueDot’s Outbreak Intelligence Platform which through the monitoring of multiple official and unofficial sources was able to identify the heightened presence of pneumonia in China six days before the World Health Organization released their official statement on the 2019 novel coronavirus (COVID-19) (Niiler, 2020). Furthermore, citizen science and crowdsourcing, enabled by the widespread use of the internet, are increasing in popularity as a means of collecting data for disease surveillance, mainly due to their low cost and high degree of population coverage (Welvaert & Caley, 2016). However, concerns exist regarding the quality of data collected through citizen science, especially in instances where there is room for subjectivity and an objective test can not be applied (Aceves-Bueno *et al.*, 2017; Kosmala, Wiggins, Swanson, & Simmons, 2016). Combining citizen science data collection with traditional surveillance at sentinel sites could therefore be beneficial in achieving a greater level of data coverage, while still maintaining a level of certainty of the data being collected (Smith *et al.*, 2021).

The crucial difference between disease surveillance and data collection is the timely analysis and dissemination of the results to stakeholders and those who can put the information into action for the sake of public health (Isere, Fatiregun, & Ajayi, 2015; Soucie, 2012; Webb *et al.*, 2017; World Health Organization, 1968). Therefore, the combination of geographic epidemiology, time series analysis, citizen science, and online information dashboards can enable the development of a comprehensive disease surveillance system towards controlling *V. destructor* populations in Ontario and beyond. This system can be used to inform policy decisions and evaluate the success of the current integrated pest management approach and intervention strategies.

1.9 Thesis Rationale and Objectives

The importance of honey bees in the global economy and agricultural sector is undeniable. Yet beekeepers have continued to experience great levels of colony loss or mortality each year. In Ontario, overwinter honey bee colony loss has reached levels as high as 58% (Canadian Association of Professional Apiculturalists, n.d.), which is nearly four times the expected and accepted level of 5-15% annual colony loss (Vidal-Naquet, 2018). Researchers, veterinarians and beekeepers have identified the parasitic *Varroa* mite as one of, if not the primary cause of these levels of loss (Claing *et al.*, 2020; E. Guzmán-Novoa, *et al.*, 2010; Rosenkranz *et al.*, 2010; Vidal-Naquet, 2018). Chemical control measures for *Varroa* are not 100% effective and the eradication of the mite is considered impossible due to reservoirs in feral bee colonies. Although efforts are being made towards the breeding of a commercially viable *Varroa*-resistant stock of bees, IPM strategies continue to be the best defence against mites and the associated colony losses through continuous monitoring efforts and the maintenance of mite levels below a critical threshold. The development and assessment of an IPM strategy requires

comprehensive knowledge of the host (honey bees), pest (*Varroa* mites) and the regional nuances that may modify their behaviours.

To date, no population-level spatial research has been conducted in Ontario regarding *Varroa* mites and their associated risk-factors. Few studies have explored the spatial relationships between apiary mite infestations and their surrounding environment. Detailed research into the population-level temporal patterns of *Varroa* mite infestations has likewise been sparse in the global literature and absent for Ontario. Detailed spatial and temporal investigations into *Varroa* mites in Ontario can produce valuable insights into the locations that may be experiencing greater or lesser than expected mite levels and aid in the development of targeted intervention and surveillance efforts.

Furthermore, it is recognized that province-specific information regarding *Varroa* is of interest not only to researchers and government officials, but also to the broad beekeeping community. Beekeepers of mixed backgrounds and experience levels can utilize tailored information to make informed, and timely management decisions and inspire future involvement in surveillance efforts, especially those that may involve self-submitted data.

The main goal of this thesis is to build upon the current literature regarding *Varroa* mites and improve the current issue of elevated honey bee colony losses. This thesis is comprised of three main objectives:

1. Understand the distribution of honey bee colonies (Chapter 2) in order to investigate the spatial patterns of *Varroa* mites in Ontario at a population level and explore the potential for an association with environmental covariates (Chapter 3).

2. Analyze *Varroa* time series data across multiple beekeeping seasons in Ontario to assess the seasonality and trends, and model potential associations with meteorologic factors (Chapter 4).
3. Develop an interactive knowledge translation tool designed for beekeepers, researchers, and government officials to monitor the state of *Varroa* in the province (Chapter 5).

1.10 References

- Aceves-Bueno, E., Adeleye, A. S., Feraud, M., Huang, Y., Tao, M., Yang, Y., & Anderson, S. E. (2017). The accuracy of citizen science data: a quantitative review. *The Bulletin of the Ecological Society of America*, 98(4), 278–290. <https://doi.org/10.1002/bes2.1336>
- Agriculture and Agri-Food Canada. (2020). *Statistical overview of the Canadian honey and bee industry, 2020*. Retrieved from <https://agriculture.canada.ca/en/canadas-agriculture-sectors/horticulture/horticulture-sector-reports/statistical-overview-canadian-honey-and-bee-industry-2020>
- Apimondia 2019 Abstract Book*. (2019). Retrieved from <https://www.apimondia2019.com/program/abstract-book/>
- Beekman, M., & Ratnieks, F. L. W. (2000). Long-range foraging by the honey-bee, *Apis mellifera L.* *Functional Ecology*, 14(4), 490–496. <https://doi.org/10.1046/j.1365-2435.2000.00443.x>
- Bees Act*, RSO 2019, c B.6, <https://canlii.ca/t/53lc3>.
- Berke, O. (2004). Exploratory disease mapping: kriging the spatial risk function from regional count data. *International Journal of Health Geographics*, 3, 1–11. <https://doi.org/10.1186/1476-072X-3-18>
- Berke, O., Romig, T., & von Keyserlingk, M. (2008). Emergence of *Echinococcus multilocularis* among Red Foxes in northern Germany, 1991–2005. *Veterinary Parasitology*, 155(3–4), 319–322. <https://doi.org/https://doi.org/10.1016/j.vetpar.2008.05.017>
- Berke, O., Sobkowich, K. E., & Bernardo, T. M. (2020). Celebration day: 400th birthday of John Graunt, citizen scientist of London. *Environmental Health Review*, 63(3), 67–69. <https://doi.org/10.5864/d2020-018>
- Berke, O., Trotz-Williams, L. A., & de Montigny, S. (2020). Good times bad times: automated forecasting of seasonal cryptosporidiosis in Ontario using machine learning. *Canada Communicable Disease Report*, 192–197. <https://doi.org/10.14745/ccdr.v46i06a07>
- Berke, O., & Waller, L. A. (2010). On the effect of diagnostic misclassification bias on the observed spatial pattern in regional count data – A case study using West Nile virus mortality data from Ontario, 2005. *Spatial and Spatio-Temporal Epidemiology*, 1(2–3), 117–122. <https://doi.org/10.1016/j.sste.2010.03.004>
- Besag, J., York, J., & Mollié, A. (1991). Bayesian image restoration, with two applications in spatial statistics. *Annals of the Institute of Statistical Mathematics*, 34, 1–20.
- Boot, W. J., Schoenmaker, J., Calis, J. N. M., & Beetsma, J. (1995). Invasion of *Varroa jacobsoni* into drone brood cells of the honey bee, *Apis mellifera*. *Apidologie*, 26(2), 109–118. <https://doi.org/10.1051/apido:19950204>
- Box, G. E. P., & Jenkins, G. M. (1976). *Time series analysis: forecasting and control*. San Francisco, CA, USA: Holden-Day.
- Butler, C. G., Callow, R. K., & Johnston, N. C. (1962). The isolation and synthesis of queen

- substance, 9-oxodec- trans -2-enoic acid, a honeybee pheromone. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 155(960), 417–432.
<https://doi.org/10.1098/rspb.1962.0009>
- Cale, G. H., & Gowen, J. W. (1956). Heterosis in the honey bee (*Apis mellifera L.*). *Genetics*, 41(2), 292–303.
- Canadian Association of Professional Apiculturalists. (n.d.). Annual colony loss reports. Retrieved December 4, 2020, from <https://capabees.com/capa-statement-on-honey-bees/>
- Canadian Institutes of Health Research. (2016). Knowledge translation - definition. Retrieved January 11, 2021, from <https://cihr-irsc.gc.ca/e/29418.html#1>
- Caron, D. M., & Connor, L. J. (2013). *Honey Bee Biology and Beekeeping* (3rd ed.). Kalamazoo, MI: Wicas Press.
- Carrat, F., & Valleron, A. J. (1992). Epidemiologic mapping using the “kriging” method: Application to an influenza-like epidemic in France. *American Journal of Epidemiology*, 135(11), 1293–1300. <https://doi.org/10.1093/oxfordjournals.aje.a116236>
- Chandler, D., Sunderland, K. D., Ball, B. V., & Davidson, G. (2001). Prospective biological control agents of *Varroa destructor* n. sp., an important pest of the European honeybee, *Apis mellifera*. *Biocontrol Science and Technology*, 11(4), 429–448.
<https://doi.org/10.1080/09583150120067472>
- Chantawannakul, P., Ramsey, S. D., VanEngelsdorp, D., Khongphinitbunjong, K., & Phokasem, P. (2018). *Tropilaelaps* mite: an emerging threat to European honey bee. *Current Opinion in Insect Science*, 26, 69–75. <https://doi.org/10.1016/j.cois.2018.01.012>
- Chauzat, M. P., Cauquil, L., Roy, L., Franco, S., Hendrikx, P., & Ribiére-Chabert, M. (2013). Demographics of the European apicultural industry. *PLoS ONE*, 8(11), e79018.
<https://doi.org/10.1371/journal.pone.0079018>
- Chen, Y. P., & Siede, R. (2007). Honey bee viruses. *Advances in Virus Research*, 70, 33–80.
[https://doi.org/10.1016/S0065-3527\(07\)70002-7](https://doi.org/10.1016/S0065-3527(07)70002-7)
- Claing, G., Kempers, M., Kennedy, K., Kozak, P., Lafrenière, R., Maund, C., ... Hoover, S. (2020). Statement on honey bee wintering losses in Canada (2020). *Canadian Association of Professional Apiculturalists*. Retrieved from <http://capabees.org/shared/2015/07/2015-CAPA-Statement-on-Colony-Losses-July-16-Final-16-30.pdf>
- Cleveland, R. B., Cleveland, W. S., McRae, J. E., & Terpenning, I. (1990). STL: a seasonal-trend decomposition procedure based on LOESS. *Journal of Official Statistics*, 6(1), 3–33.
- Coffey, M. F., & Breen, J. (2013). Efficacy of Apilife Var® and Thymovar® against *Varroa destructor* as an autumn treatment in a cool climate. *Journal of Apicultural Research*, 52(5), 210–218. <https://doi.org/10.3896/IBRA.1.52.5.07>
- Connor, L. J. (2015). *Varroa* control past and future. Retrieved January 7, 2021, from American Bee Journal website: <https://americanbeejournal.com/varroa-control-past-and-future/>
- Costa, M. A., & Kulldorff, M. (2009). Applications of spatial scan statistics: a review. In *Scan*

Statistics (pp. 129–152). https://doi.org/10.1007/978-0-8176-4749-0_6

Cullen, M. G., Thompson, L. J., Carolan, J. C., Stout, J. C., & Stanley, D. A. (2019). Fungicides, herbicides and bees: a systematic review of existing research and methods. *PLOS ONE*, 14(12), e0225743. <https://doi.org/10.1371/journal.pone.0225743>

Currie, R. W., Pernal, S. F., & Guzmán-Novoa, E. (2015). Honey bee colony losses in Canada. *Journal of Apicultural Research*, 49(1), 104–106.
<https://doi.org/https://doi.org/10.3896/IBRA.1.49.1.18>

Dainat, B., Evans, J. D., Chen, Y. P., Gauthier, L., & Neumann, P. (2012a). Dead or alive: deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. *Applied and Environmental Microbiology*, 78(4), 981–987.
<https://doi.org/10.1128/AEM.06537-11>

Dainat, B., Evans, J. D., Chen, Y. P., Gauthier, L., & Neumann, P. (2012b). Predictive markers of honey bee colony collapse. *PLoS ONE*, 7(2).
<https://doi.org/10.1371/journal.pone.0032151>

Davis, W., Dunsmuir, W., & Wang, Y. (1999). Modeling time series of count data. In S. Ghosh (Ed.), *Asymptotics, Nonparametrics, and Time Series* (pp. 63–115). New York: Marcel Dekker.

de Guzman, L. I., Williams, G. R., Khongphinitbunjong, K., & Chantawannakul, P. (2017). Ecology, life history, and management of *Tropilaelaps* mites. *Journal of Economic Entomology*, 110(2), 319–332. <https://doi.org/10.1093/jee/tow304>

DeGrandi-Hoffman, G., & Curry, R. (2004). A mathematical model of *Varroa* mite (*Varroa destructor* Anderson and Trueman) and honeybee (*Apis mellifera L.*) population dynamics. *International Journal of Acarology*, 30(3), 259–274.
<https://doi.org/10.1080/01647950408684393>

Delfinado-Baker, M., Rath, W., & Boecking, O. (1992). Phoretic bee mites and honeybee grooming behavior. *International Journal of Acarology*, 18(4), 315–322.
<https://doi.org/10.1080/01647959208683966>

Döke, M. A., Frazier, M., & Grozinger, C. M. (2015). Overwintering honey bees: biology and management. *Current Opinion in Insect Science*, 10(1), 185–193.

Ferland, J., Hoover, S., Kempers, M., Kennedy, K., Kozak, P., Lafrenière, R., ... Wilson, G. (2018). Statement on honey bee wintering losses in Canada (2018). *Canadian Association of Professional Apiculturists*, 1–18.

Ferland, J., Kempers, M., Kennedy, K., Lafrenière, R., Maund, C., Menzies, C., ... Van, P. (2019). Statement on honey bee wintering losses in Canada (2019). *Canadian Association of Professional Apiculturists*.

Forsgren, E., Locke, B., Sircoulomb, F., & Schäfer, M. O. (2018). Bacterial diseases in honeybees. *Current Clinical Microbiology Reports*, 5(1), 18–25.
<https://doi.org/10.1007/s40588-018-0083-0>

Fries, I., Camazine, S., & Sneyd, J. (1994). Population dynamics of *Varroa jacobsoni*: a model

- and a review. *Bee World*, 75(1), 5–28. <https://doi.org/10.1080/0005772X.1994.11099190>
- Giacobino, A., Cagnolo, N. B., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., ...
 Signorini, M. (2014). Risk factors associated with the presence of *Varroa destructor* in honey bee colonies from east-central Argentina. *Preventive Veterinary Medicine*, 115(3–4), 280–287. <https://doi.org/10.1016/j.prevetmed.2014.04.002>
- Giacobino, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., Bertozzi, E., ...
 Signorini, M. (2016). Key management practices to prevent high infestation levels of *Varroa destructor* in honey bee colonies at the beginning of the honey yield season. *Preventive Veterinary Medicine*, 131, 95–102.
<https://doi.org/10.1016/j.prevetmed.2016.07.013>
- Giacobino, A., Pacini, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., ...
 Signorini, M. (2017). Environment or beekeeping management: what explains better the prevalence of honey bee colonies with high levels of *Varroa destructor*? *Research in Veterinary Science*, 112, 1–6. <https://doi.org/10.1016/j.rvsc.2017.01.001>
- Gillis, D., Sobkowich, K. E., & Bernardo, T. M. (2020). Better data, more lives saved. *Canadian Science Policy Centre*. Retrieved from <https://sciencepolicy.ca/posts/better-data-more-lives-saved/>
- Giovenazzo, P., & Dubreuil, P. (2011). Evaluation of spring organic treatments against *Varroa destructor* (Acarı: Varroidae) in honey bee *Apis mellifera* (Hymenoptera: Apidae) colonies in eastern Canada. *Experimental and Applied Acarology*, 55(1), 65–76.
<https://doi.org/10.1007/s10493-011-9447-3>
- Gisder, S., Aumeier, P., & Genersch, E. (2009). Deformed wing virus: replication and viral load in mites (*Varroa destructor*). *Journal of General Virology*, 90(2), 463–467.
<https://doi.org/10.1099/vir.0.005579-0>
- Global Burden of Animal Disease. (2022). Burden of Animal Disease at a global, regional and national level: a new analytics platform providing information to guide animal health decision-makers. Retrieved January 21, 2022, from <https://animalhealthmetrics.org/informatics-portal/>
- Google Scholar. (2022). “*Varroa* Mite.” Retrieved January 14, 2022, from https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=varroa+mite&btnG=
- Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443–450. <https://doi.org/10.1051/apido/2009076>
- Hamdan, K. (2010). Robber bees. *Bee World*, 87(1), 7–9.
<https://doi.org/10.1080/0005772x.2010.11417332>
- Hansen, H., & Brødsgaard, C. J. (1999). American foulbrood: a review of its biology, diagnosis and control. *Bee World*, 80(1), 5–23. <https://doi.org/10.1080/0005772X.1999.11099415>
- Harbo, J. R. (1986). Oviposition rates of instrumentally inseminated and naturally mated queen

- honey bees (Hymenoptera: *Apidae*). *Annals of the Entomological Society of America*, 79(1), 112–115. <https://doi.org/10.1093/aesa/79.1.112>
- Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., ... Decourtey, A. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science*, 336(6079), 348–350. <https://doi.org/10.1126/science.1215039>
- Isere, E. E., Fatiregun, A. A., & Ajayi, I. O. (2015). An overview of disease surveillance and notification system in Nigeria and the roles of clinicians in disease outbreak prevention and control. *Nigerian Medical Journal*, 56(3), 161. <https://doi.org/10.4103/0300-1652.160347>
- Ivanković, D., Barbazza, E., Bos, V., Brito Fernandes, Ó., Jamieson Gilmore, K., Jansen, T., ... Kringsos, D. (2021). Features constituting actionable COVID-19 dashboards: descriptive assessment and expert appraisal of 158 public web-based COVID-19 dashboards. *Journal of Medical Internet Research*, 23(2), e25682. <https://doi.org/10.2196/25682>
- Jack, C. J., & Ellis, J. D. (2021). Integrated pest management control of *Varroa destructor* (Acari: *Varroidae*), the most damaging pest of (*Apis mellifera L.* (Hymenoptera: *Apidae*)) colonies. *Journal of Insect Science*, 21(5). <https://doi.org/10.1093/jisesa/ieab058>
- Jacques, A., Laurent, M., Ribière-Chabert, M., Saussac, M., Bougeard, S., Budge, G. E., ... Chauzat, M. P. (2017). A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. *PLoS ONE*, 12(3), 1–17. <https://doi.org/10.1371/journal.pone.0172591>
- Jay, S. C. (1971). Beekeeping techniques: how to prevent drifting. *Bee World*, 52(2), 53–55. <https://doi.org/10.1080/0005772X.1971.11097352>
- Khayli, M., Lhor, Y., Bengoumi, M., Zro, K., El Harrak, M., Bakkouri, A., ... Bouslikhane, M. (2021). Using geostatistics to better understand the epidemiology of animal rabies in Morocco: what is the contribution of the predictive value? *Heliyon*, 7(1), e06019. <https://doi.org/10.1016/j.heliyon.2021.e06019>
- Khongphinitbunjong, K., Neumann, P., Chantawannakul, P., & Williams, G. R. (2016). The ectoparasitic mite *Tropilaelaps mercedesae* reduces western honey bee, *Apis mellifera*, longevity and emergence weight, and promotes Deformed wing virus infections. *Journal of Invertebrate Pathology*, 137, 38–42. <https://doi.org/10.1016/j.jip.2016.04.006>
- Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., & Tscharntke, T. (2014). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences*, 281(1775), 20132440. <https://doi.org/10.1098/rspb.2013.2440>
- Kluser, S., & Peduzzi, P. (2007). *Global pollinator decline: a literature review* (p. 4). p. 4. United Nations Environment Programme.
- Kosmala, M., Wiggins, A., Swanson, A., & Simmons, B. (2016). Assessing data quality in citizen science. *Frontiers in Ecology and the Environment*, 14(10), 551–560. <https://doi.org/10.1002/fee.1436>
- Kozak, P. (2017). *2017 Provincial Apiarist Report*. Retrieved from

<http://www.omafra.gov.on.ca/english/food/inspection/bees/17rep.htm#3>

Kozak, P. (2021). *Notes on Varroa mites* [Unpublished lecture notes]. Ontario Ministry of Agriculture, Food and Rural Affairs.

Kozak, P., Eccles, L., Kempers, M., Rawn, D., Lacey, B., & Guzmán-Novoa, E. (2021). Ontario treatment recommendations for honey bee disease and mite control. Retrieved June 24, 2021, from Ontario Ministry of Agriculture, Food and Rural Affairs website: <http://www.omafra.gov.on.ca/english/food/inspection/bees/2017-treatment.htm#VM>

Kralj, J., Brockmann, A., Fuchs, S., & Tautz, J. (2007). The parasitic mite *Varroa destructor* affects non-associative learning in honey bee foragers, *Apis mellifera L. Journal of Comparative Physiology A*, 193(3), 363–370. <https://doi.org/10.1007/s00359-006-0192-8>

Kralj, J., & Fuchs, S. (2006). Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. *Apidologie*, 37(5), 577–587. <https://doi.org/10.1051/apido:2006040>

Kulldorff, M. (1997). A spatial scan statistic. *Communications in Statistics: Theory and Methods*, 26, 1481–1496.

Kulldorff, M., & Nagarwalla, N. (1995). Spatial disease clusters: detection and inference. *Statistics in Medicine*, 14(8), 799–810. <https://doi.org/10.1002/sim.4780140809>

Lambert, O., Piroux, M., Puyo, S., Thorin, C., Larhantec, M., Delbac, F., & Pouliquen, H. (2012). Bees, honey and pollen as sentinels for lead environmental contamination. *Environmental Pollution*, 170, 254–259. <https://doi.org/10.1016/j.envpol.2012.07.012>

Langstroth, L. L. (1847). Patent US9300A: Bee Hive. Retrieved from <https://patents.google.com/patent/US9300A/en>

Langstroth, L. L. (1853). *Langstroth on the Hive and the Honey-Bee: A Bee Keeper's Manual* (1st ed.). Northampton: Hopkins, Bridgman & Company.

Le Conte, Y., Arnold, G., Trouiller, J., Masson, C., Chappe, B., & Ourisson, G. (1989). Attraction of the parasitic mite *Varroa* to the drone larvae of honey bees by simple aliphatic esters. *Science*, 245(4918), 638–639. <https://doi.org/10.1126/science.245.4918.638>

Le Conte, Y., Ellis, M., & Ritter, W. (2010). *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie*, 41(3), 353–363. <https://doi.org/10.1051/apido/2010017>

Le Conte, Y., & Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. *Revue Scientifique et Technique (International Office of Epizootics)*, 27(2), 485–497, 499–510. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/18819674>

Lee, K. V., Moon, R. D., Burkness, E. C., Hutchison, W. D., & Spivak, M. (2010). Practical sampling plans for *Varroa destructor* (Acari: *Varroidae*) in *Apis mellifera* (Hymenoptera: *Apidae*) colonies and apiaries. *Journal of Economic Entomology*, 103(4), 1039–1050. <https://doi.org/10.1603/ec10037>

Liang, K. Y., & Zeger, S. L. (1986). Longitudinal data analysis using generalized linear models.

- Biometrika*, 73(1), 13–22.
- Lu, C., Warchol, K. M., & Callahan, R. A. (2014). Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder. *Bulletin of Insectology*, 67(1), 125–130.
- Martin, S. J. (1995). Reproduction of *Varroa jacobsoni* in cells of *Apis mellifera* containing one or more mother mites and the distribution of these cells. *Journal of Apicultural Research*, 34(4), 187–196. <https://doi.org/10.1080/00218839.1995.11100904>
- Martinez, H. (2007). Spatial epidemiology of bovine tuberculosis in Mexico. *Veterinaria Italiana*, 43(3), 629–634.
- Matheron, G. (1960). Krigeage d'un panneau rectangulaire par sa périphérie. *Note Géostatistique*, 28.
- Matheron, G. (1963). Principles of geostatistics. *Economic Geology*, 58(8), 1246–1266. <https://doi.org/10.2113/gsecongeo.58.8.1246>
- Matheron, G. (1969). *Le krigeage universel. Vol. 1*. Paris, France: École nationale supérieure des mines de Paris.
- Melhim, A., Weersink, A., Daly, Z., & Bennett, N. (2010). *Beekeeping in Canada: honey and pollination outlook* (6th ed.; H. Clay, G. Marks, T. Woodcock, & K. Richards, Eds.). Canadian Pollination Initiative.
- Messan, K., Rodriguez Messan, M., Chen, J., DeGrandi-Hoffman, G., & Kang, Y. (2021). Population dynamics of *Varroa* mite and honeybee: effects of parasitism with age structure and seasonality. *Ecological Modelling*, 440, 109359. <https://doi.org/10.1016/j.ecolmodel.2020.109359>
- Molineri, A., Giacobino, A., Pacini, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., ... Signorini, M. (2018). Environment and *Varroa destructor* management as determinant of colony losses in apiaries under temperate and subtropical climate. *Journal of Apicultural Research*, 57(4), 551–564. <https://doi.org/10.1080/00218839.2018.1475697>
- Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., ... Le Conte, Y. (2020). Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. *International Journal for Parasitology*, 50(6–7), 433–447.
- Mondet, F., Goodwin, M., & Mercer, A. (2011). Age-related changes in the behavioural response of honeybees to Apiguard®, a thymol-based treatment used to control the mite *Varroa destructor*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 197(11), 1055–1062. <https://doi.org/10.1007/s00359-011-0666-1>
- Morfin, N., Goodwin, P. H., & Guzmán-Novoa, E. (2020). The combined effects of *Varroa destructor* parasitism and exposure to neonicotinoids affects Honey Bee (*Apis mellifera L.*) memory and gene expression. *Biology*, 9(9), 237. <https://doi.org/10.3390/biology9090237>
- Mullin, C. A., Frazier, M., Frazier, J., Ashcraft, S., Simonds, R., VanEngelsdorp, D., & Pettis, J. S. (2010). High levels of miticides and agrochemicals in North American apiaries:

- implications for honey bee health. *PLoS ONE*, 5(3), e9754. <https://doi.org/10.1371/journal.pone.0009754>
- Mutinelli, F. (2011). The spread of pathogens through trade in honey bees and their products (including queen bees and semen): Overview and recent developments. *OIE Revue Scientifique et Technique*, 30(1), 257–271. <https://doi.org/10.20506/rst.30.1.2033>
- Niiler, E. (2020, January). An AI epidemiologist sent the first warnings of the Wuhan virus. *Wired*. Retrieved from <https://www.wired.com/story/ai-epidemiologist-wuhan-public-health-warnings/>
- Nwosu, A., Berke, O., Pearl, D. L., & Trotz-Williams, L. A. (2019). Exploring the geographical distribution of cryptosporidiosis in the cattle population of Southern Ontario, Canada, 2011–2014. *Geospatial Health*, 14(2). <https://doi.org/10.4081/gh.2019.769>
- OIE: World Organisation for Animal Health. (2010). *Guide to good farming practices for animal production food safety*. Retrieved from <http://www.fao.org/3/a-i0482t.pdf>
- OIE: World Organisation for Animal Health. (2021). Manual of diagnostic tests and vaccines for terrestrial animals 2021. Retrieved December 7, 2021, from <https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>
- Oliver, R. (2018). Selective Breeding for Mite Resistance: 1000 hives, 100 hours. *American Bee Journal*, (158), 297– 301.
- OMAFRA. (2021a). Best management practices (BMP) for Ontario beekeepers' in advance of winter. Retrieved January 5, 2021, from <http://www.omafra.gov.on.ca/english/food/inspection/bees/bmpwinter.htm>
- OMAFRA. (2021b). Overview of beekeeping regulations in Ontario: what you should know if you own honey bees. Retrieved December 23, 2021, from <http://www.omafra.gov.on.ca/english/food/inspection/bees/beekeepingregulations.htm>
- OMAFRA. (2021c). *Varroa mite - sampling and monitoring infestation levels*. Retrieved December 7, 2021, from Ontario Ministry of Agriculture, Food and Rural Affairs website: <http://www.omafra.gov.on.ca/english/food/inspection/bees/varroa-sampling.htm>
- Ontario Beekeepers' Association. (2014). *Drone brood removal for the management of Varroa mites*. Retrieved from <https://www.ontariobee.com/sites/ontariobee.com/files/document/Drone-Brood-Removal-for-the-Management-of-Varroa-Mites.pdf>
- Openshaw, S. (1984). *The Modifiable Areal Unit Problem*. Norwich, England: Geo Books.
- Page, R. E., & Peng, C. Y. S. (2001). Aging and development in social insects with emphasis on the honey bee, *Apis mellifera L.* *Experimental Gerontology*, 36(4–6), 695–711. [https://doi.org/10.1016/S0531-5565\(00\)00236-9](https://doi.org/10.1016/S0531-5565(00)00236-9)
- Peck, D. T. (2021). The parasitic mite *Varroa destructor*. In *Honey Bee Medicine for the Veterinary Practitioner* (pp. 235–251). <https://doi.org/10.1002/9781119583417.ch20>
- Peck, D. T., & Seeley, T. D. (2019). Mite bombs or robber lures? The roles of drifting and

- robbing in *Varroa destructor* transmission from collapsing honey bee colonies to their neighbors. *PLoS ONE*, 14(6), 1–14. <https://doi.org/10.1371/journal.pone.0218392>
- Peck, D. T., Smith, M. L., & Seeley, T. D. (2016). *Varroa destructor* mites can nimbly climb from flowers onto foraging honey bees. *PLoS ONE*, 11(12), e0167798. <https://doi.org/https://doi.org/10.1371/journal.pone.0167798>
- Pernal, S. F., & Clay, H. (2013). *Honey bee diseases and pests* (3rd ed.). Beverlodge, AB, Canada: Canadian Association of Professional Apiculturists.
- Petukhova, T., Ojkic, D., McEwen, B., Deardon, R., & Poljak, Z. (2018). Assessment of autoregressive integrated moving average (ARIMA), generalized linear autoregressive moving average (GLARMA), and random forest (RF) time series regression models for predicting influenza A virus frequency in swine in Ontario, Canada. *PLOS ONE*, 13(6), e0198313. <https://doi.org/10.1371/journal.pone.0198313>
- Philippe, J. M. (2007). *Le guide de l'apiculteur* (Edisud, Ed.). Aix-en-Provence, France.
- Posada-Florez, F., Ryabov, E., Heerman, M. C., Chen, Y. P., Evans, J. D., Sonenshine, D. E., & Cook, S. C. (2020). *Varroa destructor* mites vector and transmit pathogenic honey bee viruses acquired from an artificial diet. *PLOS ONE*, 15(11), e0242688. <https://doi.org/10.1371/journal.pone.0242688>
- Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., ... VanEngelsdorp, D. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences*, 116(5), 1792–1801. <https://doi.org/10.1073/pnas.1818371116>
- Rangel, J., & Fisher, A. (2019). Factors affecting the reproductive health of honey bee (*Apis mellifera*) drones - a review. *Apidologie*, 50(6), 759–778. <https://doi.org/10.1007/s13592-019-00684-x>
- Remolina, S. C., Hafez, D. M., Robinson, G. E., & Hughes, K. A. (2007). Senescence in the worker honey bee *Apis Mellifera*. *Journal of Insect Physiology*, 53(10), 1027–1033. <https://doi.org/10.1016/j.jinsphys.2007.05.015>
- Ricke, D. F., Lin, C. H., & Johnson, R. M. (2021). Pollen treated with a combination of agrochemicals commonly applied during almond bloom reduces the emergence rate and longevity of honey bee (Hymenoptera: Apidae) queens. *Journal of Insect Science*, 21(6). <https://doi.org/10.1093/jisesa/ieab074>
- Riessberger, U., & Crailsheim, K. (1997). Short-term effect of different weather conditions upon the behaviour of forager and nurse honey bees (*Apis mellifera carnica* Pollmann). *Apidologie*, 28(6), 411–426. <https://doi.org/10.1051/apido:19970608>
- Rinderer, T. E., Harris, J. W., Hunt, G. J., & de Guzman, L. I. (2010). Breeding for resistance to *Varroa destructor* in North America. *Apidologie*, 41, 409–424.
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103(SUPPL. 1), S96–S119. <https://doi.org/10.1016/j.jip.2009.07.016>

- Roth, M. (2020). *Varroa* mites: new guide outlines integrated pest management options. Retrieved January 10, 2022, from Entomology Today website: <https://entomologytoday.org/2020/02/07/varroa-mites-new-guide-outlines-integrated-pest-management-options/>
- Rowland, B. W., Rushton, S. P., Shirley, M. D. F., Brown, M. A., & Budge, G. E. (2021). Identifying the climatic drivers of honey bee disease in England and Wales. *Scientific Reports*, 11(1), 21953. <https://doi.org/10.1038/s41598-021-01495-w>
- Rubinigg, M., MacDonald, M., Davenport, V., Hassler, E., Hassan, A., Shala-Mayrhofer, V., & Cazier, J. (2021). Predicting Varroa: longitudinal data, micro climate, and proximity closeness useful for predicting varroa infestations. Retrieved from <https://data-for-good.pubpub.org/pub/aawkkv33>
- Rueppell, O., Bachelier, C., Fondrk, M. K., & Page, R. E. (2007). Regulation of life history determines lifespan of worker honey bees (*Apis mellifera L.*). *Experimental Gerontology*, 42(10), 1020–1032. <https://doi.org/10.1016/j.exger.2007.06.002>
- Shuel, R. W. (1992). The production of nectar and pollen. In *The Hive and the Honey Bee* (pp. 401–433). Hamilton, IL: Bookcrafters.
- Simpson, J. (1958). The factors which cause colonies of *Apis mellifera* to swarm. *Insectes Sociaux*, 5(1), 77–95. <https://doi.org/10.1007/BF02222430>
- Siviter, H., Bailes, E. J., Martin, C. D., Oliver, T. R., Koricheva, J., Leadbeater, E., & Brown, M. J. F. (2021). Agrochemicals interact synergistically to increase bee mortality. *Nature*, 596(7872), 389–392. <https://doi.org/10.1038/s41586-021-03787-7>
- Smith, S., Sewalk, K. C., Donaire, F., Goodwin, L., Zych, A., Crawley, A. W., ... Baltrusaitis, K. (2021). Maintaining user engagement in an infectious disease surveillance-related citizen science project. *Citizen Science: Theory and Practice*, 6(1). <https://doi.org/10.5334/cstp.302>
- Snow, J. (1855). *On the mode of communication of cholera* (2nd ed.). London: John Churchill.
- Soucie, J. M. (2012). Public health surveillance and data collection: general principles and impact on hemophilia care. *Hematology*, 17(sup1), s144–s146. <https://doi.org/10.1179/102453312X13336169156537>
- Stabentheiner, A., Pressl, H., Papst, T., Hrassnigg, N., & Crailsheim, K. (2003). Endothermic heat production in honeybee winter clusters. *Journal of Experimental Biology*, 206(2), 353–358. <https://doi.org/10.1242/jeb.00082>
- Staveley, J. P., Law, S. A., Fairbrother, A., & Menzie, C. A. (2014). A causal analysis of observed declines in managed honey bees (*Apis Mellifera*). *Human and Ecological Risk Assessment: An International Journal*, 20(2), 566–591. <https://doi.org/10.1080/10807039.2013.831263>
- Stephan, J. G., de Miranda, J. R., & Forsgren, E. (2020). American foulbrood in a honeybee colony: spore-symptom relationship and feedbacks between disease and colony development. *BMC Ecology*, 20(1), 15. <https://doi.org/10.1186/s12898-020-00283-w>
- Stevenson, M. A., Benard, H., Bolger, P., & Morris, R. S. (2005). Spatial epidemiology of the

- Asian honey bee mite (*Varroa destructor*) in the North Island of New Zealand. *Preventive Veterinary Medicine*, 71(3–4), 241–252. <https://doi.org/10.1016/j.prevetmed.2005.07.007>
- Tango, T., & Takahashi, K. (2005). A flexibly shaped spatial scan statistic for detecting clusters. *International Journal of Health Geographics*, 4(1), 11. <https://doi.org/10.1186/1476-072X-4-11>
- Tantillo, G., Bottaro, M., Di Pinto, A., Martella, V., Di Pinto, P., & Terio, V. (2015). Virus infections of honeybees *Apis Mellifera*. *Italian Journal of Food Safety*, 4(3). <https://doi.org/10.4081/ijfs.2015.5364>
- Thomas-Bachli, A. L., Pearl, D. L., Parmley, E. J., & Berke, O. (2020). The influence of sociodemographic factors on the engagement of citizens in the detection of dead corvids during the emergence of West Nile virus in Ontario, Canada. *Frontiers in Veterinary Science*, 6. <https://doi.org/10.3389/fvets.2019.00483>
- Tobler, W. R. (1970). A computer movie simulating urban growth in the Detroit region. *Economic Geography*, 46, 234–240.
- Traynor, K. S., Mondet, F., de Miranda, J. R., Techer, M., Kowallik, V., Oddie, M. A. Y., ... McAfee, A. (2020). *Varroa destructor*: a complex parasite, crippling honey bees worldwide. *Trends in Parasitology*, 36(7), 592–606. <https://doi.org/10.1016/j.pt.2020.04.004>
- Tukey, J. (1977). *Exploratory data analysis*. Reading, MA, USA: Addison-Wesley.
- van Alten, A., Tam, J., & Bryans, R. (2013). *Integrated Pest Management for Beekeeping in Ontario Manual* (1st ed.; L. Eccles, M. Kempers, D. Rawn, & B. Lacey, Eds.). Ontario Beekeeper's Association - Technology Transfer Program.
- Van Der Zee, R., Gray, A., Pisa, L., & De Rijk, T. (2015). An observational study of honey bee colony winter losses and their association with *Varroa destructor*, neonicotinoids and other risk factors. *PLoS ONE*, 10(7), e0131611. <https://doi.org/https://doi.org/10.1371/journal.pone.0131611>
- VanEngelsdorp, D., Cox-Foster, D. L., Frazier, M., Ostiguy, N., & Hayes, J. (2006). *Colony collapse disorder preliminary report*.
- VanEngelsdorp, D., & Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *Journal of Invertebrate Pathology*, 103, S80–S95. <https://doi.org/10.1016/j.jip.2009.06.011>
- VanEngelsdorp, D., & Pettis, J. S. (2014). Colony collapse disorder. *Bee Health and Veterinarians*, 157–159.
- VanEngelsdorp, D., Speybroeck, N., Evans, J. D., Nguyen, B. K., Mullin, C. A., Frazier, M., ... Saegerman, C. (2010). Weighing risk factors associated with bee colony collapse disorder by classification and regression tree analysis. *Journal of Economic Entomology*, 103(5), 1517–1523. <https://doi.org/10.1603/ec09429>
- Vidal-Naquet, N. (2018). *Honeybee Veterinary Medicine*: Apis mellifera L. Sheffield, UK: 5m Publishing.

- von Frisch, K. (1967). *The Dance Language and Orientation of Bees*. Cambridge, MA.: Harvard University Press.
- Waller, L. A., & Gotway, C. A. (2004). *Applied Spatial Statistics for Public Health Data*. Hoboken, NJ, USA: John Wiley & Sons Inc.
- Ward, M. P., Iglesias, R. M., & Brookes, V. J. (2020). Autoregressive models applied to time-series data in veterinary science. *Frontiers in Veterinary Science*, 7. <https://doi.org/10.3389/fvets.2020.00604>
- Watson, K., & Stallins, J. A. (2016). Honey bees and colony collapse disorder: a pluralistic reframing. *Geography Compass*, 10(5), 222–236. <https://doi.org/10.1111/gec3.12266>
- Webb, P., Bain, C., & Page, A. (2017). *Essential Epidemiology* (3rd ed.). Cambridge, U.K.: Cambridge University Press.
- Welvaert, M., & Caley, P. (2016). Citizen surveillance for environmental monitoring: combining the efforts of citizen science and crowdsourcing in a quantitative data framework. *SpringerPlus*, 5(1), 1890. <https://doi.org/10.1186/s40064-016-3583-5>
- Whittle, P. (1951). *Hypothesis Testing in Time Series Analysis*. Uppsala University.
- Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, MA.: Harvard Univeristy Press.
- Wirtz, P., & Beetsma, J. (1972). Induction of caste differentiation in the honeybee. *Entomologia Experimentalis et Applicata*, 15(4), 517–520.
- World Health Organization. (1968). *Report of the technical discussions at the 21st World Health Assembly on National and Global Surveillance of Communicable Disease*. Geneva, Switzerland.
- Woyke, J., Jasiński, Z., Prabucki, J., Wilde, J., Chuda-Mickiewicz, B., Siuda, M., ... Jojczyk, A. (2008). Onset of oviposition by honey bee queens, mated either naturally or by various instrumental insemination methods, fits a lognormal distribution. *Journal of Apicultural Research*, 47(1), 1–9. <https://doi.org/10.1080/00218839.2008.11101416>

2 CHAPTER TWO: MAPPING THE POPULATION DENSITY OF MANAGED HONEY BEE (*APIS MELLIFERA*) COLONIES IN ONTARIO, CANADA: 2018

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2.1 Abstract

Host population density as a risk factor for infectious disease transmission is an established concept in both host-parasite ecology and epidemiological disease modeling. A ‘population-at-risk’ value is a necessary denominator in epidemiological analyses to estimate absolute risk. However, local colony density values have been missing from published literature for Ontario, Canada, and crude density measures for the province do not consider the highly heterogeneous concentration of colonies in Southern Ontario. With geostatistical kriging methods, a continuous colony density map was developed from regionally aggregated apiary registration data. This study highlights the potential implications of colony population density on a macro scale and illustrates methodologies available to produce continuous density estimates over a given region with Ontario as an example. The estimation and mapping of continuous colony density values across the population provide future work with a source of data to further investigate potential associations of colony density and disease and help to inform inspection and surveillance efforts. An interactive regional colony density map was also developed as a knowledge mobilization tool to increase the accessibility of these findings to members of the beekeeping community. The results of this study are an important practical step in advancing epidemiological research on managed honey bees and may lead to further development of strategies to improve the health of honey bees.

2.2 Introduction

The number of managed honey bee, *Apis mellifera*, colonies in Canada has more than doubled in the past century (Agriculture and Agri-Food Canada, 2019; Statistics Canada, 2018). As greater numbers of colonies populate the country, it is important to understand the implications of colony density, as well as map and monitor the distribution of said colonies to provide a bearing for researchers, government officials and beekeepers.

In the province of Ontario alone, approximately 100,000 colonies are managed by, on average, about 2,900 beekeepers (Agriculture and Agri-Food Canada, 2019; OMAFRA, 2019). A mix of commercial (≥ 50 colonies) and hobbyist (< 50 colonies) beekeeping operations comprise this population. The province occupies a landmass of over one million square kilometers, but the majority of this land is inhospitable to humans, resulting in approximately 94% of the human population occupying only around 14% of the provincial landmass (Statistics Canada, 2021). Inherently, managed honey bees exist in correlation with human populations and therefore the 100,000 colonies in Ontario would also be expected to occupy a small fraction of the provincial landmass. However, population density distribution metrics of honey bee colonies are not as well documented as that of humans.

Beekeeping is a landless branch of animal husbandry and as such, there is limited control over the interactions of these managed bees and the surrounding environment. Therefore, the beekeeping community is heavily intertwined and reliant on one another to prevent disease spread and minimize the pool of bacteria and viruses in a given region. Therefore, knowledge about the geographic colony density distribution is of critical importance.

Host population density is a key component in basic host-parasite ecology (Kilpatrick & Altizer, 2010). In a density-dependent disease transmission model, the probability of a susceptible individual becoming infected is dependent on the number of other hosts in the same area that are already infected. This population density dependence is true for honey bees and the transmission of numerous viral and bacterial pathogens (Brosi, Delaplane, Boots, & De Roode, 2017).

Infectious disease modeling has demonstrated that the speed at which a disease spreads is a function of the contact rate (i.e., the rate of contacts sufficient to transmit a pathogen between infected and susceptible individuals) as well as agent (i.e., pathogen resilience and virulence), and environmental factors (i.e., climate and population density). Favourable environmental conditions, including the density of individuals around the susceptible host, and the homogeneity of individual mixing can accelerate the horizontal spread of disease (Lindahl & Grace, 2015). Increasing the density of hosts has the potential to increase the ratio of susceptible to infected individuals, thus increasing the contact rate and the likelihood of a successful transfer of the disease (Tarwater & Martin, 2001).

Humans have artificially increased the density of *A. mellifera* colonies as a means for industrializing agricultural farming and honey production. This is believed to have accelerated the disease transmission rates between colonies and thus increased the overall prevalence of various diseases and pests (Seeley & Smith, 2015). This increase in colony density, both within and between yards can be assumed to impact neighboring colonies and yards, with respect to their level of risk for disease and pests and influences their need for management strategies to mitigate their impacts.

Horizontal pathogen transmission between colonies of honey bees may occur by several mechanisms, namely, (i) robbing of honey stores, (ii) drifting, (iii) direct contact during foraging, (iv) contact with infected materials or food sources in the environment (Fries & Camazine, 2001), and (v) venereal diseases spread through mating (Chen & Siede, 2007). All of which may conceivably be influenced by the density of colonies in a region due to (i) greater competition during foraging, leading to intensified forage and robbing behaviour if nectar becomes limited, and (ii) higher inter-colony contact rates by other such means as drifting or fomites.

Although increased colony density may have significant implications on the behaviours of honey bees and the transmission of pests and diseases between colonies, an absence of information on local colony density values across the province of Ontario, Canada, prevents these associations from being investigated. Crude measures of density offer minimal practical value as they do not take into account heterogeneity or patterns of colony distribution. Geostatistical kriging methods offer a statistically sound means to convert regional counts of colonies (derived from registration data) into spatially continuous estimates of density for a larger study area (Krige, 1981; Matheron, 1963).

Detailed estimates of colony density provide a basis for epidemiological studies and practical disease intervention efforts, while routine colony inspection could also benefit from colony density estimates to implement a sampling strategy informed by the population distribution (i.e., assigning inspections to higher density locations). Ecological research may also in time reproduce such estimates to monitor the population growth and changes in distribution over multiple years. Moreover, beekeeper and community education efforts can benefit from graphic visualization of data patterns otherwise hidden in tabular presentations. Such graphics and maps regarding colony density can be part of the knowledge mobilization tools, which are

commonly used to bridge the gap between research and practical uses of results through the dissemination of research findings in an accessible form for broad audiences (Government of Canada, 2012).

While tabulated data is sufficient for basic data analysis, more advanced statistical mapping and modeling applications require data interpolated to grid locations across a study area. Estimating the spatially continuous colony density for the province allows for future research projects to extract data as raster or grid data at the necessary resolution. Because maps of the population density are of interest to both researchers and industry professionals, an objective of the present study is to develop an interactive map depicting regional colony density that serves as an online knowledge mobilization tool between beekeepers and government agencies.

The general goal of this study was to provide an accurate depiction of honey bee colony density in Ontario and identify spatial trends or patterns in the distribution of colonies. The specific objectives of this study were (1) to estimate the local density of managed *A. mellifera* colonies across Ontario through geostatistical interpolation of regional registry data, (2) to map the estimated density values as a density heatmap for applications requiring spatially continuous or high detail information (i.e. GIS analysis requiring raster data), (3) and to design an online knowledge mobilization tool of crude regional density values for applications requiring simple regional density metrics (i.e. public outreach or education requiring intuitive and interactive tools).

2.3 Materials and Methods

Records of registered bee colonies in Ontario were provided by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). The received records consisted of all registered bee yards in 2018, which was the most current data at the time of analysis. This data consists of the location to which a yard is registered by the apiarist and therefore assumes colony stationarity.

To maintain the privacy of beekeepers, all bee yard locations and their colony counts were aggregated to the Census Consolidated Subdivision (CCS) level before being released by OMAFRA. The CCS data divides the province of Ontario into 273 regions of varying sizes. The data consists of the total number of colonies and the total number of bee yards per CCS. A boundary file of the CCS subdivisions was obtained from Statistics Canada (Statistics Canada, 2019).

Within R (R Core Team, 2020), the boundary file was projected using the Lambert Equal Area (LEA) projection, because of the ability to retain the integrity of a square kilometer unit over a large geographical body, which is important for population density mapping. Bee colony densities per square kilometer were calculated and joined to the geographic centroid of each CCS in Ontario. The colony density values were heavily skewed and therefore a log-normal kriging approach was used.

Kriging is a geostatistical process that allows the production of a spatially continuous surface from distinct sampling points. In this case, a spatially continuous estimate of the population density can be estimated from a collection of regionally aggregated colony registration counts.

The process of geostatistical kriging requires that a variogram model be fit to the data to determine the overall spatial dependence and the degree to which that dependence diminishes over a given distance. A spherical variogram model was fit to the data through maximum likelihood estimation (Ribeiro & Diggle, 2018). The range of the variogram was restricted to a maximum of 500 km, assuming local but not global stationarity. The model fit was assessed via the Akaike information criterion, as well as a visual inspection of a normal QQ-plot of the model residuals.

Ordinary log-normal kriging interpolation was performed, assuming a constant unknown mean across the study area. The process of log-normal kriging includes an automated procedure to transform the data and back-transform the prediction values during kriging. The *R* package, *geoR* was used for all spatial data management and analysis (Ribeiro & Diggle, 2018).

Isopleth mapping based on the back-transformed results from geostatistical kriging was used to illustrate the provincial population density of colonies with the use of the CCS data (Berke, 2004; Carrat & Valleron, 1992). All spatial regression model parameters including the variogram were estimated through maximum likelihood estimation.

Ordinary log-normal kriging interpolation was performed at the provincial level first and then separately applied to the Southern Ontario region following the removal of data from Northern Ontario. This was deemed appropriate due to Southern Ontario's distinctively smaller regions, which are difficult to recognize on a provincial map. Furthermore, Northern and Southern Ontario differ dramatically in terms of size and crude colony density, so it cannot be assumed that a single spatial dependence structure accurately represents the entire province. The two population density maps for Ontario and Southern Ontario differ in their colour scales and are to be compared with this in mind.

Recognizing that there is a shared need for colony density information by researchers and industry professionals, a practical knowledge mobilization dashboard was developed. Using the *leaflet* package in *R* (Cheng, Karambelkar, & Xie, 2018), an interactive choropleth map at the CCS level was produced. The map allows for the exploration of the colony densities across the CCSs with the ability to zoom to finer resolutions. Upon selection of a CCS region, a pop-up window with further colony density information is displayed. This internet dashboard can be updated upon the availability of new data.

2.4 Results

The Ontario provincial dataset represents colony counts from 273 census consolidated subdivisions. The total number of colonies in Ontario in 2018 was n=87,777. A map representing the 273 CCSs and the associated crude density values is provided in Figure 2.1.

The 273 CCSs ranged in areal size from about 52km² to 446,000km² [mean: 3,613km² ± 2,930km²]. The crude colony density values for each CCS varied from 0 to 14.7 colonies/km², with an average crude colony density of 0.9 colonies/km² [0.88 ± 1.75]. The colony density distribution is visualized with a choropleth map in Figure 2.1.

The estimation of the semivariogram, using the spherical model is presented visually in Figure 2.2, juxtaposed with the empirical semivariogram of the colony density data. The spherical variogram model was determined to best represent the spatial dependence structure of the data based on visual analysis of the variogram and the shape of the spherical function, as well as the Akaike information criterium (AIC) when compared to other models (i.e., exponential, gaussian, or linear). The range of spatial dependence based on the spherical model fit to the data is estimated at 360 km (Table 2.1). The estimated variogram shows a reasonably good fit

between the model and the empirical variogram (Figure 2.2a), and the standardized model residuals moderately maintain the assumption of normality with deviations at the tails (Figure 2.2b).

An isopleth map of colony density for Ontario, derived from geostatistical kriging of the crude regional density values, is shown in Figure 2.3. The average colony density across the province was estimated at a level of 0.373 colonies/km². Location-specific colony density estimates ranged from 0 to 8.93 colonies/km². An uneven distribution of colonies appears evident based on the visual examination of the estimated density map. The colony density map provides evidence of a heterogeneous distribution of colonies in Ontario, with a majority of managed honey bee colonies residing in the south of the province (<-500N, see Figure 2.3), and more specifically in Niagara and surrounding regions (500E, -900N, see Figure 2.3).

Similar to the provincial data analysis, the colony densities for Southern Ontario CCS regions were positively skewed and thus log-transformed for variogram model fitting. This transformation was found to improve the fit of the empirical variogram estimation. Like the previous variogram estimation, a spherical model was found to be appropriate for kriging purposes (Figure 2.4a). The model residuals appear to better maintain the assumption of normality, compared to the complete province model, with an approximate mean nearing 0, based on visual inspection of the quantile-quantile plot (Figure 2.4b). A practical spatial dependence range of 191 km was estimated for Southern Ontario by the variogram model (Table 2.1). Kriging-estimated colony density estimates for Southern Ontario show a large proportion of colonies residing in the Niagara Peninsula (500E, -900N) with several less concentrated pockets in the Bruce/Grey (360E, -750N), Simcoe (450E, -760N), Wellington (425E, -850N) and Northumberland regions (600E, -760N), relative to other regions of Southern Ontario (Figure

2.5). The average colony density for Southern Ontario was estimated at 0.863 colonies/km², with grid estimates ranging from -0.023 (0) to 10.4 colonies/km². This contrasts with the average colony density for Northern Ontario independently at 0.079 colonies/km².

Choropleth and isopleth mapping of the colony densities at a regional level suggests a disproportionate distribution of colonies with higher densities in the Niagara region and lower densities in the northern regions.

A screenshot of the interactive choropleth map is presented in Figure 2.6.

2.5 Discussion

The results of this study illustrate the spatial distribution and density of managed honey bee colonies in Ontario. The mapped colony density estimates indicate an overall heterogeneous distribution across the province. When compared to the province as a whole, Southern Ontario displays a high density of colonies with an average density of around 8.6 colonies/10km², compared to Northern Ontario with a density of 0.8 colonies/10km², and the collective provincial average of approximately 3.7 colonies/10km². Because this study was focused on the managed honey bee population, this distribution of colonies is not surprising, as the Southern Ontario region comprises nearly 94% of the province's human population (Statistics Canada, 2017). However, this result may be surprising in the sense that Southern Ontario only accounts for 32% of the province's agricultural farm acreage (OMAFRA, 2020) where a sizeable proportion of commercial honey bees would be expected.

The large variation in colony density across the province gave rise to a substantial positive skewing of the data. This positive skewing is common in spatially dependent observations and has been shown to cause a proportional effect in the variogram estimates,

where variation is dependent on the magnitude of the value (Manchuk, Leuangthong, & Deutsch, 2009). In this study, despite the skewing of data and the differing sill values, a proportional effect was not detected between the Ontario and Southern Ontario variogram models as the range was also found to differ. However, the skewness of the data is thought to be the primary reason for the lack of fit of the provincial variogram model.

Neither the variogram model estimation nor the residual QQ-plot for the whole province (Figure 2.2) showed an exceptional fit to the data, and therefore the decision was made for Southern Ontario to be analyzed independently. By area, Northern Ontario comprises approximately 86% of the province but managed honey bee colonies in the region are sparse. The stark contrast between the colony densities in the two regions justified the decision for individual analyses since modeling the whole province with a single spatial dependence structure, while possible, is not ideal for accurately estimating model parameters. However, it was not possible to produce an independent model for Northern Ontario because the sample size of CCSs is too small without borrowing information from Southern Ontario. Consequently, the province-wide analysis was maintained to offer some representation to northern beekeepers.

When Southern Ontario was analyzed separately, there was less variation and skewness in the regional colony density values, and the estimated variogram was found to represent the data more closely (Figure 2.4a). This improved model fit is further evident by the QQ-plot of the standardized residuals (Figure 2.4b).

The Niagara Peninsula is most densely populated with bee colonies, with over five colonies/km² at minimum, and as high as over ten colonies/km² in the areas adjacent to New York State, USA (Figure 2.5). Several factors may be at play for the magnitude of apiculture activity in Niagara. First, and most evident, is the prominent agricultural landscape of Niagara,

and the need for commercial pollination to aid efficient crop production. Many of Niagara's top production crops are either self-pollinated, like peaches, cherries, plums, and grapes, or wind-pollinated such as grains, and therefore not insect pollinator-dependent. However, some of these self-pollinating crops are still commercially pollinated by honeybees to increase the yield and quality of the fruit (Klatt *et al.*, 2014). Therefore, the abundance of agriculture may offer a limited explanation for the high density of honey bee colonies in this region. A second factor for the high bee colony population density in Niagara is the presence of commercial beekeeping operations and registered addresses for numerous commercial pollinator operations servicing farmland outside of Niagara or Ontario. Niagara, being of the most southern regions in Canada, is a preferred home for commercial beekeepers, in part due to the relatively mild winters offering favourable conditions for overwintering and an early spring harvest, allowing for colonies to build up strength in time for eastern Canada blueberry pollination in May and June.

Some spatial misclassifications may result from the nature of the government data used for this study. The colony location data indicate the locations where colonies are registered, typically their overwintering site, but may not always be accurate to the locations where the colonies reside during the entire season. These registrations and resulting data represent a snapshot in time and the numbers of colonies and their locations can change throughout the season. This issue becomes more dramatic when out-of-province pollination demands, such as blueberry pollination in eastern provinces, significantly reduce the number of colonies residing in Ontario, as roughly 20-35% of colonies leave at this time for pollination in New Brunswick, Prince Edward Island, and Quebec (OMAFRA, 2013). This considerable movement of honey bee colonies causes the population density to be a dynamic and complex topic of study.

Another study limitation is the potential for underreporting of colony ownership. Underreporting can occur when beekeepers do not accurately declare ownership of colonies to the provincial government (OMAFRA) and instead report fewer than the actual number of colonies, or when beekeepers do not report keeping bees at all. Assuming that beekeepers have no regionally varying motivation to underreport, the underreporting effect should be non-differential and affect all areas proportionally. Thus, the spatial pattern would not be biased, but the colony density estimates have the potential for minimal underreporting. Under the Ontario Bees Act, “No person shall be a beekeeper in Ontario without a certificate of registration issued by the Provincial Apiarist” and this registration includes the disclosure of the number of colonies in possession (Bees Act, 2019). Thus, to the knowledge of the Ontario Ministry of Agriculture, Food and Rural Affairs, along with the provincial beekeeper, the data used in this study are accurate and well representative of the population of managed colonies in Ontario.

To protect the privacy of beekeepers and yard locations, this study only received access to regional data at the level of the CCS. The use of aggregated data inherently results in some loss of information for data analysis because the exact spatial distribution within each region is averaged. Regions with larger geographic areas are more likely to be affected by this regional aggregation. This is expected to be a larger issue in Northern Ontario where regions with a large landmass accounted only for a small number of colonies. For Southern Ontario, the bias from the regional aggregation of the data is considered relatively small because colonies may not be tied to a single point but moved within the region during the season. Therefore, point data would not necessarily be more accurate in regions that are small relative to the movement of colonies. Yard locations may be updated by an inspector at the time of inspection if discrepancies are found.

If this study could be based on precise locations of the colonies (i.e., spatial point data) the findings could change, which is known as the modifiable areal unit problem in geographic epidemiology (Waller & Gotway, 2004), as clustering within the CCS is likely to exist in yard-level and operation-level spatial groupings of colonies. This would be expected to impact the more remote regions of Northern Ontario, where the few existing colonies are likely operated by only a handful of beekeepers in a limited number of yards. This clustering hierarchy of yards, operations, and CCS regions contributes further complexity to the topic of honey bee population density when reliant on aggregated data. In Southern Ontario, where regions are smaller and the number of colonies is greater, the variogram model accounts for spatial correlation of colony locations and kriging can adequately accommodate and exploit these spatial correlation structures to produce continuous spatial estimates. The clustering of colonies is a spatial data pattern related to the location of placed colonies by beekeepers and does not necessarily relate to the biological flight range of bees.

From the results of this study, it follows that the density of managed honey bee colonies in select areas of Ontario is quite high (upwards of 10 colonies/km² in areas of Southern Ontario), especially when compared to the apparent natural density of feral honey bees in forests of New York at roughly 1 colony/km² (Seeley, 2007; Seeley, Tarpy, Griffin, Carcione, & Delaney, 2015). While the results from this study draw no conclusions on the health status of bees in high-density regions of Ontario, they do identify areas that may be of interest for future studies based on the known implications of population density on pathogen and pest transmission between colonies. Future work on the spatial distribution of diseases among managed honey bee colonies could consider the findings from this study to standardize disease counts based on the

population-at-risk to prevent misrepresentation of disease frequency in areas of high population density.

Based on this study, Niagara is hypothesized to possess a greater total prevalence of communicable diseases due to the higher density of colonies at risk, but disease prevalence is also dependent on management practices and treatment schedules. In instances of *Varroa destructor*-induced colony death, an abundance of colonies in close proximity to the weakened colony may increase the probability of robbing to occur and may transfer mites between colonies (Frey & Rosenkranz, 2014; Peck & Seeley, 2019). Furthermore, the transmission of *Paenibacillus larvae* by means of robbing may occur more readily in the higher density regions identified, where intercolonial distance is within the 1 km range, which is a reported risk factor (Lindström, Korpela, & Fries, 2008). These high-density regions may also selectively support more highly virulent strains of *P. larvae* that can induce more severe clinical symptoms without concern of exhausting available hosts, where pathogens in low-density regions must be more sparing of their hosts and not be lethal enough to out-pace host reproduction (Fries & Camazine, 2001). Horizontal transmission by means of drifting could also be expected to be elevated in regions of high density such as the Niagara peninsula based on previous literature on the consequences of density on drifting (Seeley & Smith, 2015). Furthermore, a region possessing a high density may not be indicative of close proximity between neighbouring yards as the number of colonies per yard can vary.

The results of this study provide insight into the distribution of colony density and distribution across Ontario, Canada. The overall population density structure across the province is heterogeneous with two main patterns. Southern Ontario is highly concentrated compared to Northern Ontario, and the Niagara Peninsula is highly concentrated compared to the remainder

of Southern Ontario. Future studies should aim to ground truth these results, such as using prevalence data of honey bee pathogens to confirm an association between high colony density and high prevalence of various pathogenic diseases and pests (e.g., *Varroa* mites). Results from this study can inform risk-based apiary health inspections through a focus on areas at potentially greater risk to transmit disease. Furthermore, this study highlights the methodologies available for similar mapping projects to be conducted elsewhere, including areas with non-uniform densities. Disease transmission is complex and multifaceted, and therefore, it should not be concluded from this study alone that the areas identified as high density possess higher than average disease or pest risk. These findings are meant to expose the potential implications of high colony density and give beekeepers, researchers, and government officials a statistically sound estimation of the densities in all regions of Ontario. Furthermore, visually intuitive, and interactive maps developed as part of this project provide adequate information distribution to members of the commercial and hobbyist beekeeping community.

2.6 References

- Agriculture and Agri-Food Canada. (2019). *Statistical overview of the Canadian honey and bee industry, 2019*.
- Bees Act (2019). Canada. Retrieved from <https://www.ontario.ca/laws/statute/90b06>
- Berke, O. (2004). Exploratory disease mapping: kriging the spatial risk function from regional count data. *International Journal of Health Geographics*, 3, 1–11.
<https://doi.org/10.1186/1476-072X-3-18>
- Broosi, B. J., Delaplane, K. S., Boots, M., & De Roode, J. C. (2017). Ecological and evolutionary approaches to managing honeybee disease. *Nature Ecology and Evolution*, 1(9), 1250–1262. <https://doi.org/10.1038/s41559-017-0246-z>
- Carrat, F., & Valleron, A. J. (1992). Epidemiologic mapping using the “kriging” method: Application to an influenza-like epidemic in France. *American Journal of Epidemiology*, 135(11), 1293–1300. <https://doi.org/10.1093/oxfordjournals.aje.a116236>
- Chen, Y. P., & Siede, R. (2007). Honey bee viruses. *Advances in Virus Research*, 70, 33–80. [https://doi.org/10.1016/S0065-3527\(07\)70002-7](https://doi.org/10.1016/S0065-3527(07)70002-7)
- Cheng, J., Karambelkar, B., & Xie, Y. (2018). *Leaflet*: create interactive web maps with the JavaScript “*Leaflet*” library. R package version 2.0.2. Retrieved from <https://cran.r-project.org/package=leaflet>
- Frey, E., & Rosenkranz, P. (2014). Autumn invasion rates of *Varroa destructor* (Mesostigmata: *Varroidae*) into honey bee (Hymenoptera: *Apidae*) colonies and the resulting increase in mite populations. *Journal of Economic Entomology*, 107, 508–515.
- Fries, I., & Camazine, S. (2001). Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie*, 32, 199–214.
- Government of Canada. (2012). Guidelines for effective knowledge mobilization. Retrieved April 20, 2019, from https://www.sshrc-crsh.gc.ca/funding-financement/policies-politiques/knowledge_mobilisation-mobilisation_des_connaissances-eng.aspx#:~:text=Knowledge%20mobilization%20is%20an%20umbrella,by%20researchers%20and%20knowledge%20users.
- Kilpatrick, A. M., & Altizer, S. (2010). Disease ecology. *Nature Education Knowledge*, 3(10), 55.
- Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., & Tscharntke, T. (2014). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences*, 281(1775), 20132440.
<https://doi.org/10.1098/rspb.2013.2440>
- Krige, D. G. (1981). *Lognormal-de Wijsian geostatistics for ore evaluation*. South African Institute of Mining and Metallurgy.
- Lindahl, J. F., & Grace, D. (2015). The consequences of human actions on risks for infectious diseases: a review. *Infection Ecology and Epidemiology*, 1, 1–11.

- Lindström, A., Korpela, S., & Fries, I. (2008). Horizontal transmission of *Paenibacillus larvae* spores between honey bee (*Apis mellifera*) colonies through robbing. *Apidologie*, 39(5), 515–522. <https://doi.org/10.1051/apido:2008032>
- Manchuk, J. G., Leuangthong, O., & Deutsch, C. V. (2009). The proportional effect. *Mathematical Geosciences*, 41(7), 799–816. <https://doi.org/10.1007/s11004-008-9195-z>
- Matheron, G. (1963). Principles of geostatistics. *Economic Geology*, 58(8), 1246–1266. <https://doi.org/10.2113/gsecongeo.58.8.1246>
- OMAFRA. (2013). 2012 Ontario Provincial Apiarist Annual Report, 1–15. Retrieved from <http://www.omafra.gov.on.ca/english/food/inspection/bees/12rep.htm>
- OMAFRA. (2019). Production and farm value of honey: 1982 - 2019. Retrieved April 20, 2019, from <http://www.omafra.gov.on.ca/english/stats/hort/index.html>
- OMAFRA. (2020). Census of agriculture and strategic policy branch. Retrieved April 23, 2019, from http://www.omafra.gov.on.ca/english/stats/county/southern_ontario.htm
- Peck, D. T., & Seeley, T. D. (2019). Mite bombs or robber lures? The roles of drifting and robbing in *Varroa destructor* transmission from collapsing honey bee colonies to their neighbors. *PLoS ONE*, 14(6), 1–14. <https://doi.org/10.1371/journal.pone.0218392>
- R Core Team. (2020). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.r-project.org/>
- Ribeiro, P. J., & Diggle, P. J. (2018). geoR: analysis of geostatistical data. R package version 1.7-5.2.1. Retrieved from <https://cran.r-project.org/package=geoR>
- Seeley, T. D. (2007). Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States. *Apidologie*, 38(1), 19–29. <https://doi.org/10.1051/apido:2006055>
- Seeley, T. D., & Smith, M. L. (2015). Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. *Apidologie*, 716–727. <https://doi.org/10.1007/s13592-015-0361-2>
- Seeley, T. D., Tarpy, D. R., Griffin, S. R., Carcione, A., & Delaney, D. A. (2015). A survivor population of wild colonies of European honeybees in the northeastern United States: investigating its genetic structure. *Apidologie*, 46(5), 654–666. <https://doi.org/10.1007/s13592-015-0355-0>
- Statistics Canada. (2017). Population size and growth in Canada: key results from the 2016 Census.
- Statistics Canada. (2018). Let's talk honey. Retrieved April 20, 2019, from <https://www150.statcan.gc.ca/n1/pub/11-630-x/11-630-x2016004-eng.htm>
- Statistics Canada. (2019). Boundary files. Retrieved September 9, 2020, from <https://www12.statcan.gc.ca/census-recensement/2011/geo/bound-limit/bound-limit-eng.cfm>
- Statistics Canada. (2021). Population estimates, July 1, by economic region, 2016 boundaries.

Retrieved July 6, 2021, from
<https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1710013701>

Tarwater, P. M., & Martin, C. F. (2001). Effects of population density on the spread of disease. *Complexity*, 6(6), 29–36. <https://doi.org/10.1002/cplx.10003>

Waller, L. A., & Gotway, C. A. (2004). *Applied spatial statistics for public health data*. Hoboken, NJ, USA: John Wiley & Sons Inc.

2.7 Tables

Table 2.1: Spherical variogram model parameters for Ontario and Southern Ontario honey bee colony density data.

	β_0	Nugget Variance (τ^2)	Partial Sill (σ^2)	Range (km) (ϕ)
Ontario				
Spherical	0.3	0.049	0.2	360
Southern Ontario				
Spherical	0.571	0.054	0.176	191

2.8 Figures

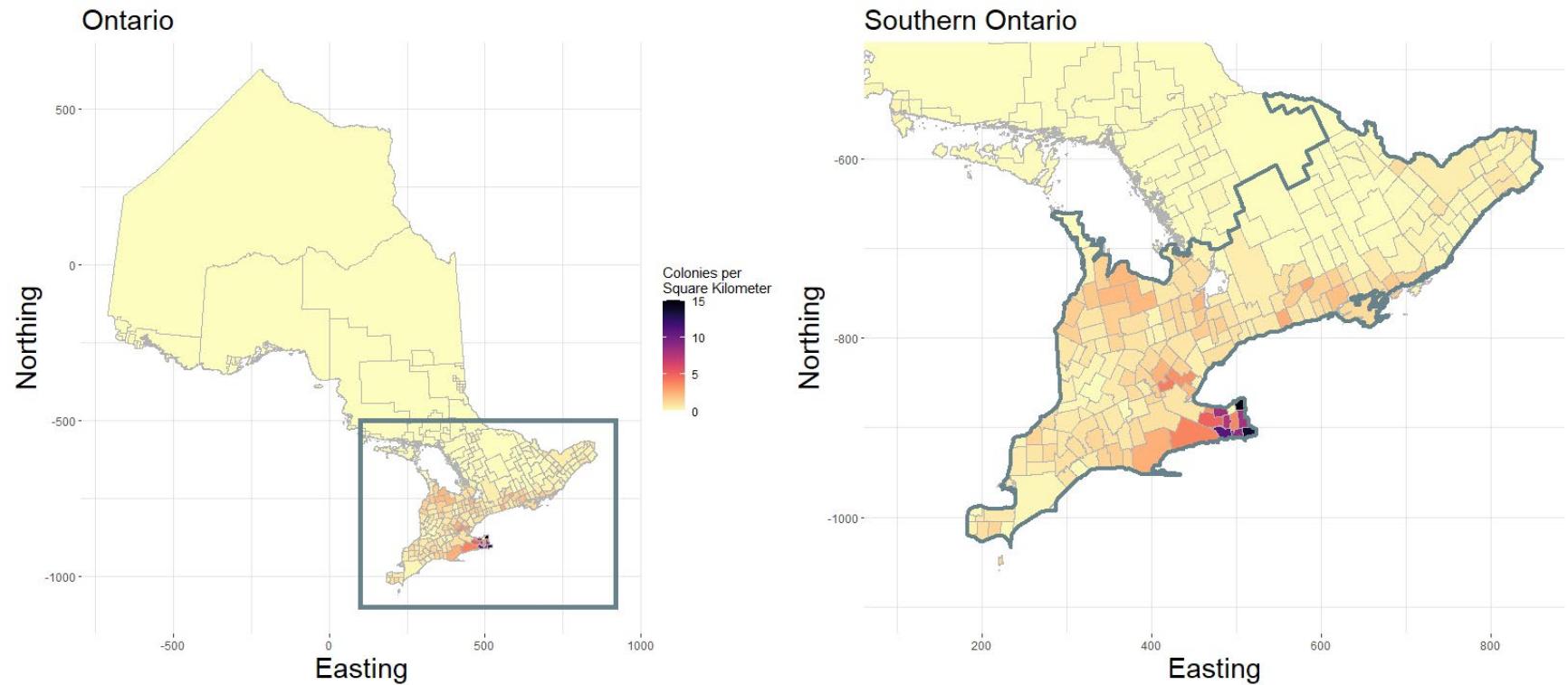


Figure 2.1: Choropleth map of the crude honey bee colony densities across census consolidated subdivisions in Ontario and Southern Ontario.

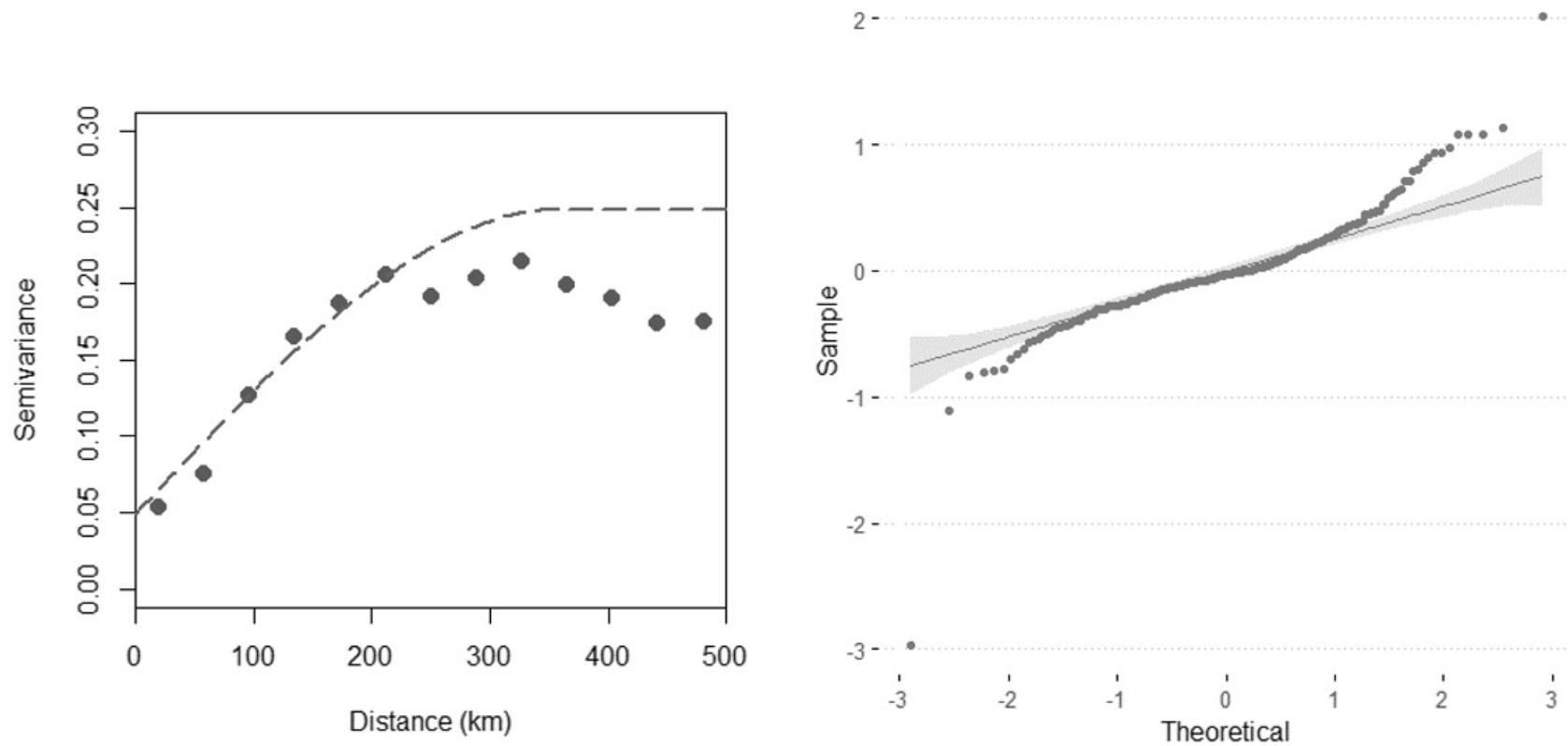


Figure 2.2: (A) Empirical semivariogram model estimation for Ontario fitted with the spherical model (dashed line). (B) Quantile-Quantile plot of the variogram model standardized residuals.

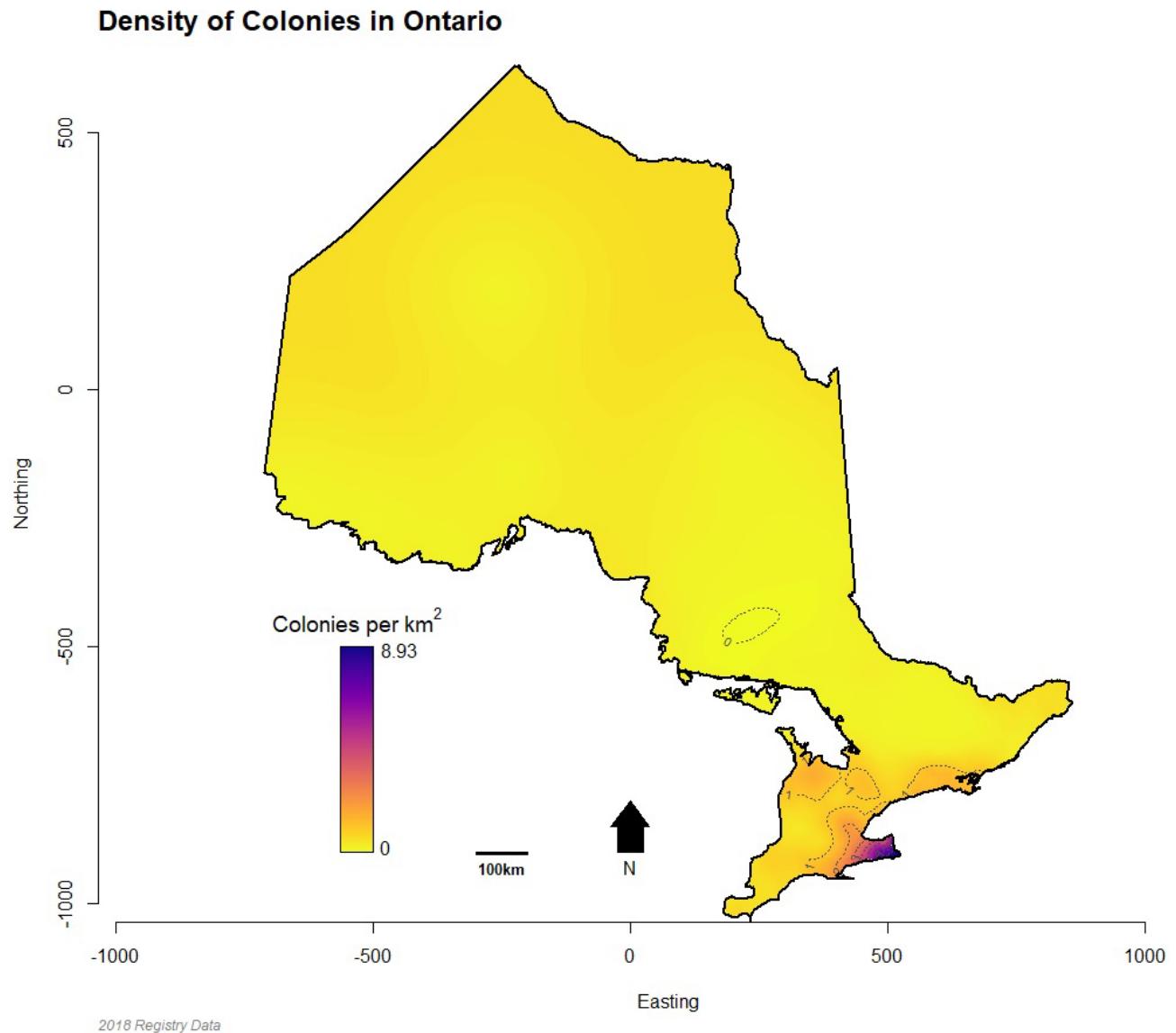


Figure 2.3: Geostatistical kriging interpolated honey bee colony density for Ontario.

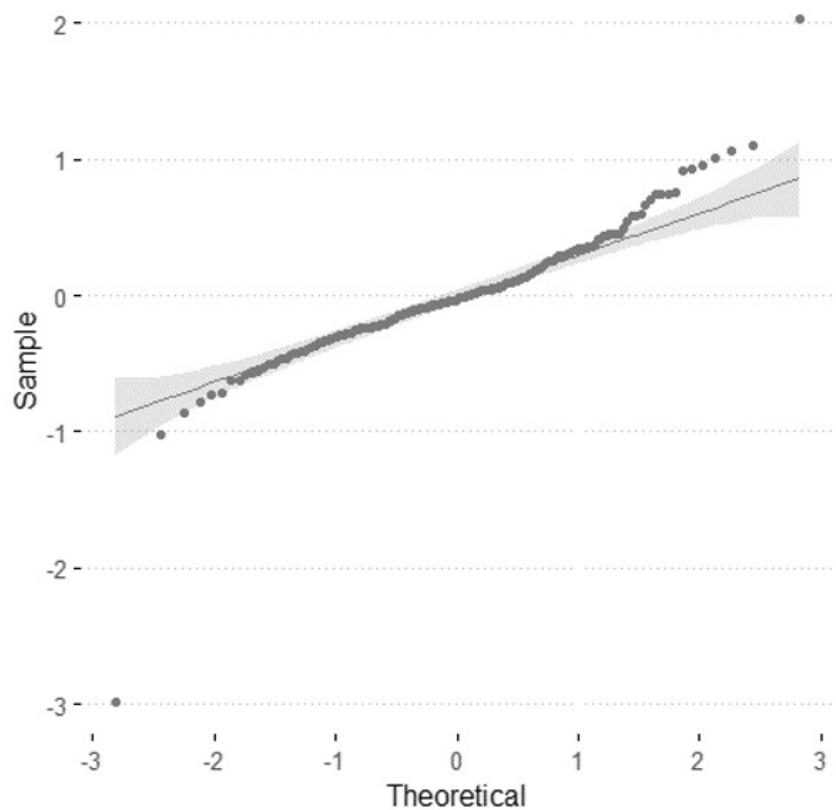
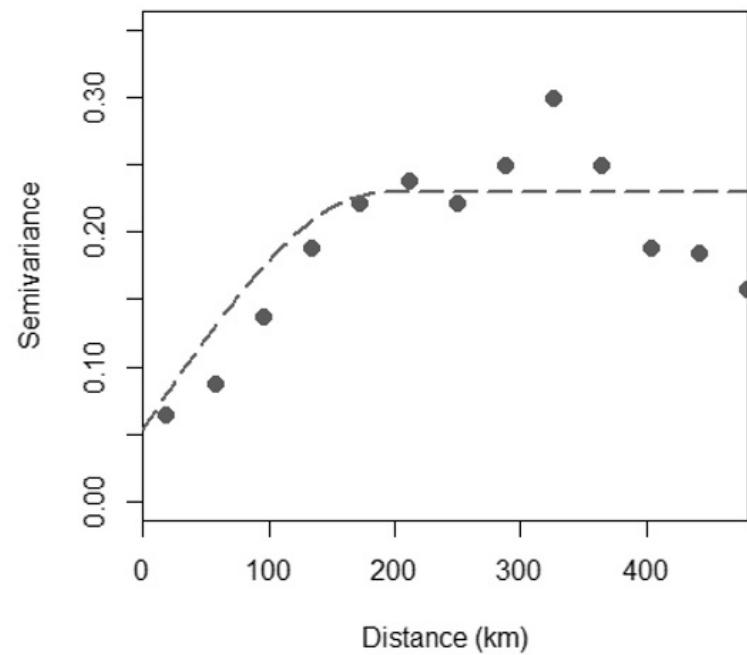


Figure 2.4: (A) Empirical semivariogram model estimation for Southern Ontario, fitted with the spherical model (dashed line). (B) Quantile-Quantile plot of the variogram model residuals.

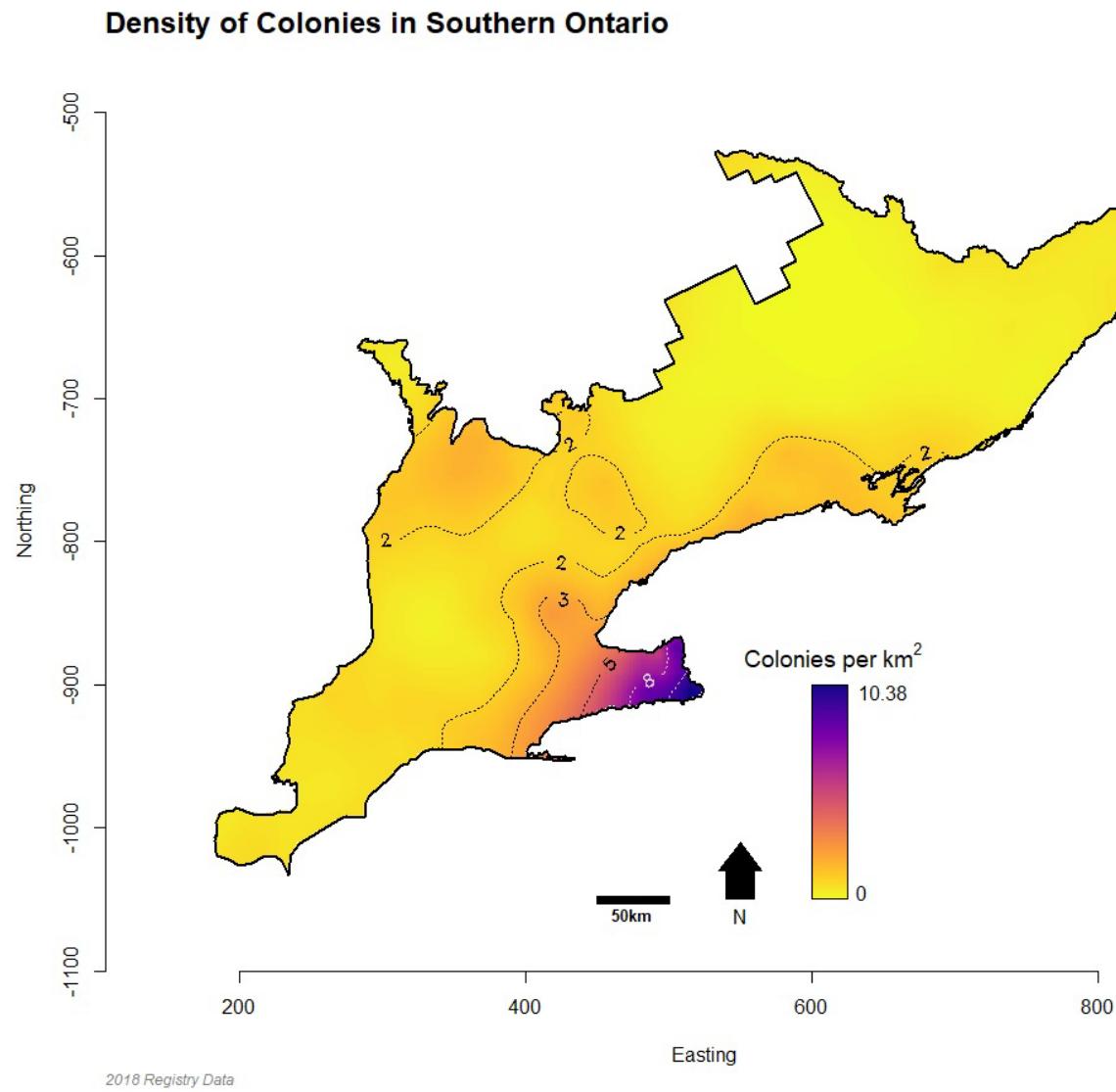


Figure 2.5: Geostatistical kriging interpolated honey bee colony density for Southern Ontario.

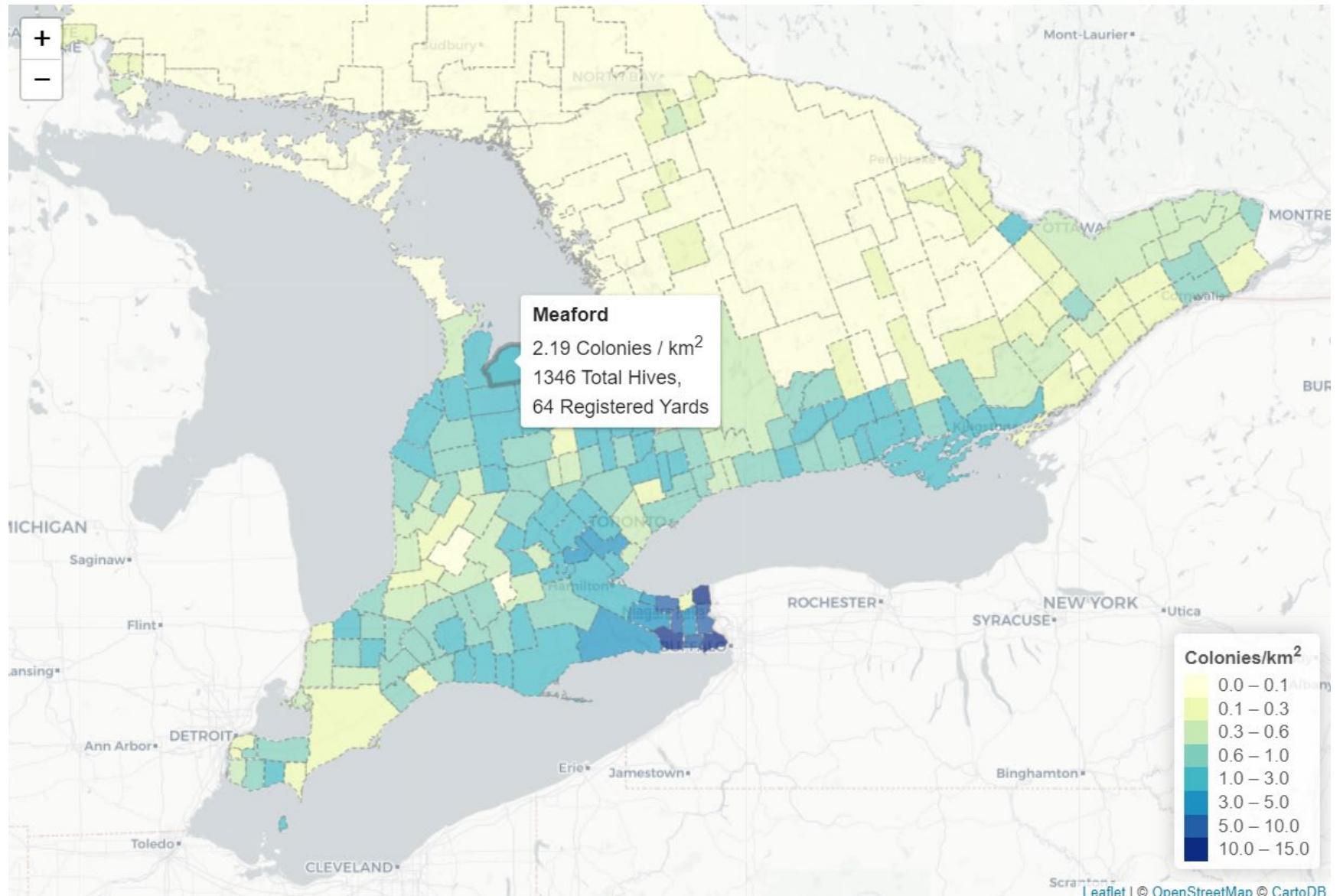


Figure 2.6: Screenshot of the interactive online dashboard developed, depicting crude regional colony densities for Ontario.

3 CHAPTER THREE: SPATIAL EPIDEMIOLOGICAL ANALYSIS OF *VARROA DESTRUCTOR* PREVALENCE IN SOUTHERN ONTARIO: 2015 – 2019

3.1 Abstract

Elevated colony losses have continued to be an issue for Canadian beekeepers for more than a decade. Numerous studies have identified unmanaged colony infestation by the *Varroa destructor* mite as a main cause of the problem. *Varroa* mites spread externally of the hive through a phoretic stage in their life cycle. Consequently, their movement outside the hive is influenced by honey bee flight behaviours, which can range to multiple kilometers from the originating hive in any direction. *Varroa* mites are therefore of regional concern as neighboring colonies and yards share nearby forage which can serve as fomites. Additionally, mites can be transmitted through bee behaviours such as robbing and drifting, thus impacting surrounding colonies. Understanding the distribution of mites across a population is key for surveillance and equitable allocation of resources. Spatial patterns of *Varroa* mite infestations in Southern Ontario, Canada, were investigated using a combination of cluster analysis, and geostatistical modelling, using five years of provincial apiary inspection data, from 2015 to 2019. A collection of disease clusters of *Varroa* mite infestations was identified and found to be stable over multiple years with several other individual clusters occurring sporadically throughout Southern Ontario during the same study period. Universal kriging was applied to the *Varroa* data in combination with regional colony density, and land use data as covariates, producing an isopleth map of the prevalence risk for *Varroa* infestation. No substantial link between *Varroa* infestation and environmental factors was found. This study highlights the need for more data and investigation to determine the cause of the identified clusters and areas of elevated risk. These results are

hypothesis-generating but simultaneously provide information for government agencies, industry organizations, and beekeepers into the spatial distribution of *Varroa* at a macro scale.

3.2 Introduction

Between 2015 and 2019, Ontario beekeepers reported an average overwinter colony loss of 30.5% (Canadian Association of Professional Apiculturalists, n.d.). Other Canadian provinces reported similarly high losses, with an average of 25.7% overwinter colony loss in 2019 across all 10 provinces. This amount of loss is beyond the accepted level of 5-15% (Vidal-Naquet, 2018). Elevated levels of colony loss have been experienced consistently since the Canadian Association of Professional Apiculturists (CAPA) began reporting on the issue of “colony collapse disorder” in 2007 (Canadian Association of Professional Apiculturalists, n.d.). Despite the high percentage of colony loss, beekeepers in Canada have managed to maintain a consistent population of colonies in the past 5 years (Agriculture and Agri-Food Canada, 2019). This paradox demonstrates the effectiveness of modern advancements in beekeeping, allowing for beekeepers to compensate continuing large losses through techniques such as hive splitting, and commercialization of queen and nucleus colonies, but the issue of long-term colony health still remains.

First reported in Canada in 1989 (McElheran, 1990), the parasitic mite, *Varroa destructor*, has continued to be one of the most serious threats to beekeeping in Canada and has spread to most beekeeping regions across the country (Currie, Pernal, & Guzmán-Novoa, 2015). *Varroa destructor* (commonly referred to as *Varroa* or *Varroa* mites) is a phoretic mite, which feeds on adult honey bees for survival, and acts as a parasite to honey bee larva during developmental stages (Rosenkranz, Aumeier, & Ziegelmann, 2010). *Varroa* mites also serve as a vector for several viruses, including deformed wing virus, and black queen cell virus (Tentcheva

et al., 2004). Clinically, the infestation of a honey bee colony by *Varroa* mites, and the associated symptoms, is referred to as varroosis. Varroosis has been found to be most detrimental when co-prevalent with other parasites and abiotic stressors (Roberts, Anderson, & Durr, 2017), and left untreated, is capable of decimating entire honey bee colonies. *Varroa* mites have been considered by numerous researchers as the greatest contributor to weakened colonies and overwinter colony losses (Barroso-Arévalo, *et al.*, 2019; Guzmán-Novoa *et al.*, 2010; Van Der Zee, *et al.*, 2015).

Varroa mites are an endemic and treatable issue in beekeeping in Canada and around the majority of the world. Therefore, the effects of an infestation can usually be mitigated when detected early. However, the presence of *Varroa* may go undetected due to sampling error or an absence of testing. If detected, chemical and non-chemical treatment options are available. Some chemical treatment regimens for *Varroa* may be detrimental to the colony's health if administered incorrectly, though not all have been shown to have negative effects (Giovenazzo & Dubreuil, 2011). *Varroa* mites have also demonstrated resistance to various chemical treatment options due to improper administration or rotation (Rawn *et al.*, 2019). Non-chemical treatment options against *Varroa* infestations exist, but have been shown to be less effective at reducing *Varroa* load (Haber, *et al.*, 2019). Flaws in both *Varroa* detection and *Varroa* treatment could influence the regional *Varroa* abundance, as neighboring yards may contract *Varroa* as a result of bees robbing from a weakened colony possessing a high *Varroa* load (Peck & Seeley, 2019), or other means of transmission. Because no treatment is 100% effective, and the eradication of mites is not possible, integrated pest management (IPM) strategies are important to keep mite levels below critical thresholds. Adequate knowledge on the pest of interest and their distributions across the population is key for effective surveillance and IPM.

The phoretic nature of *Varroa* mites and the flight behavior of honey bees, implies that the presence of *Varroa* is a landscape-wide issue and is not localized to single bee yard outbreaks unless geographically isolated. Increased *Varroa* load in a single yard may result in subsequent transmission to nearby colonies as *Varroa* is transmitted by means of robbing, drifting (if colonies within yards are not adequately spaced), or through fomites in the environment (Peck & Seeley, 2019; Peck, *et al.*, 2016). Therefore, regional population levels of *Varroa* should be considered when making management decisions. Limitations in *Varroa* detection and treatment suggest a need to switch from reactive to proactive population medicine for *Varroa* management, for which enhanced surveillance is necessary.

Geospatial epidemiological studies can address all aspects of the epidemiologic triad: agent, host, and environmental risk factors of disease (Berke, 2005). Spatial autocorrelation can indicate if the disease agent's prevalence is spatially related, while the detection of clusters can give an indication of whether the host's susceptibility and behaviours are influencing the distribution of disease. Furthermore, spatial regression and trend analysis can help identify which environmental risk factors may be contributing to the prevalence of the disease. This approach can therefore provide insight into all major aspects of *Varroa* distribution and spread mechanics at a population level. To date, few spatial epidemiological studies have investigated the prevalence of *Varroa destructor*, and none have been identified in the literature for Ontario or Canada. In one geospatial study of varroosis in New Zealand, Stevenson *et al.* (2005) identified clusters of *Varroa* infestations, as well as a spatial dependence structure that decays over distance from an infected yard. Similar patterns may exist in Ontario and should be investigated.

Previous studies have explored the impacts of surrounding landscape on the health of managed honey bees, but found no association with *Varroa* (Dolezal, Carrillo-Tripp, Miller,

Bonning, & Toth, 2016). However, this study by Dolezal *et al.* (2016) investigated only two landscape categories: high cultivation and low/no cultivation. Further investigation into more landscape classifications is therefore warranted to confirm that this choice of binary classification is not suppressing a true association. Surrounding land-use may influence mite loads due to variations in diversity and quantity of available forage, as well as potential for mite transfer from feral bee colonies in natural landscapes or managed bees in higher colony density areas. The diet of honey bees has previously been linked to health issues such as immunocompetence (Alaux, Ducloz, Crauser, & Le Conte, 2010) and surrounding land-use type has been shown to impact the quantity and quality of forage and food accumulation, and bee health in general (Dolezal *et al.*, 2016; Sponsler & Johnson, 2015). Landscapes with higher colony densities, could possess greater mite prevalence because of the increase in density of susceptible colonies for mite transfer to occur, a theory accepted in human epidemiology (where population density is related to disease transmission (Tarwater & Martin, 2001)), but not yet accepted in bee research.

Honey bee colonies surrounded by natural landscapes are more likely to forage on a more diverse diet and have access to ample food sources but simultaneously may interact more with feral colonies, potentially spreading and contracting mites more frequently. Bees located in heavily cultivated landscapes are less likely to interact with untreated feral colonies, but have access to a less diverse diet and may spread mites between other managed colonies because of the increased density observed in areas of farmland in Ontario (Sobkowich, Berke, Bernardo, Pearl, & Kozak, 2021). In locations where colonies exist beside a large body of water, the immediate foraging landscape is effectively reduced and may lead to less available forage resulting in a greater competition for nectar sources, which may contribute to mite spread due to

shared forage or increased robbing (Peck & Seeley, 2019; Peck *et al.*, 2016). In contrast, bees in an urban setting may experience similar issues of reduced forage quantity but may face less competition because of decreased colony density (Sobkowich *et al.*, 2021). These scenarios are the justification for a five-category landscape classification system to be evaluated for an association with *Varroa* prevalence. These five landscape categories are: natural land, forageable agricultural land, non-forageable agricultural land, urban/developed land, and water bodies.

Geostatistical kriging allows for spatial prediction of prevalence even in locations or areas where the sample size is otherwise too small. Kriging can be used to predict the prevalence over the entire study area which can inform policy decisions, aid in the efficient allocation of resources, and provide a basis for a risk-based sampling model for future inspections (Berke, 2004; Carrat & Valleron, 1992). Universal kriging is based on a spatial general linear model (GLM) to study the impact of potential risk factors, such as land-use types derived from satellite imagery (as applied in this study) in the presence of spatial dependence.

The goal of this study was to explore the spatial distribution of *Varroa* infestations in managed honey bee colonies of Southern Ontario, using a population-level epidemiologic approach, over a 5-year study period. This study has three objectives to achieve this goal: (1) explore the spatial distribution of *Varroa* and the tendency for spatial clustering of varroosis cases; (2) locate high-risk clusters of varroosis; and (3) use geostatistical modelling to assess the effects of the five various land-use types on *Varroa* infestation to estimate and map the prevalence-risk.

3.3 Materials and Methods

Varroa data were received from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). The data were collected by trained inspectors. Inspections occur mainly for three reasons: regulatory inspections, confirmation of *Varroa* status of commercialized queens or nucleus colonies, or to address beekeeper concern of poor colony health. *Varroa* inspection data are based on the standard alcohol wash method (Dietemann *et al.*, 2013) and reported as a total count per 300 bees. These counts were converted to a value of mites per 100 bees, referred to subsequently as the “*Varroa* rate”. Inspected colonies were recorded with their GPS location, date of inspection, and the observed *Varroa* rate. Geographic coordinates of yard locations were truncated to maintain the privacy of exact yard locations. The cleaned dataset contained 3,786 colony-level observations in Southern Ontario between 2015 and 2019. Regional colony density values were derived from OMAFRA registry data from the 2018 beekeeping season, aggregated by census consolidated subdivision (CCS) (Statistics Canada, 2018) to maintain beekeeper’s privacy. The 2018 registry dataset was the most recent and complete at the time of analysis. Each inspection location was assigned a colony density value based on the CCS region that the inspection occurred within.

Land usage data were acquired from the Government of Canada, and the Agriculture and Agri-Food division, through their Annual Crop Inventory program (Government of Canada, 2020). The data were in raster file format, produced by optical and radar-based satellites. The raster file contained land use information at a spatial resolution of 30m with a reported accuracy of 85%. Seventy-two distinct land-use types were used to define the provincial landscape of Ontario. These 72 categories were then aggregated into the 5 categories of interest for this study: natural land, developed land, forageable agriculture, non-forageable agriculture, and water.

Agricultural land was deemed forageable if included in the *Pollinator Partnership Canada Guide for Planting Forage for Honeybees* (Pollinator Partnership Canada, 2017).

3.3.1 Spatial distribution of *Varroa* and determination of varroa mite clustering

A sampled *Varroa* rate of greater than 3 mites per 100 bees was considered a case colony, as outlined by the OMAFRA treatment threshold guidelines (Kozak *et al.*, 2021). Further reference to cases in this study is with respect to a colony found to have a *Varroa* rate at or above the threshold of 3 mites per 100 bees. Approximate locations of cases and controls (colonies observed to have a *Varroa* rate below the threshold of 3 mites per 100 bees) were plotted on a map of the province for data exploration.

As proposed by Diggle and Chetwynd (1991), the D-function was applied to assess spatial clustering of cases. Estimation of the D-function further provides an approximation for the spatial range at which clustering may be occurring. A confidence band derived from the standard errors was used to determine the presence of spatial clustering.

3.3.2 Detection of high-risk clusters of *Varroa* mite prevalence

Clusters of *Varroa* case locations were detected and point estimates of the standardized morbidity ratios were calculated using the spatial scan statistic (implemented in the *SatScan* software (Kulldorff & Information Management Services Inc., 2009). The scan statistic was applied for each of the 5 study years individually, and the results were then overlayed onto a map to check for temporal stability of *Varroa* case clusters across beekeeping seasons. The spatial scan analysis used the Bernoulli model (Kulldorff, 1997), with a purely spatial method to detect regions of high rates. A circular scanning window was used, with a maximum cluster size of 20% of the population at risk. A maximum of 20% was used in place of the standard 50% maximum to uphold biological relevance, owing to the scale of the study area (the distribution of

colonies) in relation to the typical movement and flight ranges of bees. This reduced maximum cluster size has been used previously by researchers looking to account for low levels of data, spatial discontinuity or to look specifically for smaller clusters (Ma, Yin, Zhang, Zhou, & Li, 2016). The standard Monte-Carlo method, with 999 replications, was used as a means to estimate the p-value for detected clusters. All clusters identified at a 5% significance level were highlighted on a map of Southern Ontario. Only non-overlapping secondary clusters were reported. The 95% confidence intervals of the SMR were estimated using the Vandenbroucke method (Vandenbroucke, 1982) in the *epiR* package for *R* (Stevenson and Sergeant, 2022).

3.3.3 Spatial regression modelling

For spatial regression modelling, the Ontario land use data were merged with the *Varroa* rate data using a buffer analysis in *QGIS* software (QGIS Development Team, 2020). A Lambert azimuthal equal-area projection was applied to preserve the study's area size and minimize distance distortions. The locations of bee yards inspected for *Varroa* from all five study years were used as centroids for a buffer analysis. Buffers with a 3km radius (approximating the average foraging range of honey bees around their hives (Pollinator Partnership Canada, 2017; Visscher & Seeley, 1982)) to link the land-use raster data to the inspection data. A summary of the percentage of each of the five land use categories within each buffer was calculated and merged with the *Varroa* inspection data. The five land use categories and regional colony density values were considered as covariates in the model building process. For spatial modelling, counts of the number of mites for each inspection were used (mites per 100 bees sampled). Repeat inspection observations at the same geographical location were addressed by averaging the mite counts.

The general linear model component of regression-kriging was fit by comparing the results of regression models for each covariate. A Gaussian family GLM model was used to model the continuous *Varroa* rate, and an iteratively reweighted least squares approach was used to fit the GLM model. The Akaike information criterium (AIC) from each regression model was used as an indication of model fit. Simple and multiple regression models were considered using the land-use types, and colony density as covariates. The covariate(s) with the lowest AIC value was selected. This model would then be put forward in the regression-kriging model building process. Because this study is hypothesis generating, p-values were considered as exploratory metrics only (Matthews, Wasserstein, & Spiegelhalter, 2017). Estimated regression coefficients (β) for the simple regression models with their 95% Wald confidence intervals were presented in a forest plot to visualize the magnitude and direction of their potential effect on *Varroa* prevalence.

Universal (regression) kriging is a two-part process which combines a general regression model of the dependent variable with kriging interpolation of the residuals over a geographic area. The regression model, to estimate the influence of an independent variable(s), is fit first using ordinary least squares, then the covariance function of the residuals is used to derive generalized least squares coefficients from which the residuals can be re-estimated iteratively (Hengl, Heuvelink, & Rossiter, 2007). The variogram is then modelled for the residuals and kriging is performed to predict the regression model residuals over the study area. The predicted residuals are then combined with the regression output, using a spatially continuous raster of the independent variable(s), to produce a continuous prediction of the *Varroa* rate. Residuals from the final selected GLM model were obtained and the corresponding variogram of residuals was estimated through weighted least squares estimation (WLSE) using initial nugget, sill, and range

parameters from visual inspection of the empirical variogram. A spherical variogram model was used to represent the GLM residual variogram. Following the fit of the regression model, and variogram model, universal kriging was applied to predict the prevalence of *Varroa* mites onto a grid covering the entire study area for mapping.

All analyses, unless otherwise stated, were performed using the open-source software *R* (R Core Team, 2020). The package “*gstat: Spatial and Spatio-Temporal Geostatistical Modelling, Prediction and Simulation*” was used to perform kriging (Gräler & Pebesma, 2016; Pebesma, 2004).

3.4 Results

A total of 3,786 observations were collected over the 5-year study period from 2015 to 2019 at 1,082 unique locations. The annual number of observations declined from 1,030 in 2015, to a total of 939, 757, 551, and 509 inspections conducted in 2016 to 2019, respectively. The observed annual prevalence of *Varroa* cases (≥ 3 mites per 100 bees) varied during the 5-year study period around an average of 13.6% of colonies sampled. From 2015 to 2019, the prevalence estimates of *Varroa* cases in Ontario were 21.1%, 8.9%, 16.3%, 4.2% and 15.2% respectively. Complete descriptive statistics of the dependent and independent variables used in regression modelling is presented in Table 3.1.

3.4.1 Spatial distribution of *Varroa* and determination of *Varroa* mite clustering

Producing a point map of the locations of cases (colonies infected by ≥ 3 mites per 100 bees) and controls illustrates that the locations of inspections in Southern Ontario during the study period are geographically diverse. Furthermore, sample sites are representative of the provincial colony density, with a greater apparent number of inspections in the Niagara

Peninsula and fewer observations in the northeast. Cases appear to be present across the entire study area (Figure 3.1).

Plots of the D-function for each of the study years, and the entire study period combined, indicate the presence of spatial autocorrelation of cases (Figure 3.2). The distance at which spatial autocorrelation was detected is not consistent over the study period, with 2015 demonstrating the largest range at approximately 100km. Subsequent years to 2015 demonstrated noticeably lesser degrees of spatial autocorrelation with 2018 indicating negligible amounts present. The 2016, 2017, and 2019 years of data all showed relatively equal results of autocorrelation at an approximate range of 10km. When the data from the 5-year study period were combined, spatial autocorrelation of *Varroa* cases was detected by the D-function at a range of approximately 25km (Figure 3.2).

3.4.2 Detection of high-risk clusters of *Varroa* mite prevalence

At least one and up to three spatial clusters of *Varroa* cases were detected for each year in the study period (Table 3.2).

Figure 3.3 shows the locations of high-risk clusters detected in each year of the study period combined to a single map of the province. The map gives an indication of a temporal stability of clusters in the northwestern quadrant of Southern Ontario, with some satellite clusters occurring sporadically elsewhere throughout the study area. All observed clusters presented a standardized morbidity ratio (SMR) of greater than 2 with a maximum observed SMR of 12.19 (95% CI: 3.85, 25.23) (Table 3.2).

3.4.3 Spatial regression modelling

For regression modelling, the annual data were aggregated over time. Preliminary simple GLMs indicated no evidence of an association between *Varroa* rate and any of the five land-use types. The estimated regression coefficients (β) for the 5 land-use variables all possessed large confidence intervals at the 95% level, and p-values larger than 0.5. Regional colony density provided minimal evidence for a small negative association, with an estimated regression coefficient of $\beta = -0.05$ (95% CI: -0.11, 0.01, $p= 0.09$), indicating a decrease in *Varroa* rate by 0.05 for an increase of 1 colony per square kilometer. The degree of northing showed little evidence for an association with *Varroa* rate ($\beta = 1.5$; 95% CI: (-0.23, 3.23); $p= 0.09$) and likewise no association was observed for easting ($\beta = 0.34$; 95% CI: (-0.93, 1.61); $p= 0.60$), indicating insufficient evidence of a large-scale spatial trend across the study area. A forest plot of the results from the preliminary simple regression models is presented in Figure 3.4. The model with regional colony density as the sole independent covariate produced the lowest AIC and was put forward in the regression kriging process. Multiple regression modelling, by backwards model selection, did not result in a better fitting model.

The variogram estimated from the final GLM model residuals is presented in Figure 3.5. A spherical variogram model with parameters; nugget = 2.98, partial sill = 4.17, and range = 27.58km sufficiently represents the spatial correlation structure of the residuals of the GLM model (Figure 3.5).

The predicted values of the *Varroa* rate derived from the spatial regression model ranged from 0 to 15.9 ($\mu: 0.11$), compared to the observed *Varroa* prevalence range of 0 to 51 ($\mu: 0.86$). Model fit was assessed using leave-one-out cross-validation; no evidence for lack of fit was indicated by the histogram of residuals or map of residuals. The RMSE = 2.7 appears large

compared to the *Varroa* prevalence but this is an effect of a few outliers (MAE = 0.007).

Predicted values from the model for the whole study area are presented as an isopleth map in Figure 3.6. The map indicates a heterogenous spread of *Varroa* across the study area with several areas of increased risk. The locations with the greatest estimated risk both reside in the mid-north-east region of Southern Ontario near the municipalities of Peterborough and Bancroft. Several other areas across the study area showed high *Varroa* rates compared to their surroundings. Most of Southern Ontario was predicted to have an overall low rate of *Varroa* mites (Figure 3.6).

3.5 Discussion

This is the first study to comprehensively assess the spatial distribution of *Varroa destructor* in managed Ontario bee colonies at a population level. This study provides insight into all three aspects of the epidemiological triad: host, agent, and environmental risk factors for *Varroa* prevalence.

Spatial clustering of *Varroa* infestations were detected using the D-function (case-control data) and similarly through the estimation of the variogram (*Varroa* count data). Both methods presented results of clustering occurring up to a range of around 25km. In this context, clustering is indicative of the geographic extent to which *Varroa* mites are communicated between colonies of bees, be it through natural contact and exchange during foraging or through the relocation of colonies throughout the season. The nature of mite exchange was not identified in this study.

The distance at which clustering was observed in individual years varied noticeably, ranging from 100km in 2015 to 10km in 2016, 2017, and 2019. Only in 2018 was no spatial clustering observed. This discrepancy might be attributed to small sample sizes or low *Varroa*

case prevalence observed in 2018 (4.2%) compared to the 5-year average case prevalence (13.6%). Without an adequate representation of both cases and controls, in terms of numbers and spatial sampling intensity, there may be a lack of power to detect spatial clustering. When all inspection data were aggregated over the five-year study, effectively increasing the sample size and spatial representation of cases and controls, the D-function provided evidence of spatial clustering upwards of 25km (Figure 3.2). According to the geospatial epidemiologic triad, clustering can be thought of as a representation of agent factors, and the agent's tendency to spread within localized areas, which is common for infectious diseases. *Varroa* mites are communicable between bees through the environment and within yards (Rosenkranz *et al.*, 2010). This passing of mites between colonies is limited by the foraging range of the honey bees, and the number of contacts (with other bees, or colonies) in the vicinity of an infested colony (Rosenkranz *et al.*, 2010), and thus spatial clustering might occur in a semi-localized range, as observed of around 25km. While a single bee may have a limited flight radius of up to 10km (Beekman & Ratnieks, 2000), mites may be passed along a chain of colonies to reach further distances during the year. Furthermore, colonies and equipment may be moved even further distances during the beekeeping season, extending the possible range of transmission.

This finding of spatial clustering of *Varroa* mite infestations offers the basis for a *Varroa* notification system where beekeepers could be notified if elevated mite levels are detected in the immediate vicinity of their colonies (25km). Thus, allowing for more intensive monitoring of their colonies to detect an increase in mite load early, and allow for risks to be mitigated to prevent further spread and colony weakening.

Several high-risk clusters were identified in this study for all years studied. All but 3 of the 10 observed clusters were found to have a SMR with a lower 95% confidence interval of

greater than 2, indicating at least a doubling of the rate of *Varroa* cases than expected. The specific locations of these clusters varied from year to year, but recurring patterns were seen as well. Most notable was the reoccurrence of clusters of cases in the northwestern quadrant of Southern Ontario for 4 of the 5 years studied (Figure 3.3). This grouping of observed clusters covers a large area but provides evidence to suggest that there is temporal stability of *Varroa* clusters in this region. This region possesses one of the higher honey bee colony densities in Ontario (Sobkowich *et al.*, 2021) which could explain the higher than expected rates of *Varroa*, as population density has been suggested to play a role in *Varroa* transmission (Rosenkranz *et al.*, 2010). An increase in regional colony density would inherently result in an increase of susceptible colonies and an increased occurrence of robbing, drifting, and other intra-colony bee interaction events which have all been suggested as viable means of mite transmission (Kulhanek, Garavito, & VanEngelsdorp, 2021; Peck & Seeley, 2019; Peck *et al.*, 2016). However, conflicting to this is the lack of observed clusters in the Niagara peninsula (southeastern most region of Southern Ontario), which possesses the highest colony density in the province (Sobkowich *et al.*, 2021).

No clusters of *Varroa* infested colonies were identified in the Niagara region in the 5 study years, which could provide evidence against the hypothesis linking population density to *Varroa* prevalence. Similarly, the regression analysis showed a mild negative correlation between *Varroa* rate and colony density, which is contrary to what would be expected in support of this hypothesis. However, the colony density values used are based upon self-reports from colony registration and therefore may be representative of stationary colonies or overwinter locations, but not the locations in which colonies spend the majority of the season.

Potential bias might exist since a large proportion of honeybee colonies in Niagara belong to large-scale commercial operations, offering mobile pollination services to other provinces throughout the beekeeping season. Colonies are screened for *Varroa* before being moved for pollination services, and therefore there may be an inflation of low *Varroa* count observations, and a simultaneous overestimation of colony density, as commercial operations treat their colonies before the inspection to ensure a satisfactory result for travel. This is largely, but not always the case. Therefore, the hypothesis of a relationship between *Varroa* prevalence and colony density can not be rejected considering the nature of the current data (i.e., based on registration locations rather than foraging locations of colonies).

Natural land had been hypothesized to increase the odds of varroosis due to transmission of mites from feral colonies (Peck *et al.*, 2016), but was not found to be associated with *Varroa* rate in the regression analysis. Chemurot *et al.* (2016), in Uganda, proposed a relationship between colony placement in farmland and *Varroa* prevalence which was also not observed in the current study. None of the land-use covariates examined in this study showed sufficient evidence of an association with *Varroa* rate, suggesting that other factors have stronger effects on *Varroa* prevalence such as beekeeping management practices, including control measures, abiotic factors that fluctuate over time such as weather, or biotic factors such as mite and bee behaviours. Time-dependent factors, such as temperature or precipitation, were not accounted for in this analysis but may lend themselves well to time-series modelling approaches.

The isopleth map of *Varroa* rates (Figure 3.6) illustrates an overall low rate for Southern Ontario with sporadic high-rate areas throughout the province. Notably, the high-rate area south of Bancroft (Figure 3.6) exists in an area of low sampling as seen in Figure 3.1 and therefore may be an overprediction of the true rate. The North-western quadrant of the study area exhibited

several clusters over the 5-year study period (Figure 3.2) when using varroosis case locations based on the 3-mite threshold. This pattern is similarly illustrated by the isopleth map where the *Varroa* rate is shown to be greater overall compared to the rest of the study area by comparison (Figure 3.6). In the region south of Peterborough, a high rate was estimated by spatial modelling, which contrasts to the findings seen through cluster detection as no cluster of varroosis cases were observed in this area. This contradiction is likely the result of a repeat of high *Varroa* count samples each year, but not multiple high *Varroa* count samples in a single given season. Furthermore, the difference in the data structure used in this study (i.e., binary case and control data used for cluster detection and *Varroa* rates for spatial modelling) could have led to differences in data analysis results. This problem has been termed the modifiable areal unit problem and is a common source of bias in geostatistical studies such as the present work (Waller & Gotway, 2004).

Studies such as this are reliant on large sample sizes, accurately recorded, and serving as a representative sample for the target population. Inspections are not truly random samples and may be biased in some cases towards beekeepers with higher *Varroa* loads or beekeepers better skilled at treating for pests and diseases (i.e., commercial operations). In cases where an inspection is requested by the beekeeper to address recent issues with their colonies, there is a greater likelihood that *Varroa* may be present, as *Varroa* is recognized as a common pest and is responsible for colony weakening and reduced hive activity (Barroso-Arévalo *et al.*, 2019). Whereas in cases that an inspection was requested for the purpose of verifying the disease-free status of colonies to be sold as queens and nuclei, there may be a bias towards lower levels of *Varroa*. Routine apiary inspections also occur and are expected to be more representative of the

true population but are still prone to sampling and measurement bias. The reason for colony inspection was not explored in the present work but should be explored in future studies.

A limitation of this study is inconsistent sampling locations from year to year. To sample Ontario beekeepers representatively, inspections are not guaranteed to occur in the same geographical locations every year. For this reason, one region may be over-sampled one year and under-sampled in the next as inspectors may choose to group inspections by proximity. This limitation was the primary reason for the decision to treat the annual data as a whole during geospatial modelling rather than 5 distinct years. This also suggests that there may be years where a high-risk cluster exists but is not detected, since sufficient repeat sampling did not occur in that region over the year. This could explain the absence of clusters in the Peterborough region despite a high predicted risk through modelling. The supposed grouping of inspections may also explain some of the clusters observed in this study, but the consistency and significance of clusters observed over 5 years suggest that a true effect may be in place. Further studies could address this limitation through the use of a continuous cohort of colonies spread across the study area, rather than the repeated cross-sectional sampling approach used in this study.

With advancements in communications and the low cost and absence of necessary technical tools to sample bees for *Varroa* mites, the collection of these data lends itself well to a citizen science approach (Khayli *et al.*, 2021; Thomas-Bachli, Pearl, Parmley, & Berke, 2020). With the implementation of citizen science and self-reported *Varroa* mite counts by beekeepers, agencies can achieve a greater number of observations per year, and cover a greater spatial area, without the need to increase inspector resources. Furthermore, this approach frees up inspectors to allow for more strategic sampling and respond to inspection requests from operations experiencing difficulties. Skepticism exists around the quality of self-reported data, but evidence

exists to suggest that citizen science approaches can produce data that are equal to or greater than the quality obtained by professionals (dependent on the difficulty of data collection, upon other factors) (Kosmala, Wiggins, Swanson, & Simmons, 2016).

3.6 Conclusion

This study provides evidence for temporally stable clusters of varroosis throughout Southern Ontario, which were not explained sufficiently by the environmental factors considered in this study but suggest that there are environmental (i.e., meteorological) and management influences at play. A spatial clustering effect was also observed, suggestive of the transmission patterns of *Varroa* mites and the influence that neighbouring yards have on each other's mite counts. The results of this study provide a launch point to further assess the spatial patterns of *Varroa* identified. Intervention efforts should focus on areas of Southern Ontario exhibiting clusters of excess *Varroa*, and especially the regions in the northwest, where clusters appear over multiple years. The predicted risk map identifies areas where *Varroa* is likely to exist at elevated levels and therefore highlights the need for more data and investigation to identify the cause of these increased *Varroa* rates. It is recommended that inspections and intervention programs focus their efforts on these areas, while citizen science efforts could provide data elsewhere in the province, resulting in an enhanced province wide *Varroa* surveillance system.

3.7 References

- Agriculture and Agri-Food Canada. (2019). *Statistical Overview of the Canadian Honey and Bee Industry, 2019*.
- Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, 6(4), 562–565. <https://doi.org/10.1098/rsbl.2009.0986>
- Barroso-Arévalo, S., Fernández-Carrión, E., Goyache, J., Molero, F. P., & Sánchez-Vizcaíno, J. M. (2019). High load of deformed wing virus and *Varroa destructor* infestation are related to weakness of honey bee colonies in southern Spain. *Frontiers in Microbiology*. <https://doi.org/https://doi.org/10.3389/fmicb.2019.01331>
- Beekman, M., & Ratnieks, F. L. W. (2000). Long-range foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology*, 14(4), 490–496. <https://doi.org/10.1046/j.1365-2435.2000.00443.x>
- Berke, O. (2004). Exploratory disease mapping: kriging the spatial risk function from regional count data. *International Journal of Health Geographics*, 3, 1–11. <https://doi.org/10.1186/1476-072X-3-18>
- Berke, O. (2005). Exploratory spatial relative risk mapping. *Preventive Veterinary Medicine*, 71(3–4), 173–182. <https://doi.org/10.1016/j.prevetmed.2005.07.003>
- Canadian Association of Professional Apiculturalists. (n.d.). Annual colony loss reports. Retrieved December 4, 2020, from <https://capabees.com/capa-statement-on-honey-bees/>
- Carrat, F., & Valleron, A. J. (1992). Epidemiologic mapping using the “kriging” method: Application to an influenza-like epidemic in France. *American Journal of Epidemiology*, 135(11), 1293–1300. <https://doi.org/10.1093/oxfordjournals.aje.a116236>
- Chemurot, M., Akol, A. M., Masembe, C., de Smet, L., Descamps, T., & de Graaf, D. C. (2016). Factors influencing the prevalence and infestation levels of *Varroa destructor* in honeybee colonies in two highland agro-ecological zones of Uganda. *Experimental and Applied Acarology*, 68, 497–508. <https://doi.org/DOI 10.1007/s10493-016-0013-x>
- Currie, R. W., Pernal, S. F., & Guzmán-Novoa, E. (2015). Honey bee colony losses in Canada. *Journal of Apicultural Research*, 49(1), 104–106. <https://doi.org/https://doi.org/10.3896/IBRA.1.49.1.18>
- Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., ... Ellis, J. D. (2013). Standard methods for varroa research. *Journal of Apicultural Research*, 52(1), 1–54. <https://doi.org/10.3896/IBRA.1.52.1.09>
- Diggle, P. J., & Chetwynd, A. G. (1991). Second-order analysis of spatial clustering for inhomogeneous populations. *Biometrics*, 47, 1155–1163.
- Dolezal, A. G., Carrillo-Tripp, J., Miller, W. A., Bonning, B. C., & Toth, A. L. (2016). Intensively cultivated landscape and *Varroa* mite infestation are associated with reduced honey bee nutritional state. *PLOS ONE*, 11(4), e0153531. <https://doi.org/10.1371/journal.pone.0153531>

- Giovenazzo, P., & Dubreuil, P. (2011). Evaluation of spring organic treatments against *Varroa destructor* (Acari: Varroidae) in honey bee *Apis mellifera* (Hymenoptera: Apidae) colonies in eastern Canada. *Experimental and Applied Acarology*, 55(1), 65–76.
<https://doi.org/10.1007/s10493-011-9447-3>
- Government of Canada. (2020). Annual crop inventory. Retrieved October 29, 2020, from
<https://open.canada.ca/data/en/dataset/ba2645d5-4458-414d-b196-6303ac06c1c9>
- Gräler, B., & Pebesma, E. J. (2016). Spatio-temporal interpolation using gstat. *The R Journal*, 8(1), 204–218.
- Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443–450. <https://doi.org/10.1051/apido/2009076>
- Haber, A. I., Steinhauer, N. A., & VanEngelsdorp, D. (2019). Use of chemical and nonchemical methods for the control of *Varroa destructor* (Acari: Varroidae) and associated winter colony losses in U.S. beekeeping operations. *Journal of Economic Entomology*, 112(4), 1509–1525. <https://doi.org/https://doi.org/10.1093/jee/toz088>
- Hengl, T., Heuvelink, G. B. M., & Rossiter, D. G. (2007). About regression-kriging: From equations to case studies. *Computers & Geosciences*, 33(10), 1301–1315.
<https://doi.org/10.1016/j.cageo.2007.05.001>
- Khayli, M., Lhor, Y., Bengoumi, M., Zro, K., El Harrak, M., Bakkouri, A., ... Bouslikhane, M. (2021). Using geostatistics to better understand the epidemiology of animal rabies in Morocco: what is the contribution of the predictive value? *Helijon*, 7(1), e06019.
<https://doi.org/10.1016/j.heliyon.2021.e06019>
- Kosmala, M., Wiggins, A., Swanson, A., & Simmons, B. (2016). Assessing data quality in citizen science. *Frontiers in Ecology and the Environment*, 14(10), 551–560.
<https://doi.org/10.1002/fee.1436>
- Kozak, P., Eccles, L., Kempers, M., Rawn, D., Lacey, B., & Guzmán-Novoa, E. (2021). Ontario treatment recommendations for honey bee disease and mite control. Retrieved June 24, 2021, from <http://www.omafra.gov.on.ca/english/food/inspection/bees/2017-treatment.htm#VM>
- Kulhanek, K., Garavito, A., & VanEngelsdorp, D. (2021). Accelerated *Varroa destructor* population growth in honey bee (*Apis mellifera*) colonies is associated with visitation from non-natal bees. *Scientific Reports*, 11(1), 7092. <https://doi.org/10.1038/s41598-021-86558-8>
- Kulldorff, M. (1997). A spatial scan statistic. *Communications in Statistics: Theory and Methods*, 26, 1481–1496.
- Kulldorff, M., & Information Management Services Inc. (2009). SatScan v8.0: Software for the spatial and space-time scan statistics. Retrieved from <http://www.satscan.org/>
- Ma, Y., Yin, F., Zhang, T., Zhou, X. A., & Li, X. (2016). Selection of the maximum spatial cluster size of the spatial scan statistic by using the maximum clustering set-proportion

- statistic. *PLOS ONE*, 11(1), e0147918. <https://doi.org/10.1371/journal.pone.0147918>
- Matthews, R., Wasserstein, R., & Spiegelhalter, D. (2017). The ASA's p -value statement, one year on. *Significance*, 14(2), 38–41. <https://doi.org/10.1111/j.1740-9713.2017.01021.x>
- McElheran, B. (1990). *National Varroa Survey*. Winnipeg, Manitoba, Canada: Canadian Association of Professional Apiculturists.
- Pebesma, E. J. (2004). Multivariate geostatistics in R: the gstat package. *Computers & Geosciences*, 30, 683–691.
- Peck, D. T., & Seeley, T. D. (2019). Mite bombs or robber lures? The roles of drifting and robbing in *Varroa destructor* transmission from collapsing honey bee colonies to their neighbors. *PLoS ONE*, 14(6), 1–14. <https://doi.org/10.1371/journal.pone.0218392>
- Peck, D. T., Smith, M. L., & Seeley, T. D. (2016). *Varroa destructor* mites can nimbly climb from flowers onto foraging honey bees. *PLoS ONE*, 11(12), e0167798. <https://doi.org/https://doi.org/10.1371/journal.pone.0167798>
- Pollinator Partnership Canada. (2017). *Planting forage for honey bees in Canada: a guide for farmers, land managers, and gardeners*. Retrieved from <https://honeycouncil.ca/wp-content/uploads/2017/06/Planting-Guide-FINAL-ISBN-June-2017-for-Web-English.pdf>
- QGIS Development Team. (2020). *QGIS Geographic Information System*. Open Source Geospatial Foundation Project.
- R Core Team. (2020). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.r-project.org/>
- Rawn, D., Guzmán-Novoa, E., Chaput, J., Eccles, L., Morfin, N., Kozak, P., ... Pasma, T. (2019). *Surveillance on resistant Varroa destructor mite population to three synthetic acaricides in Ontario*. Retrieved from <https://www.oahn.ca/wp-content/uploads/2019/07/OAHN-Varroa-resistance-report-19-07-16.pdf>
- Roberts, J. M. K., Anderson, D. L., & Durr, P. A. (2017). Absence of deformed wing virus and *Varroa destructor* in Australia provides unique perspectives on honeybee viral landscapes and colony losses. *Scientific Reports*, 7(6925). <https://doi.org/https://doi.org/10.1038/s41598-017-07290-w>
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103(SUPPL. 1), S96–S119. <https://doi.org/10.1016/j.jip.2009.07.016>
- Sobkowich, K. E., Berke, O., Bernardo, T. M., Pearl, D. L., & Kozak, P. (2021). Mapping the population density of managed honey bee (*Apis mellifera*) colonies in Ontario, Canada: 2018. *Journal of Apicultural Science*, 65(2), 303–314. <https://doi.org/10.2478/jas-2021-0023>
- Sponsler, D. B., & Johnson, R. M. (2015). Honey bee success predicted by landscape composition in Ohio, USA. *PeerJ*, 3, e838. <https://doi.org/10.7717/peerj.838>
- Statistics Canada. (2018). Census consolidated subdivisions (CCS). Retrieved December 7,

2021, from <https://www150.statcan.gc.ca/n1/pub/92-195-x/2011001/geo/ccs-sru/ccs-sru-eng.htm>

- Stevenson, M. A., Benard, H., Bolger, P., & Morris, R. S. (2005). Spatial epidemiology of the Asian honey bee mite (*Varroa destructor*) in the North Island of New Zealand. *Preventive Veterinary Medicine*, 71(3–4), 241–252. <https://doi.org/10.1016/j.prevetmed.2005.07.007>
- Stevenson, M. A., & Sergeant, E. (2022). epiR: Tools for the Analysis of Epidemiological Data. R package version 2.0.48. <https://CRAN.R-project.org/package=epiR>
- Tarwater, P. M., & Martin, C. F. (2001). Effects of population density on the spread of disease. *Complexity*, 6(6), 29–36. <https://doi.org/10.1002/cplx.10003>
- Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2004). Prevalence and seasonal variations of six bee viruses in *Apis mellifera L.* and *Varroa destructor* mite populations in France. *Applied and Environmental Microbiology*, 70(12), 7185–7191. <https://doi.org/10.1128/AEM.70.12.7185-7191.2004>
- Thomas-Bachli, A. L., Pearl, D. L., Parmley, E. J., & Berke, O. (2020). The influence of sociodemographic factors on the engagement of citizens in the detection of dead corvids during the emergence of West Nile virus in Ontario, Canada. *Frontiers in Veterinary Science*, 6. <https://doi.org/10.3389/fvets.2019.00483>
- Van Der Zee, R., Gray, A., Pisa, L., & De Rijk, T. (2015). An observational study of honey bee colony winter losses and their association with *Varroa destructor*, neonicotinoids and other risk factors. *PLoS ONE*, 10(7), e0131611. <https://doi.org/https://doi.org/10.1371/journal.pone.0131611>
- Vandenbroucke, J. P. (1982). A shortcut method for calculating the 95 per cent confidence interval of the standardized mortality ratio. *American Journal of Epidemiology*, 115(2), 303–304. <https://doi.org/10.1093/oxfordjournals.aje.a113306>
- Vidal-Naquet, N. (2018). *Honeybee Veterinary Medicine*: Apis mellifera L. Sheffield, UK: 5m Publishing.
- Visscher, P. K., & Seeley, T. D. (1982). Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology*, 63(6), 1790. <https://doi.org/10.2307/1940121>
- Waller, L. A., & Gotway, C. A. (2004). *Applied Spatial Statistics for Public Health Data*. Hoboken, NJ, USA: John Wiley & Sons Inc

3.8 Tables

Table 3.1: Descriptive statistics of the dependent and independent variables.

Variable	Obs. ⁱ	Mean	Std. Dev.	Min.	Max.
Dependent					
Varroa Rate	1082	0.863	2.65	0	51
Independent					
Regional Colony Density	1,370,880	2.17	2.82	0.01	14.7
Developed Land (%)	1,370,880	0.09	0.12	0.01	0.95
Forageable Land (%)	1,370,880	0.35	0.13	0.002	0.76
Non-Forageable Land (%)	1,370,880	0.23	0.17	0	0.84
Natural Land (%)	1,370,880	0.3	0.18	0.01	0.98
Water Coverage (%)	1,370,880	0.02	0.07	0.03	0.69

ⁱ independent variable observation counts represent the grid resolution of the raster.

Table 3.2: Results from spatial scan analysis for clusters of high-risk of *Varroa* cases in Southern Ontario (2015-2019).

Year	Cluster	High-Risk Clusters				
		Standardized			SMR (95% CI)	p-value
		Radius (km)	morbidity ratio			
2015	1	(773.9, -587.9)	71.80	2.25	(1.75, 2.81)	0.001
	2	(338.1, -806.7)	28.80	3.95	(2.41, 5.86)	0.001
	3	(402.1, -918.5)	2.20	4.58	(1.95, 8.29)	0.018
2016	1	(370.1, -852.1)	16.89	12.19	(3.85, 25.23)	0.002
	2	(730.0, -698.7)	33.09	4.33	(2.15, 7.27)	0.019
2017	1	(326.3, -771.3)	13.61	7.14	(3.67, 11.76)	0.001
	2	(401.0, -757.1)	50.50	2.57	(1.52, 3.90)	0.046
2018	1	(429.3, -792.9)	48.96	9.58	(5.07, 15.46)	0.001
2019	1	(315.8, -920.2)	11.27	6.28	(3.77, 9.41)	0.001
	2	(675.7, -775.1)	48.19	4.38	(2.45, 6.89)	0.001

3.9 Figures

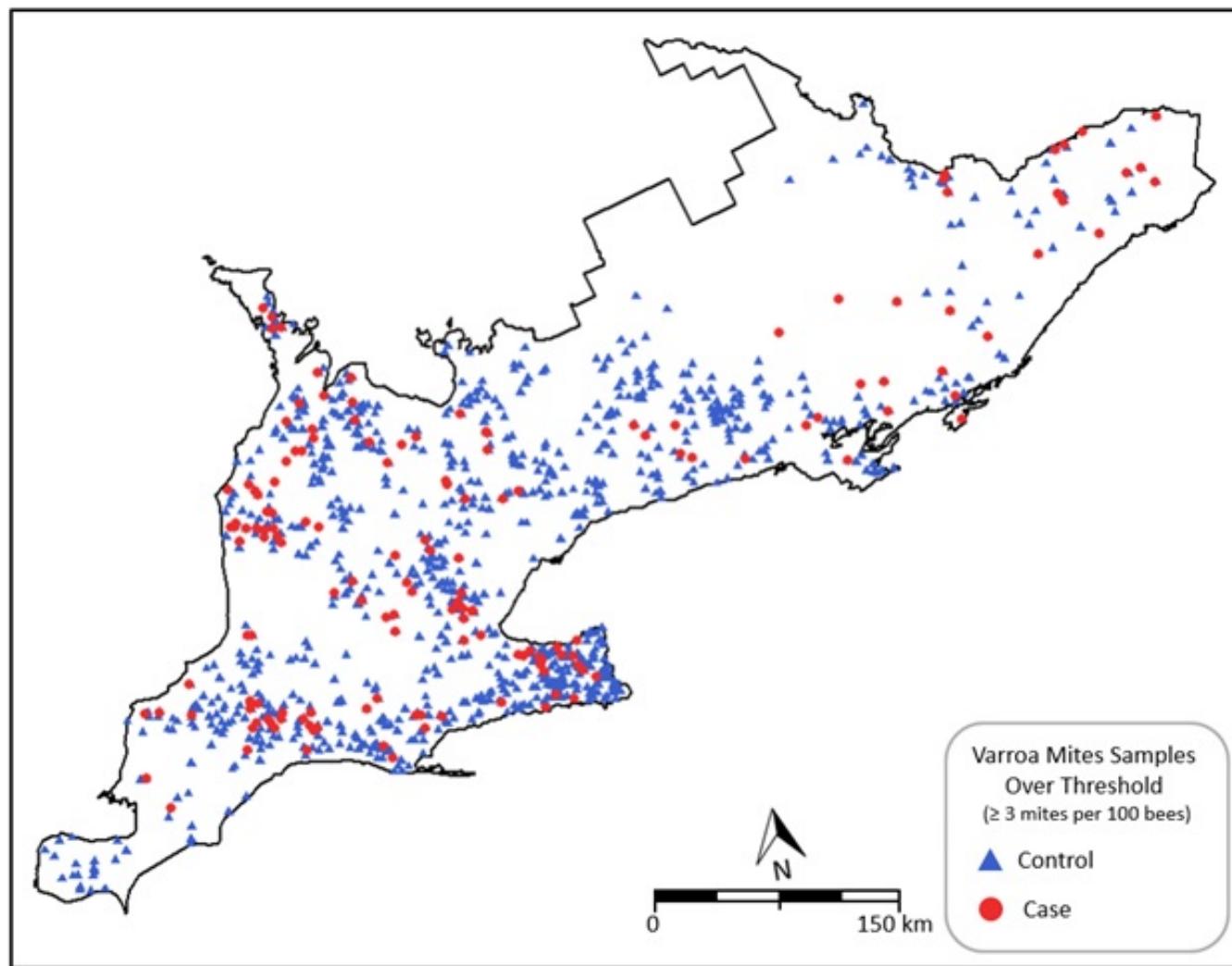


Figure 3.1: Point map of Southern Ontario indicating *Varroa destructor* counts above threshold (≥ 3 mites per 100 bees) as cases in red circles and controls in blue triangles, 2015 - 2019.

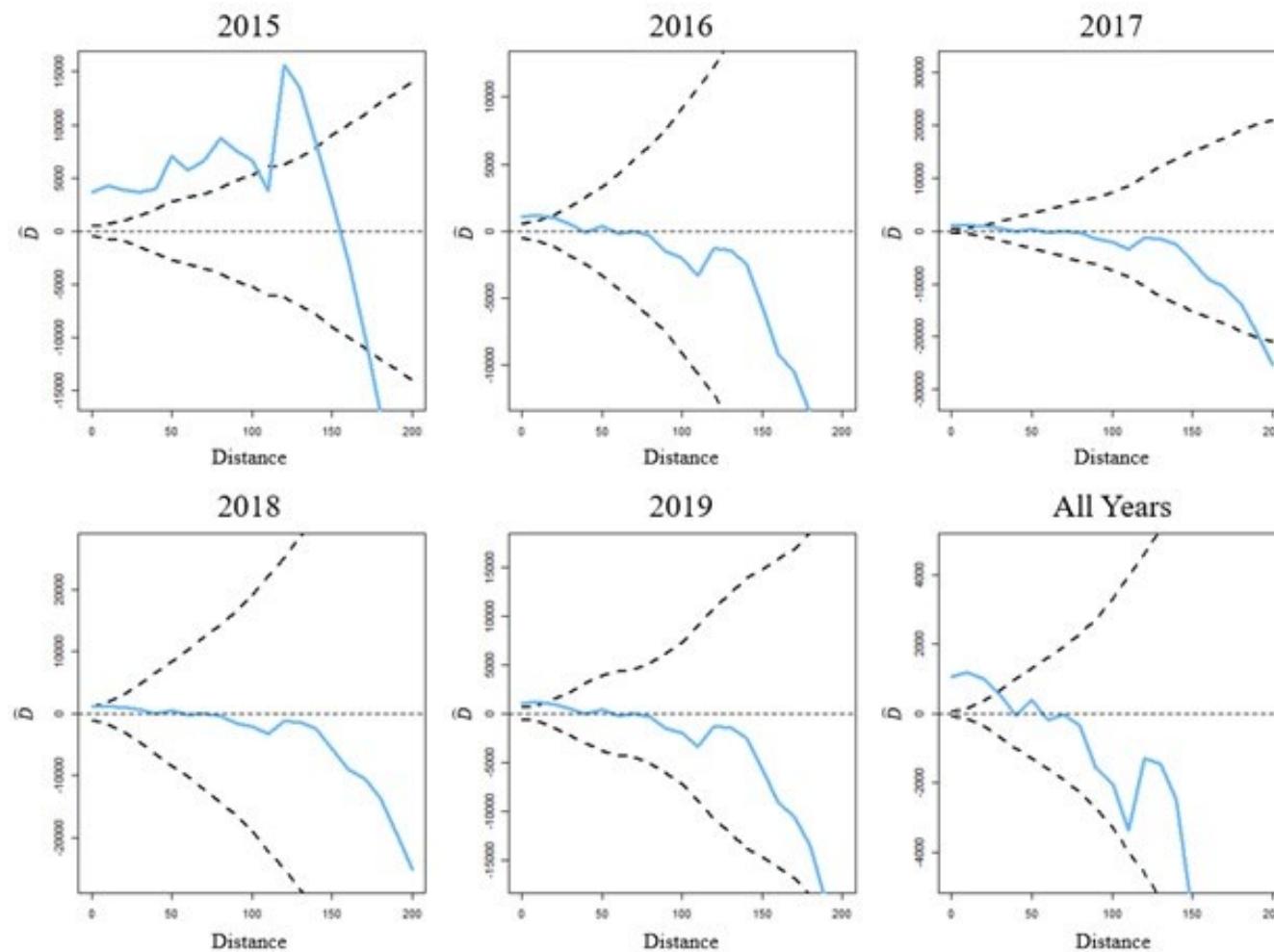


Figure 3.2: D-Functions for inspected colony locations in Southern Ontario with *Varroa* counts exceeding 3 mites per 100 bees for each of the study years and the 5-year study period combined.

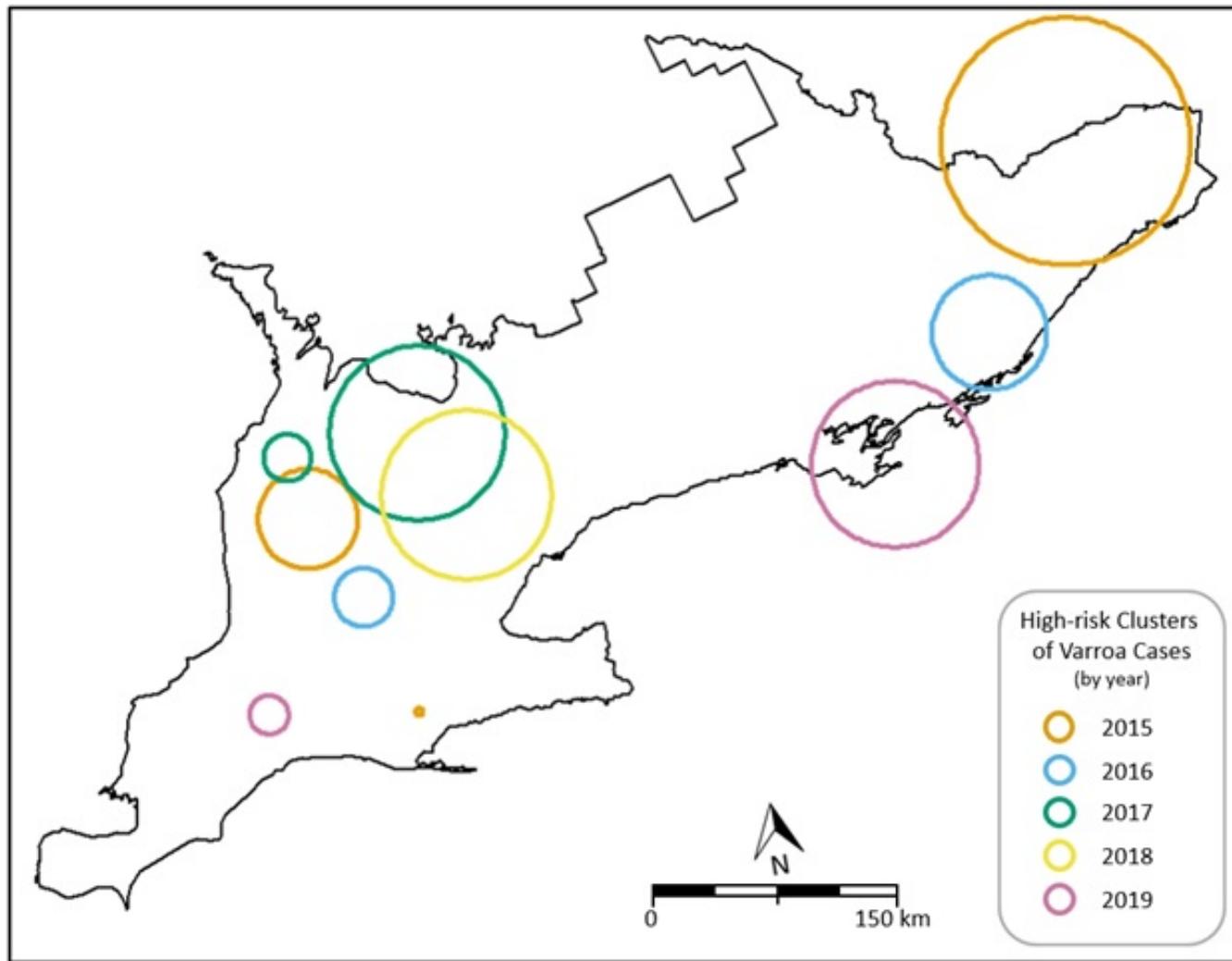


Figure 3.3: Map of the detected high-risk clusters of *Varroa* cases by year in Southern Ontario using the spatial scan statistic.

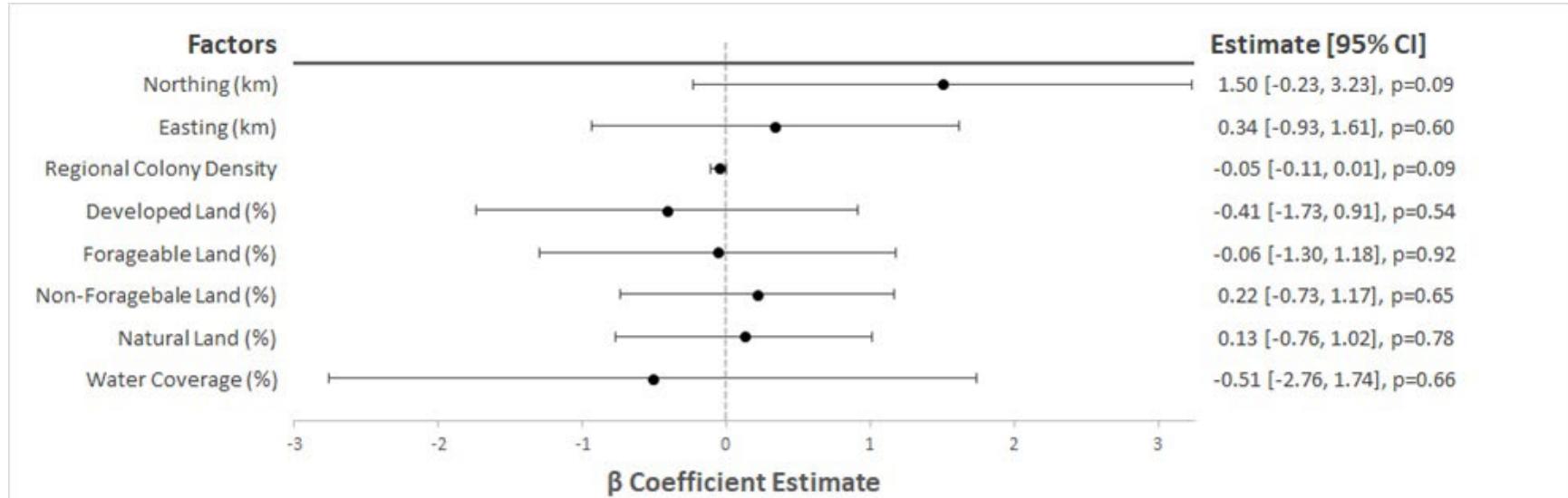


Figure 3.4: Forest plot of estimated beta coefficients from preliminary simple linear modelling of *Varroa* rate in Southern Ontario managed honey bee colonies (2015-2019).

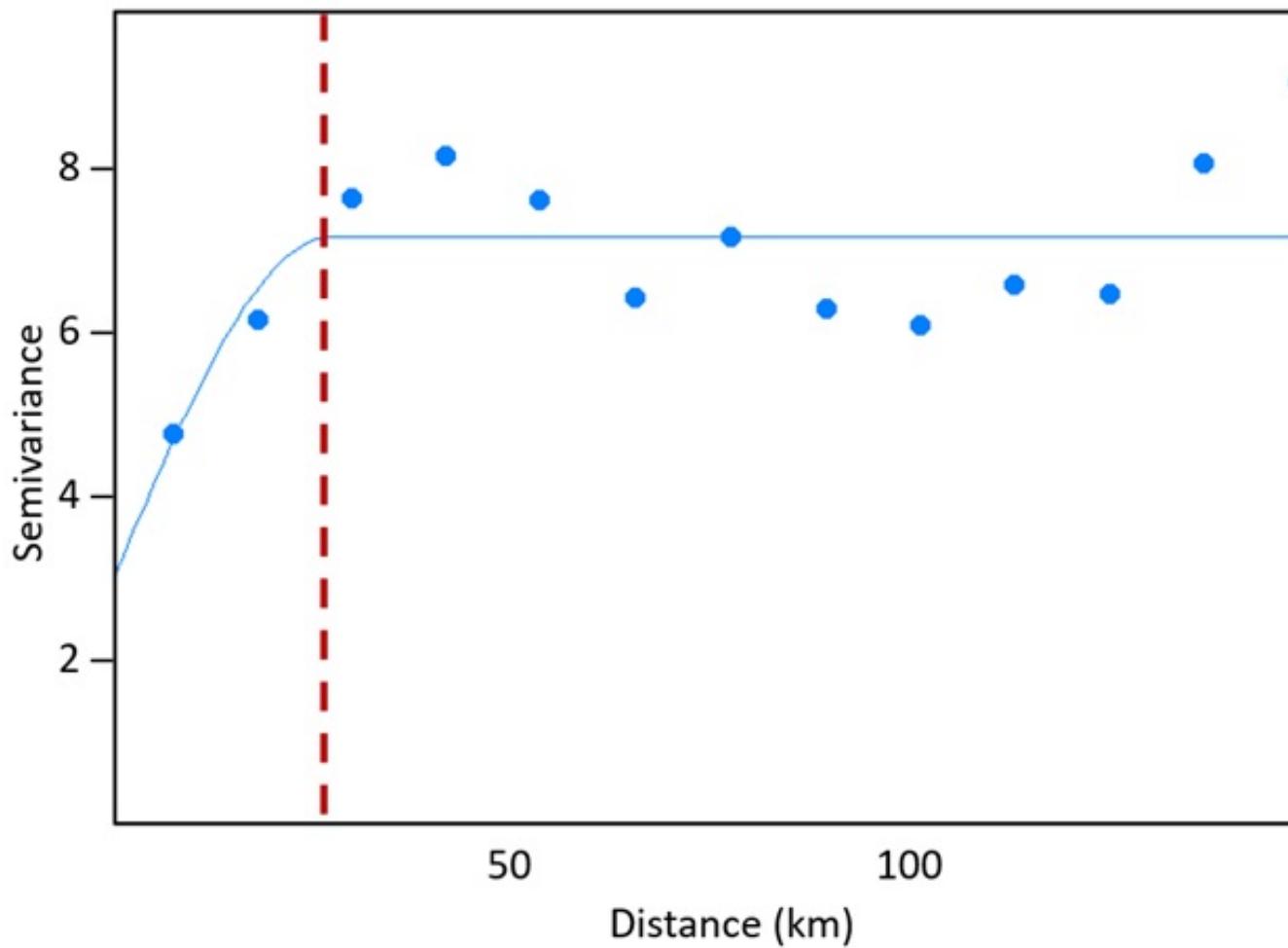


Figure 3.5: Variogram of generalized linear model residuals (points), with spherical variogram model (solid blue line) and spatial correlation range (red dashed line) for sampled rates of *Varroa* mites in managed honey bee colonies in Southern Ontario (2015-2019).

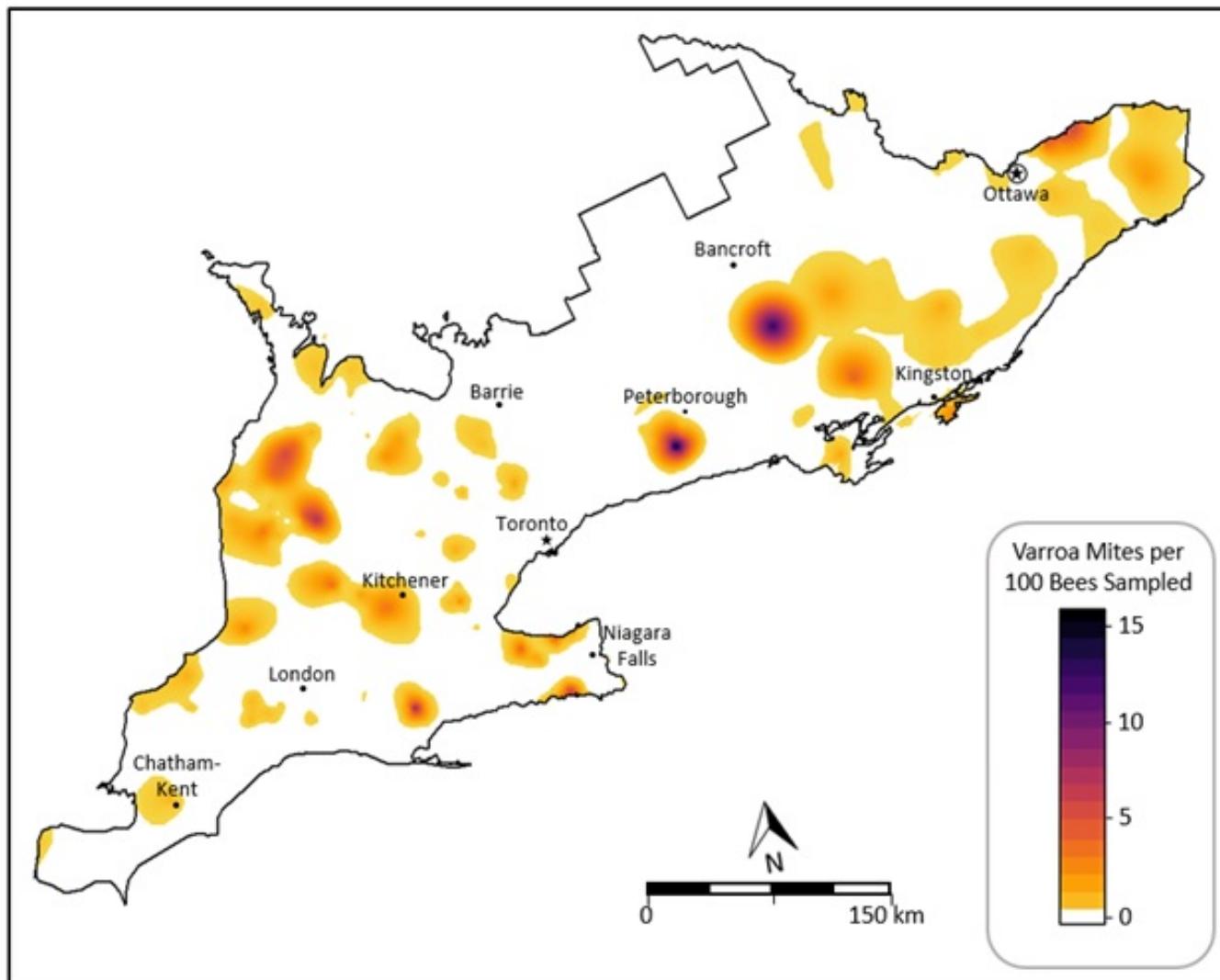


Figure 3.6: Isopleth map of predicted *Varroa* rates for Southern Ontario derived from spatial regression modelling.

4 CHAPTER FOUR: EPIDEMIOLOGIC TIME SERIES ANALYSIS OF *VARROA DESTRUCTOR* INFESTATIONS IN RELATION TO WEATHER ACROSS ONTARIO HONEY BEE COLONIES: 2015-2019

4.1 Abstract

The *Varroa destructor* mite is the most important risk factor for honey bee colony health in Canada, having been repeatedly linked to elevated levels of colony loss and poor colony health. Therefore, it is in the interest of beekeepers, farmers, and consumers to focus on mitigating the impacts of *Varroa* to prevent disruptions in food production. Understanding spatial and temporal patterns and trends is a necessary but understudied topic for the development and implementation of an effective *Varroa* integrated pest management system, in Ontario, Canada. This study assesses temporal patterns of *Varroa* mite prevalence over a 5-year study period (2015-2019) to complement recent findings of spatial patterns of mites in Ontario (Chapter 3). Using time-series decomposition and Generalized Linear Autoregressive Moving Average (GLARMA) modeling, seasonal patterns and long-term trends of mite counts in managed bee colonies were identified and found to be associated with ambient temperature and dew point at a 7-week lag interval. This study found counts of *Varroa* mites in colonies to follow a predictable seasonal pattern with a marginally decreasing trend over the five years studied. These patterns were not fully explained by the weather factors considered (average temperature, maximum temperature, minimum temperature, dew point, humidity, and precipitation) indicating that additional factors might have been at play. Lagged and instantaneous effects of the weather variables were considered. These results offer an epidemiologic perspective of *Varroa* mite infestations over time and can benefit surveillance efforts by providing a comparative basis for expected mite loads in future seasons.

4.2 Introduction

Approximately one-third of global crops rely directly on pollinators (Klein *et al.*, 2007), and the European honeybee (*Apis mellifera*) contributes the greatest pollination impact from an economic perspective (Johnson, 2010). In Canada alone, the economic contribution of honey bees is upwards of \$5 billion (CAD), with over \$2.5 billion (CAD) stemming from additional crop production due to pollination services (Agriculture and Agri-Food Canada, 2020). High annual overwinter colony losses of around 25% or more (Ferland *et al.*, 2021) present an issue for the beekeeping industry as well as national and global food production.

The *Varroa* mite (*Varroa destructor*), is acknowledged as the most significant parasite and veterinary concern to bees (Boecking & Genersch, 2008; Guzmán-Novoa *et al.*, 2010; Vidal-Naquet, 2018), and is a considerable factor in western honey bee colony loss. These mites are the greatest contributor to colony damage and economic cost compared to all other apicultural diseases (Boecking & Genersch, 2008). *Varroa* mites have spread worldwide and are endemically present in all colonies, with few regional exceptions (Vidal-Naquet, 2018). Left untreated, *Varroa* mite populations can severely weaken and decimate a colony, while simultaneously spreading to nearby uninfected host colonies (Peck & Seeley, 2019; Stevenson, Benard, Bolger, & Morris, 2005; Vidal-Naquet, 2018). *Varroa* mites are fully dependent on the brood of honey bees for reproduction and the movements of adult workers for inter-colony transmission (Rosenkranz, Aumeier, & Ziegelmann, 2010), and are therefore influenced by both the behaviours of the colonies they infest and the environment the bees occupy (including surrounding colonies and their behaviours).

Varroa mite reproduction is a climate-sensitive process within the hive and will not proceed outside of a specific temperature range of 31-37°C (Vidal-Naquet, 2018), which is slightly more temperature tolerant than the optimal range for bee development (33-36°C) (Petz, Stabentheiner, & Crailsheim, 2004). Furthermore, at an internal colony humidity of greater than 80%, *Varroa* reproduction is not possible (Vidal-Naquet, 2018). *Varroa* mites are also indirectly affected by hive temperature and humidity since these factors can alter honey bee cleaning behaviours (Tahmasbi, 2009). Honey bees are particularly good at maintaining inter-colony temperature homeostasis both in the winter, through shivering (Stabentheiner, Pressl, Papst, Hrassnigg, & Crailsheim, 2003), and summer, through evaporative cooling (Lindauer, 1955), and have likewise been shown to have success in regulating the humidity, but this has not been as extensively studied (Human, Nicolson, & Dietemann, 2006). External temperature has not been demonstrated to substantially impact the internal hive temperature, with the exception of extreme temperatures (Corkins, 1932; Simpson, 1961), but may impact *Varroa* populations indirectly due to behaviours of the host bees. Honey bee behaviours including foraging, mating, and swarming have been linked to weather variables (Abou-Shaara, Owayss, Ibrahim, & Basuny, 2017), and may have indirect implications on *Varroa* movement and spread among bee populations. Too much or too little rainfall may also impact *Varroa* mite spread due to changes in the foraging behaviour of honey bees (Akala, Makindi, & Esilaba, 2018) or increased instances of robbing in cases of reduced nectar availability under drought conditions (Winston, 1987). Previous modelling of honey bee foraging activity based on weather factors provided evidence of a correlation with atmospheric pressure, temperature, solar radiation and humidity (Clarke & Robert, 2018). Therefore, weather is believed to impact inter-colony mite transmission due to modulation of bee activities external to the hive. Robbing, drifting, and foraging are all viable

routes of mite transmission (Peck & Seeley, 2019; Peck, Smith, & Seeley, 2016), and may be influenced by the weather.

Temporal dynamics of *Varroa* mite populations during the beekeeping season follow a predictable pattern of exponential mite growth (Noireterre, 2011; OIE: World Organisation for Animal Health, 2021), wherein mite populations double every 20 days when no intervention is administered, and the proliferation of mites is greater with higher initial mite counts at the beginning of the season (Calderón, van Veen, Sommeijer, & Sanchez, 2010; DeGrandi-Hoffman & Curry, 2004; Vidal-Naquet, 2018). In the absence of treatment, mite population growth in colonies will surpass a critical threshold within 5 years and the colony will collapse (Vidal-Naquet, 2018). Because the eradication of *Varroa* is not deemed possible, integrated pest management strategies use a multidisciplinary approach to keep *Varroa* mites below a threshold value to mitigate instances of weakened colonies and colony loss. In Ontario, treatment against *Varroa* is typically administered in June, August and early fall, but is recommended whenever infestation exceeds 2% (i.e., 2 mites per 100 bees) in spring or 3% in summer or fall (Kozak *et al.*, 2021). To aid in the development and implantation of an effective integrated pest management strategy, the general population dynamics of *Varroa* should be studied, specific to the region where the program is being implemented. Furthermore, potential risk factors for *Varroa* mite population growth should be identified to inform surveillance and intervention strategies. Recognizing if particular weather factors correlate with mite infestation intensity can allow for predictive modelling and preparation for outbreaks.

To understand the impact of *Varroa* mites on Ontario honey bee populations, it is important to identify spatial and temporal trends of infestation intensity, the current status, as well as the expected long and short-term trajectories. Previously, *Chapter 3* highlighted the

geographic distribution of *Varroa* in Southern Ontario and identified several clusters and high-risk areas for infestations. However, an equivalent study identifying temporal patterns of *Varroa* infestations has not been reported.

Varroa is known to be a multi-factorial problem and is influenced by many environmental factors (including weather), as well as management and biological factors of the honey bees (Chemurot *et al.*, 2016; Correia-Oliveira *et al.*, 2018; Rosenkranz *et al.*, 2010). The goal of this study is to explore the status of *Varroa* in Ontario over time and identify whether meteorological conditions explain temporal variation and patterns in *Varroa* infestations. The objectives of this study were to: (1) identify temporal patterns of *Varroa* infestations in Ontario over a five-year study period; (2) explore possible correlations between immediate and lagged weather variables (including temperature, humidity, precipitation, atmospheric pressure, and dew point); and (3) develop a time-series model to explain the variations in *Varroa* infestation intensities over time, considering weather variables (lagged and instantaneous) as covariates.

4.3 Materials and Methods

The data for this study were collected by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) over five years between 2015 and 2019 via apiary inspections. Included for each data point is a count of observed *Varroa* mites in the colony, and the date of inspection. All *Varroa* counts were collected using the standard alcohol wash method on a sample of approximately 300 bees per colony (OMAFRA, 2021b). The reason for these apiary inspections is a combination of routine and beekeeper requested colony health check-ups. Beekeeper requested inspections may be to address undiagnosed colony health issues, or to

verify suitability for travel or for the sale of queens and nucleus colonies. All inspections in this dataset were performed by trained professionals.

Historical weather data from 2015 to 2019 were obtained from the Government of Canada open data repository (“Historical Data,” 2021). The weather station at Pearson International Airport in Toronto was selected as a representative source of weather data for the entire dataset for two main reasons: the data is expected to be complete and accurate because of its use for international passenger and commercial air traffic control, and the geographic location of Toronto is central to the majority of the colonies registered in Ontario (Sobkowich *et al.*, 2021a) as well as representative of the average location of *Varroa* inspections used in this study (calculated by the mean latitude and longitude values). Average daily temperature, maximum daily temperature, minimum daily temperature, average daily dew point temperature, average daily relative humidity, average daily atmospheric pressure, and total daily volume of precipitation were downloaded and merged by date to the *Varroa* inspection data (“Historical Data,” 2021). The combined data (i.e., *Varroa* counts and the weather data) were then aggregated by week. Weekly aggregates resulted from totalling the counts of *Varroa*, number of inspections and volume of precipitation, while averaging the remaining weather variables by mean. In instances of unexplainable outliers in the *Varroa* count observations, Winsorizing to the 98th percentile was applied.

Varroa mite inspections were not conducted during the overwintering period of the beekeeping season and therefore the data do not contain *Varroa* counts for roughly 17 weeks of the year. To address this missing data issue, the dataset was converted to a standard 35-week year format, excluding the overwintering period. The active beekeeping season for this study was defined as the period between March 15 and November 15, based on the average first and last

observations from each year. The total *Varroa* counts per week were divided by the total number of inspections (colonies) occurring in the same week to produce a standardized metric across weeks of varying numbers of inspections. Henceforth, this standardized value will be referred to as the *Varroa* rate, whereas the undivided total number of *Varroa* mites observed in each week will be referred to as the *Varroa* count.

Seasonal and Trend Decomposition using Loess (STL) was applied to the weekly *Varroa* rate data using robust fitting and season and trend windows of 35 and 105 weeks respectively. STL allows for the dissection of time series data into its component parts of trend, seasonality, and random noise (Cleveland, Cleveland, McRae, & Terpenning, 1990). This exploratory procedure allows for the identification of patterns in the data before more complex model building, using the remainder to provide insight into the remaining variation in the data that may be explainable by exogenous factors.

It is hypothesized that the *Varroa* counts are influenced by weather factors, although the effect may not be instantaneous and may instead be lagged in time. To assess the potential for a lagged correlation between the weather covariates and the observed *Varroa* counts, a Vector Autoregressive (VAR) process was used to select the lag at which the greatest correlation exists for each covariate with the *Varroa* counts over the five-year study. The ‘VARselect’ function in the *vars* package of *R* was used to estimate the final prediction error (FPE) and information criteria (Akaike information criterium (AIC)) for each weather variable at sequentially increasing lags, using an ordinary least squares process and identifying the lag at which these information criteria are optimized in correlation with *Varroa* counts (Pfaff, 2008). Each weather variable was subjected to lag selection independently using a maximum lag of 8 weeks (allowing for several mite and bee reproduction cycles). The optimal lag order was selected using the AIC by

application of the ‘VARselect’ function in *R* (Pfaff, 2008; R Core Team, 2020). AIC and FPE are considered to be superior to other criteria when using smaller sample sizes (Liew, 2004). To address the overwintering period, the VAR lag selection between the weekly *Varroa* count (outcome) and the weekly weather measurements (covariates) was conducted before the exclusion of the overwintering weeks. Then each variable was lagged by the identified number of weeks before the removal of the winter period. This was done to maintain the lagged relationship between the covariates and *Varroa* counts in the early weeks of the beekeeping season which would otherwise have been lost if the winter weeks were removed before lag selection.

To further assess the plausibility of an association between the selected lagged variables and the *Varroa* counts, a Granger predictive causality test (Granger, 1969) was applied to examine the usefulness of each lagged covariate to predict *Varroa* counts. The Granger Causality Test is borrowed from economics and is used to determine whether lagged values of one variable can predict later values of another variable, in this case, weather covariates predicting *Varroa* counts. A variable is said to “Granger cause” another if it shows a significant predictive ability to the other. Granger causality, unlike causality, is a misnomer and does not imply a true causal relationship between the two variables, but rather a metric of correlation. The Granger test was conducted using the “grangertest” function in *R* (Zeileis & Hothorn, 2015). A p-value from each Granger test was reported to provide statistical context of the likelihood of a Granger causality relationship.

To assess the correlation of the weather variables with the *Varroa* counts with no lag introduced, a Pearson correlation test was applied to estimate the relationship between the

Varroa and weather variables independently. P-values estimated from the Pearson correlation tests were used as an exploratory tool (Matthews, Wasserstein, & Spiegelhalter, 2017).

To model the *Varroa* count time series data a negative binomial generalized linear autoregressive moving average (GLARMA) model was used (Davis, Dunsmuir, & Wang, 1999). Plots of the autocorrelation function (ACF) and partial autocorrelation function (PACF) were used to determine the initial autoregressive (AR) and moving average (MA) orders of the model by examination of residual autocorrelation. The GLARMA model was fit to the total *Varroa* counts as the dependent variable using the log number of colony inspections per week as an offset. To introduce the independent weather covariates, a forward model building process was used. Both lagged and instantaneous weather variables were considered in the model building process. Interaction terms between variables were also considered in the model. Forward model selection using the AIC was employed to find the best fitting GLARMA model. The use of a time-series model, as opposed to a simple regression model, allows for the inclusion of intrinsic autocorrelation of *Varroa* counts over time as well as the seasonal and long-term trends in the model in addition to explanatory covariates. A GLARMA model was selected to accommodate *Varroa* count data following a negative binomial distribution but does not allow for observation clustering.

ACF and PACF plots were used to determine if autocorrelation still existed in the model residuals. Residuals were also plotted over time and to a QQ-plot to assess homoskedasticity. To assess the presence of over-dispersion, the probability integral transformation (PIT) residual histogram (Czado, Gneiting, & Held, 2009) was examined. A likelihood ratio test was used to compare the likelihood of the fitted GLARMA model to the likelihood of a GLM model with the same structure.

4.4 Results

A total of 13,170 inspections were included in the OMAFRA apairy inspection dataset. These comprised 733 unique colony inspection dates over the five-year study period between 2015 and 2019. The number of inspections recorded from 2015 to 2019 were 3493, 3502, 2396, 2118, and 1661 respectively over 146, 154, 164, 137, and 132 unique inspection dates. The geographic centroid of the observation locations, found by averaging the latitude and longitude, is at Brampton, Ontario (43.768, -79.778), located east of Toronto and within the Greater Toronto Area, 16km from Toronto's Pearson International Airport weather station. Minimum, maximum and median distances of observations from Toronto's Person International Airport were 5.63km, 1305.5km, and 130.4km respectively. Following data cleaning and aggregation of daily observations to weeks, 175 (35 per year) unique records of weekly *Varroa* counts and weather data existed, excluding the winter season, during which no inspections occurred. The week of March 26, 2015, and the week of November 5, 2015, possessed average *Varroa* counts of 37.5 mites per 100 bees and 52 mites per 100 bees, respectively, and were Winsorized to the 98th percentile of 16.2 mites per 100 bees. The weekly *Varroa* rates followed a positively skewed distribution with a mean of 3.71 and 25% and 75% quartiles of 0 and 1.72 respectively.

STL decomposition provided evidence of a regular annual seasonal pattern described by an initial spike in *Varroa* counts in the early spring inspections, followed by a sudden reduction and gradual increase to a second, larger peak in the late fall. The trend component showed a slight downwards tendency until 2019 when a slight positive rebound was observed (Figure 4.1). The remainder indicates evidence that time series decomposition was completed successfully. The range of the remainder component suggests that the seasonal and trend components describe

a considerable amount of the variation in the data but do not fully explain the weekly *Varroa* rates (mean = 0.49, σ = 3.52) (Figure 4.1). The positive mean of the remainder indicates a slight bias towards underestimation in the decomposition. Plotting the signal (trend and season components) derived from STL decomposition atop the original time series shows an appreciable level of fit (Figure 4.2).

Table 4.1 presents the results from a correlation analysis between the weekly *Varroa* counts and the instantaneous weather covariates. The instantaneous temperature-based covariates (maximum, minimum, average, and dew point temperature) indicated evidence for correlation with *Varroa* counts based on the magnitude of correlation and p-value. Evidence for an association between average humidity and *Varroa* count was also found to exist. The maximum weekly temperature was found to show the strongest evidence of a positive correlation with mite counts with a Pearson correlation coefficient of 0.415 ($p < 0.001$). The strongest evidence for negative correlation was found to exist between *Varroa* counts and weekly average humidity (-0.174, $p < 0.01$) (Table 4.1).

Results of vector autoregressive (VAR) lag selection of the weather covariates suggests a correlation between *Varroa* counts and lagged temperature covariates (minimum, maximum, and average) and dew point, all at a lag of 7 weeks ($p < 0.05$), based on lag selection by the AIC. The remaining covariates (average humidity, total precipitation and average pressure) did not provide evidence for an association with *Varroa* mite counts at any lag up to a maximum of 8 weeks (Table 4.2).

The final model for the *Varroa* count data was fit as a GLARMA (0, 2), with a trend component and offset of the log number of inspections occurring during the week. Two weather covariates were also included in the final model as linear components: 7-week lagged weekly

average dew point (0.172, $p < 0.001$) and 7-week lagged weekly average temperature (-0.121; $p < 0.001$) (Table 4.3). This combination of weather variables was found to possess the lowest AIC value compared to other combinations. None of the tested interactions between weather variables were found to be significant contributors to the final model.

Examination of the ACF and PACF plots of the model residuals indicated no substantial remaining autocorrelation in the prediction errors of the model, and thus no evidence for a lack of fit. The histogram of the probability integral transformation (PIT) residuals also shows no evidence of a lack of fit. Visual examination of the predicted values plotted atop the original data suggest that the model performs well at predicting *Varroa* counts within a certain range, failing mostly in instances of sudden spikes in the data (Figure 4.3). The final negative binomial GLARMA (0,2) model is represented by the following equation:

$$V(t) = 1.746 - 1.395(t) + 0.172(D_{t-7}) - 0.121(T_{t-7}) + 0.114(V_{t-35}) + 0.158(V_{t-71}) + \log(s)$$

Where 't' represents the time in weeks, D_{t-7} represents the average weekly dew point ($^{\circ}$) at time 't-7', T_{t-7} represents the weekly average temperature ($^{\circ}$) at time 't-7', V_{t-35} represents the total weekly *Varroa* count from one year (35 weeks) previous, V_{t-71} represents the total weekly *Varroa* count from two years (71 weeks) previous, and $\log(s)$ represents the offset of the number of observations.

4.5 Discussion

The development and execution of an effective integrated pest management strategy requires considerable knowledge of the pest's distribution patterns and population dynamics. Both spatial and temporal distribution metrics are equally important to understand and evaluate the intensity as well as the trajectory of population-level *Varroa* mite infestation levels over time

and space. This study complements previous spatial epidemiological studies of *Varroa* mite infestations in managed bee colonies in Ontario (Sobkowich *et al.*, 2022), by providing an examination of the temporal patterns and trends of infestations over the same 5-year study period. The goal of this study was to investigate the intensity of *Varroa* infestations in Ontario over time and explore the link between the changing mite intensity and changing weather variables.

STL decomposition of the data provided strong evidence that *Varroa* mite counts in Ontario follow a predictable seasonal pattern each year. An initial elevated level of *Varroa* was observed in the early spring around the time of initial hive opening, followed by a sharp decline and then a gradual build throughout the summer months until a period of exponential growth in the early fall. This exponential growth has previously been showcased through modelling of *Varroa* population dynamics in temperate climates (Noireterre, 2011; OIE: World Organisation for Animal Health, 2021). This pattern is apparent in all the years studied and is consistent with global findings based on the varroosis reported to the World Animal Health Organisation (OIE), which found a consistent seasonal pattern with greater levels in the second half of the beekeeping season (Fanelli & Tizzani, 2020). The data from 2019 lacked an initial spike in *Varroa* counts in the early spring, supposedly due to the overall lower *Varroa* counts observed in the previous year. During the study, the lowest *Varroa* counts were reported in 2018, which could explain the lack of a spike in mite populations in the spring of 2019. In the early spring months, the initial mite counts are representative of the mite levels carried over in the colony from the previous season and would be expected to be higher if not effectively treated before overwintering.

Fries *et al.* (1994), found elevations in mite counts in the previous year have a substantial impact on the growth rate and peak *Varroa* levels in subsequent years if adequate treatment is

not administered. Although this study acquired no information on treatment or control strategies implemented, the slight evidence towards a decreasing trend over the study period suggests that the control measures in Ontario are seemingly adequate for population-level mite control. The general trend of *Varroa* counts over the 5-year study was found to slightly decline from 2015 to 2019 based on the STL decomposition and final GLARMA model. This decreasing trend compliments the results of a global study on *Varroa* infestations, where continental climates experienced a net decrease in intensity between 2015 and 2019 (Fanelli & Tizzani, 2020). Results from the STL decomposition of the *Varroa* shows some evidence of the trend reversing marginally from negative to positive in 2019.

Modeling using a GLARMA model and weather covariates was moderately successful in describing *Varroa* counts over the five-year study period. A final model including the 7- week lagged average dew point temperature, and 7- week lagged average temperature best fit the data based on lowest AIC. *Varroa* counts were found to increase marginally with an increase in dew point temperature and decrease with an increase in average temperature. These results may have several biological explanations. Dew point is a measure of the amount of moisture present in the air, and is defined as the temperature at which the air would become fully saturated (i.e., 100% relative humidity). In 2003, Harris *et al.* found temperature to display a positive correlation with *Varroa* mite population growth rates, and a negative correlation between relative humidity and *Varroa* population growth (Harris *et al.*, 2003). While dew point and relative humidity are not equivalent measures, they are both indicative of the amount of ambient moisture in the air, indicating a level of agreeance between the findings of this study and the findings of Harris *et al.* (2003). However, while studies in neotropical environments have also showed temperature to be an important factor in varroosis they have found rainfall to be more of a contributor than

humidity or dew point to infestation levels (Correia-Oliveira *et al.*, 2018); suggesting that environmental covariate importance may depend on climatic zones or region specificity in general. Later research supported the findings between temperature, humidity, and *Varroa*, and noted that the likely cause of these relationships is reproductive limitations due to weather and increased mortality of the mites under certain conditions (Harris, Villa, & Danka, 2004).

Several explanations are plausible for the relationship of temperature and air moisture content with *Varroa* mite counts, ranging from the impact of weather on the behaviours and biology of bees, to reproduction and mortality of the mites, and temperature effects on treatment efficacy. Formic acid and oxalic acid are recognized treatments by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and are encouraged for use in the fall season, but have a known temperature dependency (Aldea *et al.*, 2013; Underwood & Currie, 2003). Furthermore, the impacts of temperature and humidity on honey bee behaviours has been extensively studied and reviewed (Abou-Shaara, Owayss, Ibrahim, & Basuny, 2017). The behavioural changes due to temperature and humidity can influence brood rearing, foraging behaviour, worker survival, swarming and absconding (Abou-Shaara, Owayss, Ibrahim, & Basuny, 2017) and could play a role in modulating *Varroa* mite population dynamics. Although no mechanism is described in this study, the time for the effects of temperature and air moisture content to materialize into a noticeable change in the *Varroa* mite population appears to be approximately 7 weeks. However, important to note is that the temperature follows a predictable seasonal pattern, and therefore could be serving as a proxy for time, and potentially acting as a confounding variable for other relationships with *Varroa* mite counts.

A limitation of this study is the use of a single weather station in Toronto to represent the province-wide study area. However, from the results of a spatial analysis of the distribution of

registered colonies in Ontario by Sobkowich *et al.*, (2021a), a large number of colonies in Ontario reside in the Niagara Peninsula (roughly 70km from Toronto), and nearly all colonies reside in the broad region of Southern Ontario. Furthermore, the geographic centroid of the observations present in the dataset used for this study is located in Brampton, Ontario, only 16km away from Toronto's Pearson International Airport. Pearson Airport's weather station was therefore selected for two main reasons: (1) the weather station services a major international airport and is therefore expected to be held to a high degree of accuracy and consistency, and (2) the location of the weather station exists in the approximate geometric median of both the observed colonies in the data and the registered colonies in the province (Sobkowich *et al.*, 2021).

Subdividing the province into five distinct regions, each with its own station for weather data for more local specificity was considered. However, the decision to model the observations as a single larger time series rather than as four smaller time series was made as to not dilute the data to the point of being unable to detect associations if present. When analyzing time-series data, it is not possible to assess multiple groups with a single model, since all dates require a single observation. Therefore, to address multiple regions, multiple distinct models would be required. With only 13,000 observations, divided already across a five-year study period (~2,600 per year on average), further subdividing by the five beekeeping regions in Ontario (OMAFRA, 2021a), would result in only a few hundred observations available in each region per year. For this reason, and because this study has a focus on exploratory modelling, it was decided to investigate a population-level model. Future work may decide to isolate a more localized area more intensively, however, more data would be necessary.

Another limitation of this study is the nature of the *Varroa* data utilized, in terms of sampling and spatial distribution. The observations used in this study likely exist clustered in time to some degree, both by beekeeping operation or location. This clustering is due to the clumping of apiary locations across the province and the practice of sampling multiple colonies per yard per inspection. However, GLARMA models are not inherently able to account for clustering. For the present study, a GLARMA model was selected as it is able to effectively model temporal autocorrelation while simultaneously modeling potential risk factors. Subsequent studies looking to investigate the potential impacts of this observation clustering, may choose to use a simpler generalized linear model. However, the use of a non-time-series model will forfeit the ability to model the long and short-term temporal autocorrelation commonly present in time-series data.

This study utilized inspection data with a standardized sampling procedure, where *Varroa* counts are the result of an alcohol wash using an approximate 300 bee sample. Thus, the *Varroa* counts are representative of the number of living mites, currently within the colony and not in the brood. The counts are therefore not necessarily representative of the number of dropped mites, mites external to the hive (i.e., on foraging bees) or present in the capped brood. Similar studies looking into correlations of *Varroa* with weather utilizing a sticky board sampling method (sampling of dropped mites) found a similar correlation between maximum temperature and the number of mites dropped (Poonia, Gulati, & Sharma, 2014), which provides evidence that the correlation of mite counts and temperature is observable regardless of the sampling method.

A major consideration of this study is the absence of testing during the winter months. Opening colonies during the deep winter months is generally discouraged, and therefore sampling for *Varroa* is not undertaken once colonies enter the winter months. This leads to a gap

in the continuity of time series data, providing a challenge for modelling. This was overcome by creating an artificial 35-week year to represent the beekeeping season, but some information loss is inevitable. Furthermore, if a colony is overwintered with a high *Varroa* load, there is a substantial increase in the likelihood of collapse before spring (Guzmán-Novoa *et al.*, 2010), and collapsed colonies are typically not sampled for mites in the spring. Therefore, further continuity can be lost from year to year. This selection bias may prevent the true relationship between high fall mite loads and spring mite loads from being observed in the current data.

4.6 Conclusion

The results of this study provide some further indication of a lagged relationship between temperature and dew point with *Varroa* mite prevalence. Some evidence for an association between *Varroa* counts and several other weather variables exists, but only these two variables were found to be useful in predicting *Varroa* counts in a GLARMA model where autocorrelation is defined. The overall growth of *Varroa* mite populations can be reasonably and simply described by a consistent seasonal pattern of exponential growth in the mid-summer to late fall, which has been documented previously in the literature and is now further documented in Ontario specifically. The current integrated pest management strategies in Ontario appear to be successful in keeping *Varroa* mites at hypoendemic levels and there appears to be a decreasing trend this five-year study. Through this epidemiological time series evaluation of *Varroa* in Ontario, in combination with similar spatial studies (Sobkowich *et al.*, 2022), information on the patterns and trajectories of *Varroa* mite prevalence are more accessible and can be used to further refine intervention strategies, surveillance and develop educational tools to inform members of the beekeeping community.

4.7 References

- Abou-Shaara, H. F., Owayss, A. A., Ibrahim, Y. Y., & Basuny, N. K. (2017). A review of impacts of temperature and relative humidity on various activities of honey bees. *Insectes Sociaux*, 64(4), 455–463. <https://doi.org/10.1007/s00040-017-0573-8>
- Agriculture and Agri-Food Canada. (2020). *Statistical overview of the Canadian honey and bee industry, 2020*. Retrieved from <https://agriculture.canada.ca/en/canadas-agriculture-sectors/horticulture/horticulture-sector-reports/statistical-overview-canadian-honey-and-bee-industry-2020>
- Akala, H., Makindi, S. M., & Esilaba, M. O. (2018). Effects of climate variability on foraging behaviour of bees: a case study of Marigat and Rata locations in Baringo County , Kenya. *Earth Science*, 122, 51690–51693.
- Aldea, P., Rodriguez, R., Olivares, A., Farffin, M., Riveros, D., Nfifiez, F., & Trivelli, L. (2013). Effect of ambient temperature and humidity conditions on the efficacy of organic treatments against *Varroa destructor* in different climatic zones of Chile. *Journal of Agricultural Science and Technology*, 3(6), 474–483.
- Boecking, O., & Genersch, E. (2008). Varroosis - the ongoing crisis in bee keeping. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, 3(2), 221–228. <https://doi.org/10.1007/s00003-008-0331-y>
- Calderón, R. A., van Veen, J. W., Sommeijer, M. J., & Sanchez, L. A. (2010). Reproductive biology of *Varroa destructor* in Africanized honey bees (*Apis mellifera*). *Experimental and Applied Acarology*, 50(4), 1–17. <https://doi.org/10.1007/s10493-009-9325-4>
- Chemurot, M., Akol, A. M., Masembe, C., de Smet, L., Descamps, T., & de Graaf, D. C. (2016). Factors influencing the prevalence and infestation levels of *Varroa destructor* in honeybee colonies in two highland agro-ecological zones of Uganda. *Experimental and Applied Acarology*, 68, 497–508. <https://doi.org/DOI 10.1007/s10493-016-0013-x>
- Clarke, D., & Robert, D. (2018). Predictive modelling of honey bee foraging activity using local weather conditions. *Apidologie*, 49(3), 386–396. <https://doi.org/10.1007/s13592-018-0565-3>
- Cleveland, R. B., Cleveland, W. S., McRae, J. E., & Terpenning, I. (1990). STL: a seasonal-trend decomposition procedure based on LOESS. *Journal of Official Statistics*, 6(1), 3–33.
- Corkins, C. L. (1932). The temperature relationships of the honey bee cluster under controlled external temperature conditions. *Journal of Economic Entomology*, 25(4), 820–825. <https://doi.org/10.1093/jee/25.4.820>
- Correia-Oliveira, M. E., Mercês, C., Mendes, R. B., Neves, V., Silva, F., & Carvalho, C. (2018). Can the environment influence varroosis infestation in Africanized honey bees in a neotropical region? *Florida Entomologist*, 101(3), 464–469. <https://doi.org/10.1653/024.101.0304>
- Czado, C., Gneiting, T., & Held, L. (2009). Predictive model assessment for count data. *Biometrics*, 65(4), 1254–1261. <https://doi.org/10.1111/j.1541-0420.2009.01191.x>

- Davis, W., Dunsmuir, W., & Wang, Y. (1999). Modeling time series of count data. In S. Ghosh (Ed.), *Asymptotics, Nonparametrics, and Time Series* (pp. 63–115). New York: Marcel Dekker.
- DeGrandi-Hoffman, G., & Curry, R. (2004). A mathematical model of *Varroa* mite (*Varroa destructor* Anderson and Trueman) and honeybee (*Apis mellifera L.*) population dynamics. *International Journal of Acarology*, 30(3), 259–274.
<https://doi.org/10.1080/01647950408684393>
- Fanelli, A., & Tizzani, P. (2020). Spatial and temporal analysis of varroosis from 2005 to 2018. *Research in Veterinary Science*, 131(January), 215–221.
<https://doi.org/10.1016/j.rvsc.2020.04.017>
- Ferland, J., Claing, G., Kempers, M., Kennedy, K., Kozak, P., Lafrenière, R., ... Hoover, S. (2021). Preliminary report on honey bee wintering losses in Canada (2021). *Canadian Association of Professional Apiculturists*.
- Fries, I., Camazine, S., & Sneyd, J. (1994). Population dynamics of *Varroa jacobsoni*: a model and a review. *Bee World*, 75(1), 5–28. <https://doi.org/10.1080/0005772X.1994.11099190>
- Granger, C. W. J. (1969). Investigating causal relations by econometric models and cross-spectral methods. *Econometrica*, 37(3), 424. <https://doi.org/10.2307/1912791>
- Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443–450. <https://doi.org/10.1051/apido/2009076>
- Harris, J. W., Harbo, J. R., Villa, J. D., & Danka, R. G. (2003). Variable population growth of *Varroa destructor* (Mesostigmata: Varroidae) in colonies of honey bees (Hymenoptera: Apidae) during a 10-year period. *Environmental Entomology*, 32(6), 1305–1312.
<https://doi.org/10.1603/0046-225X-32.6.1305>
- Harris, J. W., Villa, J. D., & Danka, R. G. (2004). Environmental effects on the growth of *Varroa* mite populations. *Bee Culture*, 132(5), 23–25.
- Historical Data. (2021). Retrieved May 12, 2021, from
https://climate.weather.gc.ca/historical_data/search_historic_data_e.html
- Human, H., Nicolson, S. W., & Dietemann, V. (2006). Do honeybees, *Apis mellifera scutellata*, regulate humidity in their nest? *Naturwissenschaften*, 93(8), 397–401.
<https://doi.org/10.1007/s00114-006-0117-y>
- Johnson, R. M. (2010). *Honey Bee Colony Collapse Disorder*. Washington, D.C.
- Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274(1608), 303–313.
<https://doi.org/10.1098/rspb.2006.3721>
- Kozak, P., Eccles, L., Kempers, M., Rawn, D., Lacey, B., & Guzmán-Novoa, E. (2021). Ontario treatment recommendations for honey bee disease and mite control. Retrieved June 24,

2021, from <http://www.omafra.gov.on.ca/english/food/inspection/bees/2017-treatment.htm#VM>

Liew, V. K. . (2004). Which lag length selection criteria should we employ? *Economics Bulletin, Access Economics*, 3(33), 1–9.

Lindauer, M. (1955). The water economy and temperature regulation of the honey bee colony. *Bee World*, 36(5), 81–92. <https://doi.org/10.1080/0005772X.1955.11094876>

Matthews, R., Wasserstein, R., & Spiegelhalter, D. (2017). The ASA's p -value statement, one year on. *Significance*, 14(2), 38–41. <https://doi.org/10.1111/j.1740-9713.2017.01021.x>

Noireterre, P. (2011). Biologie et pathogénie du *Varroa destructor*. *Bulletin Des GTV*, 62, 101–106.

OIE: World Organisation for Animal Health. (2021). Manual of diagnostic tests and vaccines for terrestrial animals 2021. Retrieved December 7, 2021, from <https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>

OMAFRA. (2021a). Annual apiculture winter loss reports. Retrieved January 29, 2022, from ontario.ca/document/annual-apiculture-winter-loss-reports#figure1

OMAFRA. (2021b). *Varroa* mite - sampling and monitoring infestation levels. Retrieved December 7, 2021, from <http://www.omafra.gov.on.ca/english/food/inspection/bees/varroa-sampling.htm>

Peck, D. T., & Seeley, T. D. (2019). Mite bombs or robber lures? The roles of drifting and robbing in *Varroa destructor* transmission from collapsing honey bee colonies to their neighbors. *PLoS ONE*, 14(6), 1–14. <https://doi.org/10.1371/journal.pone.0218392>

Peck, D. T., Smith, M. L., & Seeley, T. D. (2016). *Varroa destructor* mites can nimbly climb from flowers onto foraging honey bees. *PLoS ONE*, 11(12), e0167798. <https://doi.org/https://doi.org/10.1371/journal.pone.0167798>

Petz, M., Stabentheiner, A., & Crailsheim, K. (2004). Respiration of individual honeybee larvae in relation to age and ambient temperature. *Journal of Comparative Physiology B*, 174(7), 511–518. <https://doi.org/10.1007/s00360-004-0439-z>

Pfaff, B. (2008). *Analysis of Integrated and Cointegrated Time Series with R* (2nd ed.). New York: Springer.

Poonia, A., Gulati, R., & Sharma, S. K. (2014). Effect of environmental factors on the population of *Varroa destructor* in *Apis mellifera L.* Colonies. *The Ecoscan*, 8(1&2), 23–25.

R Core Team. (2020). *R*: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.r-project.org/>

Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103(SUPPL. 1), S96–S119. <https://doi.org/10.1016/j.jip.2009.07.016>

Simpson, J. (1961). Nest climate regulation in honey bee colonies. *Science*, 133(1), 1327–1333.

- Sobkowich, K. E., Berke, O., Bernardo, T. M., Pearl, D. L., & Kozak, P. (2021). Mapping the population density of managed honey bee (*Apis mellifera*) colonies in Ontario, Canada: 2018. *Journal of Apicultural Science*, 65(2), 303–314. <https://doi.org/10.2478/jas-2021-0023>
- Stabentheiner, A., Pressl, H., Papst, T., Hrassnigg, N., & Crailsheim, K. (2003). Endothermic heat production in honeybee winter clusters. *Journal of Experimental Biology*, 206(2), 353–358. <https://doi.org/10.1242/jeb.00082>
- Stevenson, M. A., Benard, H., Bolger, P., & Morris, R. S. (2005). Spatial epidemiology of the Asian honey bee mite (*Varroa destructor*) in the North Island of New Zealand. *Preventive Veterinary Medicine*, 71(3–4), 241–252. <https://doi.org/10.1016/j.prevetmed.2005.07.007>
- Tahmasbi, G. (2009). The effect of temperature and humidity on grooming behaviour of honeybee, *Apis mellifera* (Hym.: Apidae) colonies against *Varroa* mite, *Varroa destructor* (Acari: Varroidae). *Journal of Entomological Society of Iran*, 28(2), 7–23.
- Underwood, R. M., & Currie, R. W. (2003). The effects of temperature and dose of formic acid on treatment efficacy against *Varroa destructor* (Acari: Varroidae), a parasite of *Apis mellifera* (Hymenoptera: Apidae). *Experimental and Applied Acarology*, 29.
- Vidal-Naquet, N. (2018). *Honeybee Veterinary Medicine*: *Apis mellifera* L. Sheffield, UK: 5m Publishing.
- Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, MA.: Harvard Univeristy Press.
- Zeileis, A., & Hothorn, T. (2015). Diagnostic checking in regression relationships. *R News*, 2(3), 7–10.

4.8 Tables

Table 4.1: Pearson correlation coefficients of weather covariates related to average *Varroa* count per colony inspection.

	Pearson Correlation Coefficient	Lower 95% Confidence Limit	Upper 95% Confidence Limit	p-value
Average Temperature	0.407	0.300	0.503	<0.001
Maximum Temperature	0.415	0.309	0.511	<0.001
Minimum Temperature	0.377	0.268	0.476	<0.001
Average Humidity	-0.174	-0.289	-0.053	<0.010
Volume of Precipitation	0.144	0.023	0.261	0.020
Average Atmospheric Pressure	0.076	-0.045	0.196	0.219
Average Dew Point Temperature	0.392	0.284	0.490	<0.001

Table 4.2: Indication of optimal lag (in weeks) for weather covariates based on Akaike information criteria and Granger predictive causality test.

	Optimal Lag (max. 8)		Granger Test p-value
	AIC	FPE	
Average Temperature	7	7	<0.01
Maximum Temperature	7	7	<0.01
Minimum Temperature	7	7	<0.01
Average Humidity	4	4	0.142
Volume of Precipitation	2	2	0.886
Average Atmospheric Pressure	4	4	0.543
Average Dew Point Temp.	7	7	<0.01

AIC: Akaike information Criterion; FPE: Akaike final prediction error.

Table 4.3: Results of GLARMA (0,2) regression model for the total *Varroa* counts per week in Ontario between 2015 and 2019.

	Estimate	Standard Error	z-Ratio	P-Value
Negative Binomial Parameter				
α	1.096	0.139	7.897	<0.001
GLARMA Coefficients				
Θ_1	0.114	0.069	1.669	0.095
Θ_2	0.158	0.070	2.266	0.023
Linear Model Coefficients				
Intercept	1.746	0.236	7.411	<0.001
Trend	-1.395	0.283	-4.928	<0.001
7-Week Lagged Average Dew Point ($^{\circ}$)	0.172	0.040	4.315	<0.001
7-Week Lagged Average Temperature ($^{\circ}$)	-0.121	0.036	-3.311	<0.001

4.9 Figures

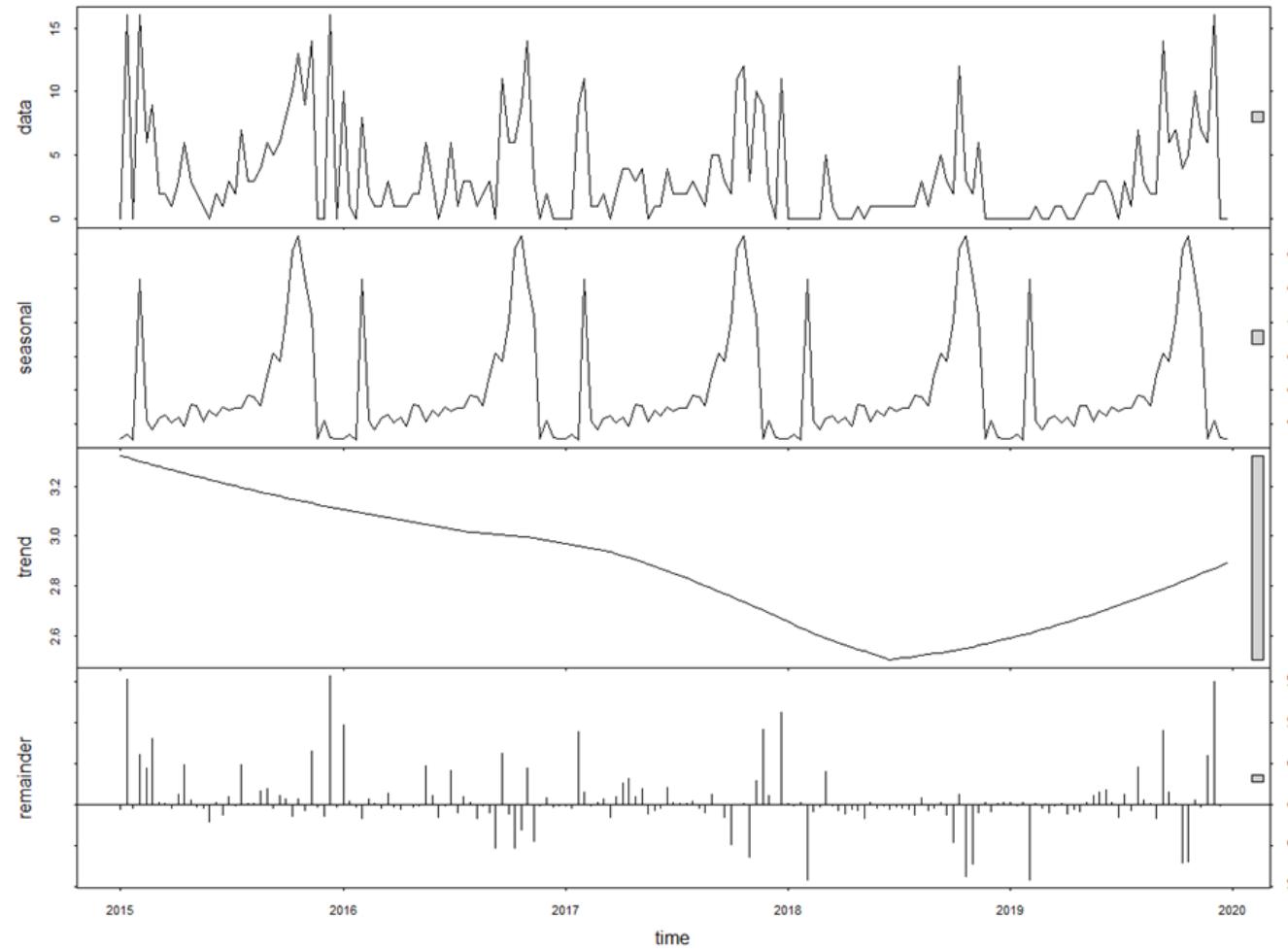


Figure 4.1: Time series components plot resulting from a seasonal and trend decomposition by LOESS (STL) of the average *Varroa* count per 300 bees sampled from Ontario colonies between 2015 and 2019 (winters removed).

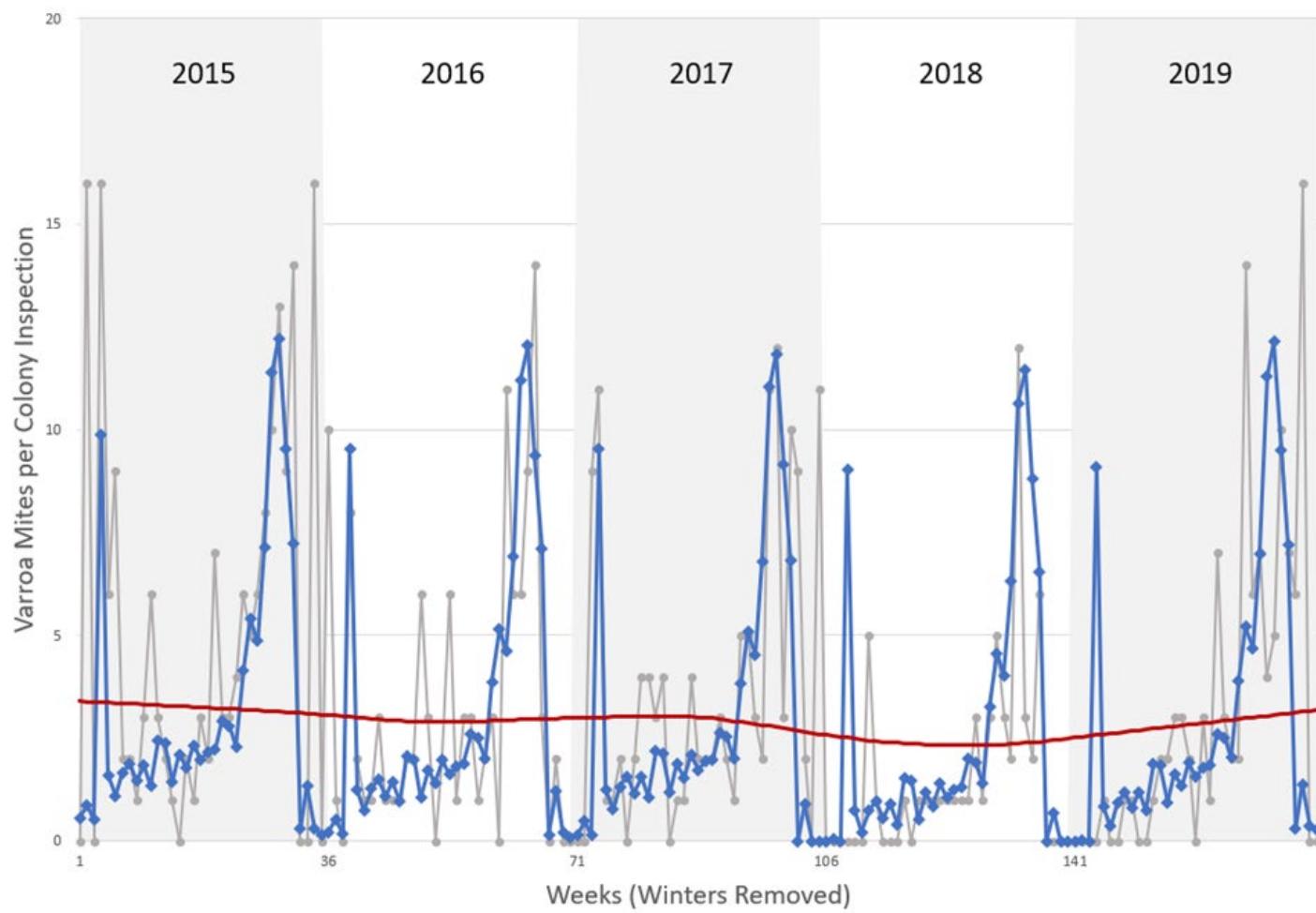


Figure 4.2: The signal and trend (blue/diamonds) and isolated trend (red) components juxtaposed over the average *Varroa* count per 300 bees sampled from Ontario colonies by week (gray/circles) between 2015 and 2019.

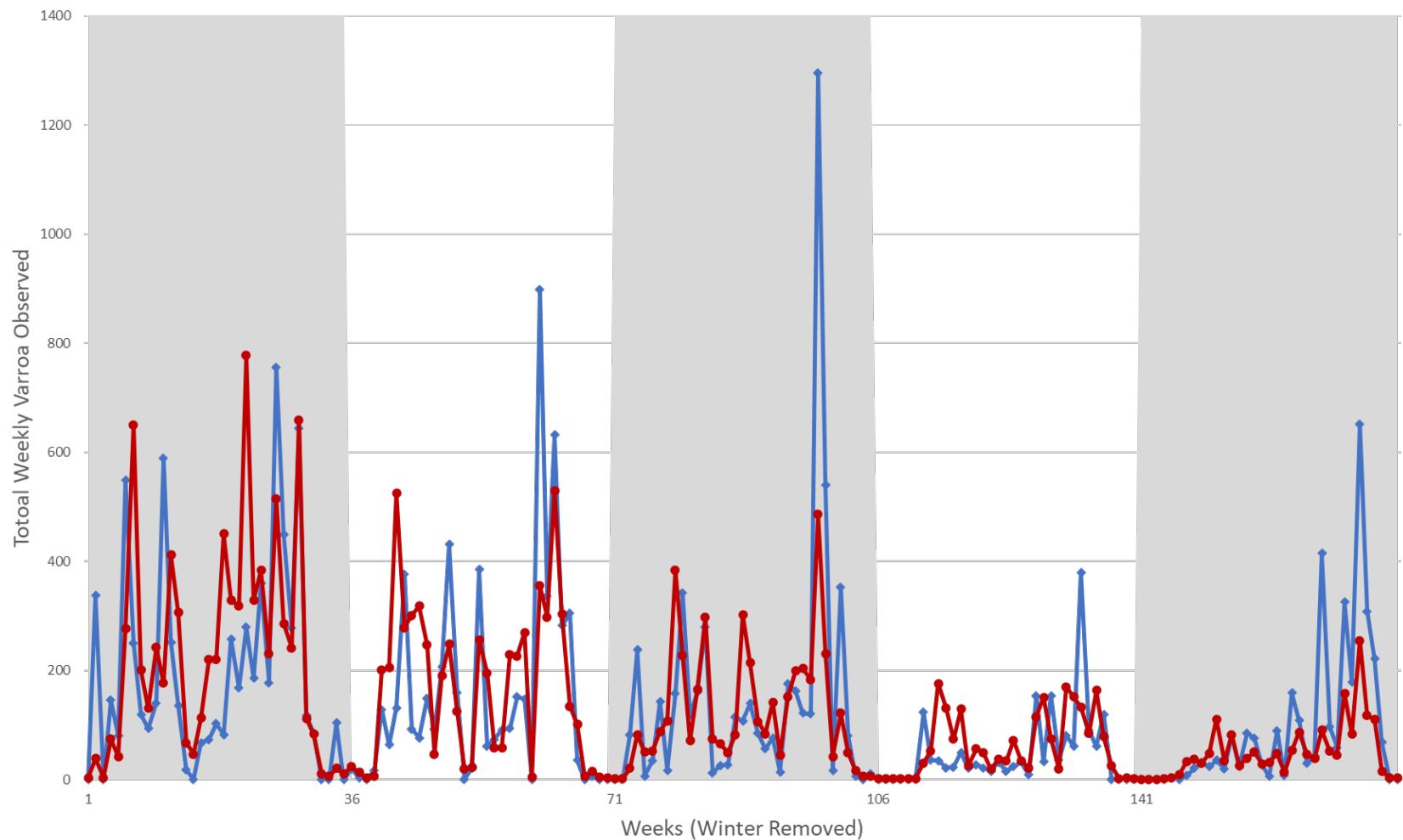


Figure 4.3: GLARMA model estimated *Varroa* counts (red/circles) and observed *Varroa* counts (blue/diamonds) for Ontario honey bee colonies between 2015 and 2019.

5 CHAPTER FIVE: DEVELOPMENT AND ASSESSMENT OF THE *HIVE*: AN EPIDEMIOLOGIC DASHBOARD FOR SURVEILLANCE OF *VARROA* *DESTRUCTOR* IN ONTARIO APIARIES.

5.1 Abstract

Varroosis (caused by the *Varroa destructor* mite) is the most prominent health issue in beekeeping. Because these mites can exist in reservoirs of feral honey bee colonies, eradication is impossible. Therefore, integrated pest management strategies are critical in controlling their populations, for which adequate regional surveillance is necessary. This project aims to build on the success of epidemiological data dashboards developed throughout the 2019-coronavirus pandemic and showcase how this technology can improve surveillance of *Varroa* mite infestations in Ontario. Dashboards provide a consistent source of information and epidemiologic metrics through data visualizations, and mobilize data otherwise bound to tables and intermittent reports. A prototype of an interactive dashboard for the surveillance of *Varroa* mite infestations across the province is presented. This dashboard was developed using routine ministry inspection data to depict the spatio-temporal distribution of mites over a five-year data collection period. Through interactive figures and plots, able to be disaggregated to a specific region and time frame, this dashboard allows for members of the beekeeping community to monitor provincial mite observations continuously throughout the season. Seven criteria found to be common across highly successful and actionable COVID-19 dashboards were used to assess the quality of the proposed *Varroa* dashboard, and critically reflect on its strengths and weaknesses. Furthermore, future directions for surveillance dashboards are explored, including their integration with emerging health informatics (i.e., artificial intelligence, big data, and sensors) and citizen science data collection, to develop a comprehensive province-wide surveillance system. The outcome of

this project is a functional dashboard for population-level monitoring of *Varroa* mites and a model for the design and assessment of future dashboards for other species, and diseases.

5.2 Introduction

Varroa mites (*Varroa destructor*) continue to be the predominant issue for beekeeping globally (Dahle, 2010; Guzmán-Novoa *et al.*, 2010; Vidal-Naquet, 2018). The effect of these parasitic mites is a weakening of bees, vectoring of viruses (Le Conte, Ellis, & Ritter, 2010; Rosenkranz, Aumeier, & Ziegelmann, 2010), a reduction in worker bee efficiency (Kralj, Brockmann, Fuchs, & Tautz, 2007; Kralj & Fuchs, 2006), and ultimately a decrease in honey production, pollination, and an increased burden on beekeepers due to lost colonies. In 2020, beekeepers in Ontario identified *Varroa* as the perceived leading cause for colony loss, specifically listing infestations of mites originating from neighbouring yards as the greatest contributor to loss (Claing *et al.*, 2020). This has led to an interest in population-level epidemiologic research on *Varroa* mites within managed apiaries of Ontario (Chapters 2 & 3). Elevated mite levels in one apiary can trigger subsequent infestations in nearby yards via mite transmission routes such as robbing, drifting, and transmission through fomites such as shared foraging sites (Peck & Seeley, 2019; Peck, Smith, & Seeley, 2016; Vidal-Naquet, 2018). Experts in the beekeeping field firmly believe that colony density plays a role in regional *Varroa* loads, yet research to back this claim is lacking.

Because *Varroa* mites are transmitted between beekeeping operations and are harbored within feral colonies, complete eradication is not deemed possible, and therefore keeping mite levels below a critical threshold is the main goal of integrated pest management (IPM) strategies (Vidal-Naquet, 2018). A crucial step within effective IPM is the monitoring of incidence and prevalence metrics to gauge success, recognize anomalies, and trigger the implementation of

intervention strategies. This requires continuing, consistent, and location-specific data achieved through a surveillance system. Acquiring, managing, and analyzing *Varroa* surveillance data typically results in a single report at the end of the beekeeping season. In such a scenario, the current year's data are only useful in informing and modifying the following year's IPM strategy rather than tailoring to the current status of mite loads based on various risk modifiers that may change throughout the season. This implies a need for rapid data collection and analysis to occur continuously throughout the season to monitor the effectiveness of current strategies and adjust as necessary.

During the COVID-19 (SARS-CoV-2) pandemic, epidemiologic information dashboards became a popular means for rapid information sharing between organizations and the general public (Bernardo *et al.*, 2021; Ivanković *et al.*, 2021). These dashboards consolidate and distill published data into digestible graphics offering crucial pandemic information to the public. As new data are collected, they can then be automatically analyzed and published, providing near real-time insights with less human involvement, providing a dynamic source of information at a lower resource cost. This method of data sharing offers several benefits: increased data transparency, improved surveillance (multiple eyes on the issue), facilitation of informed decision making, and enablement of comparisons across regions (Barbazza *et al.*, 2021; Ivanković *et al.*, 2021). Many dashboards also include filtering functionality in which information can be focused by the user to specific regions or timeframes. All of these features employed by COVID-19 dashboards could prove useful in monitoring *Varroa* mites in Ontario and offer substantial improvement compared to traditional reporting and communication to the public.

Previous attempts have been made to produce a *Varroa*-centric honey bee dashboard, but none have provided sufficient information to beekeepers for sustained use. *MiteCheck* (Bee Informed Partnership, 2021b) is a notable example of the currently existing *Varroa* dashboards, but lacks common functionality seen in successful COVID-19 dashboards identified by Ivanković *et al.* (2021). Missing functions of previous dashboards, such as the ability to filter either spatially, temporally, or provide locally specific information on *Varroa* management, limit the relevance for an individual beekeeper or operation. Furthermore, past dashboards have relied on simple figures such as bar charts rather than time series plots, which reduce the ability to compare mite populations over time and can introduce bias to the data presented (Talbot, Setlur, & Anand, 2014). To date, no *Varroa* surveillance dashboard has been produced specifically for Ontario. The benefit of a regionally specific dashboard is the tailoring of information both regarding the status of *Varroa* as well as the communication of government updates on location specific IPM strategies and interventions.

The goal of this project was to design and develop a *Varroa* surveillance dashboard targeted towards all stakeholders of the beekeeping community within Ontario using lessons learned from the successful dashboards of the COVID-19 pandemic. Seven criteria for an effective dashboard were considered, derived from a review of 158 COVID-19 dashboards from 53 countries: (1) knowledge of the audience, (2) information management, (3) clarity of data sources, (4) reporting of data over time, (5) regional specificity, (6) stratification of information into distinct subgroups, and (7) narrative and use of visuals (Bos, Jansen, Klazinga, & Kringos, 2021; Ivanković *et al.*, 2021). The developed dashboard aimed to be simple to use yet sophisticated in content, easily adaptable to greater volumes of data, and provide sufficient information to encourage repeated use. Individual objectives of this project include: (1) the

development of a functional data dashboard to enhance the surveillance of *Varroa* mites in Ontario, (2) an assessment of the developed dashboard using actionable criteria to identify the strengths and weaknesses, and (3) review future technological extensions for disease surveillance.

5.3 Materials and Methods

Apiary inspection data from Ontario (2015-2019) were used as an initial source of *Varroa* data. This dataset was obtained from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), which oversees apiculture in the province. The nature of the inspections are a combination of routine surveillance and beekeeper-initiated inspection requests. Each apiary inspection record included the date and region in which the inspection took place, as well as a *Varroa* count per 100 bees. Census consolidated subdivision (CCS) regions were used to divide the province, with a total of 273 regions of various shapes and sizes (Statistics Canada, 2018). Additionally, a total count of the number of registered colonies within each CCS was obtained from OMAFRA to derive a crude colony density value by dividing by regional land coverage (colonies per km²). Observations were excluded if one or more of the following were missing: date, region of observation, or *Varroa* count. All *Varroa* mite counts were obtained using the standard alcohol wash procedure on a sample of 300 bees (OMAFRA, 2021).

To visually depict *Varroa* data for Ontario, three main visualizations were selected: choropleth maps, time series plots, and data tables. Choropleth maps were generated and included to visualize the geographical distribution of *Varroa* mites across the province. Each CCS region was coloured on the basis of the average number of mites per 100 bees sampled. Likewise, a colour-based choropleth map was generated to depict the density of registered colonies per CCS region, based on 2018 government registry data. A density map was included

to address the growing concern from Ontario beekeepers and researchers regarding the potential implications of colony density on regional mite levels (Claing *et al.*, 2020; Sobkowich, Berke, Bernardo, Pearl, & Kozak, 2021). The density map serves to offer contextual information regarding the population-at-risk across the province and allows for potential correlations between colony density and *Varroa* counts to be observed. The choice of colour palette used in the development of the choropleth maps was selected from the *RColorBrewer* package, with consideration given to users with colour vision deficiency (Neuwirth, 2014). Basic interactive functions were included to allow for panning and zooming across the mapped data in addition to the ability to filter the maps by year. Both maps were implemented using the *Leaflet* package in *R* (Cheng, Karambelkar, & Xie, 2018).

Interactive time series plots were designed and developed using the *Varroa* count data and date of inspection. Two plots were generated, one depicting weekly average *Varroa* counts for the province, and a second depicting weekly average *Varroa* counts by individual CCS region. Weekly data were used in place of daily data to aid in maintaining beekeeper privacy and also to address the issue of sparse daily observations in select CCS regions. To assist in identifying temporal patterns in the data, a LOESS (locally estimated scatterplot smoothing) line was added, with an interactive smoothing parameter input. A smoothing parameter input ranging from 1 to 10, with a default value of 2 was included. Based on user input, the number of weeks over which the LOESS line is evaluated is changed, and the plot is regenerated with the new line. Time series plots were equipped with filtering functionality by year and by CCS region for further data exploration. Through the *Plotly* package (Sievert, 2020) further interactive functionality was added, allowing for zooming, panning, and cropping.

An interactive data table was generated for filtering and exploration of the raw numerical data in tabular format. For privacy protection of apiaries and beekeepers, observations were aggregated to monthly values while also remaining at the CCS regional level. Average *Varroa* counts, the number of observations, and the regional colony density were included in the data table along with the year, month, and CCS region name. The data table was developed to include the ability to search, filter and sort by any or several variables using the *DT* package in *R* (Xie, Cheng, & Tan, 2021).

In addition to the data presented, several information sections were included in the dashboard to provide material to the beekeepers on how to sample mite counts, how to treat colonies for mites, and the expected mite population dynamics in an average season. Information on mite treatment, and mite sampling methods were adapted from the OMAFRA website (Kozak *et al.*, 2021; OMAFRA, 2021) with supplementary videos from the University of Guelph Honey Bee Research Centre YouTube channel (University of Guelph, 2021) and the Ontario Beekeepers' Association – Technology Transfer Program YouTube Channel (OBA, 2021). A typical timeline of *Varroa* mite population dynamics in comparison to honey bee population dynamics was modified from OIE resources (OIE: World Organization for Animal Health, 2021) and combined with *Varroa* sampling and treatment guidelines to create a basic '*Varroa* calendar' specific to Ontario to consolidate the various information sources for beekeepers. Links to reputable, Ontario-specific web resources were included to provide supplementary information to the dashboard: (1) OMAFRA webpage for ministry policy and protocols, (2) University of Guelph Honey Bee Research Centre YouTube page for additional beekeeping methodology, (3) the Ontario Beekeepers' Association webpage, and (4) the Ontario Animal Health Network webpage, where beekeepers can submit their own mite counts.

Following the development of the dashboard, seven criteria were used to assess its actionability. These seven criteria were adapted from a review of successful COVID-19 dashboards conducted by Ivanković *et al.* (2021), and a modified version of the scoring procedure used by Bos *et al.* (2021) was applied, utilizing a third-party review by members of the Ontario beekeeping community. A three-score system was used to evaluate each of the seven criteria: “not present”, “somewhat present”, and “clearly present”, scoring zero, one, and two points respectively. The seven criteria used were: (1) knowledge of the audience, (2) information management, (3) clarity of data sources, (4) reporting of data over time, (5) regional specificity, (6) stratification of information into distinct subgroups, and (7) narrative and use of visuals (Ivanković *et al.*, 2021). The criteria are comprehensively listed and defined in Table 5.1.

The structure and basic functionality of the dashboard was developed in R (R Core Team, 2020) using the *Shiny* package (Chang *et al.*, 2021).

5.4 Results

A complete breakdown of the OMAFRA *Varroa* dataset utilized in the development of the dashboard is presented in Table 5.2. The data contained 4,058 inspections across five beekeeping seasons, but inspections were not distributed equally across the years, with a decreasing number of observations occurring across the study period. On average, 45.2% of the CCS regions in Ontario received at least one inspection visit per year, with 76.2% of the regions in the province being included at least once over the five-year period. *Varroa* count data followed a right skewed distribution in all five years of sampling data. The source of data was acknowledged and described in a dedicated section of the “Information” tab of the dashboard.

The developed dashboard (named the *HIVE* – Honeybee & Interactive *Varroa* Epidemiology dashboard) mainly displayed epidemiologic information regarding the spatial and temporal distribution of mites across the province, supplemented by relevant information for Ontario beekeepers. Six tabs comprised the navigation structure of the *HIVE* dashboard: Overview, Maps, Trends, Historical Data, *Varroa* Timeline, and Information. The “Information” tab was further subdivided into five sub-tabs: General Information, *Varroa* Sampling, *Varroa* Treatment, About This Dashboard, and Resources.

5.4.1 Sections of the *HIVE* Dashboard

A link to the *HIVE* dashboard is offered in *Appendix A*. Screen captures of each of the developed sections of the dashboard are presented in *Appendix B*.

5.4.1.1 Homepage

A dedicated landing page appears for users opening the dashboard (*Appendix B*. Figure 5.2). This page serves to orient the audience to the purpose and aim of the *HIVE* dashboard along with a brief description of each of the data and information sections.

5.4.1.2 Maps

The ‘Maps’ section primarily aids in the exploration of geographical patterns and distributions of *Varroa* mite and honey bees across the province (*Appendix B*. Figure 5.3). Two choropleth maps comprise this section, with one depicting the colony density and the other dedicated to the *Varroa* count data. Both maps utilize the same CCS region structure, subdividing the province into 273 subdivisions, offering localized information. Clear patterns of *Varroa* counts and density are discernable from these maps. A dropdown menu interface allows for the user to visualize the data by a single year or aggregated across all years sampled.

Accompanying each map is a supplementary description of what a choropleth map is, how to interpret a choropleth map, and things to be aware of when interpreting a choropleth map.

5.4.1.3 Trends

Temporal trends are portrayed through a dot plot over time with a supplementary smoothing line derived using LOESS (Appendix B. Figure 5.4). Two time series plots, depicting either the provincial *Varroa* status or the status disaggregated by CCS are included. Accompanying the regional time series plot is an interactive data table to offer further context and quantitative information. Like the maps, the time series plots are fully interactive allowing for full zoom, pan, and hover controls. Hovering over a single point on the plot reveals the date and *Varroa* count associated with it. Using the zoom tool, a select area of the plot can be enlarged to aid in instances of visual clutter. The data in this section can be filtered by year and CCS region which produces a new plot and data table for the selected criteria. Accompanying each plot is a supplementary description of what a timeseries plot is, how to interpret a timeseries plot and things to be aware of when interpreting a timeseries plot.

5.4.1.4 Historical Data

The data (post-processing) used in the *HIVE* dashboard can be accessed under this section (Appendix B. Figure 5.5). An interactive data table allows for sorting, filtering, and searching of data using one or more variables as the criteria, while still maintaining the privacy of a given observation.

5.4.1.5 Varroa Timeline

This section provides a consolidation of policy information put forth by OMAFRA regarding when and how to sample and treat for mites along with typical mite population dynamics adapted from the OIE Terrestrial Manual (OIE: World Organization for Animal

Health, 2021)(Appendix B. Figure 5.6). The graphic displays different mite population dynamic curves, one with and one without the application of treatment, and offers recommended treatment options specific to Ontario organized on a monthly timeline to account for regional climate effects on treatment efficacy (Aldea *et al.*, 2013; Underwood & Currie, 2003). This graphic is presented in Figure 5.1.

5.4.1.6 Information

The information section is dedicated to providing supplementary information to consolidate materials presented across various platforms and organizations. Basic information regarding what *Varroa* mites are, how they spread, and why they are an important issue are presented first with subsequent sections outlining OMAFRA recommended methods of sampling and treating (Figure 5.7). Video clips complement the text to aid in accessibility and support a diversity of learning styles (Figure 5.8). More details pertaining to the purpose and outlook of this dashboard along with a brief overview of the contributing members was included to improve personability and transparency of the project. Links to further reputable sources of information are also provided (Appendix B. Figure 5.9).

5.4.2 Dashboard Actionability Assessment

The third-party actionability assessment was completed by 14 members of the Ontario beekeeping community, including beekeepers and industry stakeholders. A complete breakdown of the responses to the assessment of the seven actionability criteria can be seen in Table 5.3. The *HIVE* dashboard scored highest in four of the seven actionability criteria (criteria #1, #2, #5, & #7), but was scored highly in all seven criteria. Several areas of improvement were identified for future iterations by the reviewers. The overall survey scores indicated that the current version

of the dashboard possesses a high degree of perceived actionability as defined by Ivanković et al. (2021) (Table 5.3).

Of the seven criteria, the three that received the lowest scores were criterions 3, 4, and 6. Criterion #3 “Reporting data sources and methods clearly” was scored with a tied mode of 2, and 1 out of possible 2 points (median score: 1). Criterion #4 “Linking time trends to policy and policy decisions” was scored with a mode of 2 out of 2 possible points (median score: 2), with a breakdown of 8 respondents feeling that this criterion was clearly met, 3 feeling that it was somewhat met, and 3 feeling that it was not met. The lower scores of this criterion were mainly reported as being due to the absence of time stamps on the time series plots regarding the initiation of new policy or intervention strategies. Criterion #6 “Disaggregating the information into relevant subgroups” scored by the reviewers with a mode of 2 points out of a possible 2 (median score: 1.5), where 7 respondents found this criterion to be clearly met, 6 found it to be somewhat met, and 1 did not feel that it was met. From the responses by the reviewers, the main note regarding this criterion was the low levels of data in some of the beekeeping regions. Furthermore, reviewers noted that a breakdown by operation type (i.e., commercial vs. hobbyist) and management strategies (i.e., treatment or sampling method) would be beneficial.

5.5 Discussion

IPM strategies are reliant on consistent and continuing data to track the status and spread of a pest over time and space, and ensure it remains at, or below, hypoendemic levels. Being able to compare current data to previous data is key to recognizing if current actions are effective or if modifications should be made. Furthermore, having comparative data can help to identify anomalies and outbreaks. Collection and analysis of large amounts of surveillance data surpass the realistic limits of traditional inspection methodologies which are typically limited to annual

reporting. A system to automatically record data, analyze them and disseminate basic epidemiologic metrics and figures, as proposed here, can delegate repetitious data management and basic analysis to computer automation and liberate manpower for more intensive data analysis, academic writing, and hypothesis generation (Kankanhalli, 2020). Such a system can analyze data in near real-time (at a rate similar to it being collected) allowing for monitoring of provincial *Varroa* levels continuously rather than annual analysis and reporting. During the COVID-19 pandemic, there was a surge of information dashboards translating numeric values of cases into digestible and intuitive graphics for reference by the general public and officials. At least twenty-seven COVID-19 dashboards were produced by volunteers in Canada alone (“COVID-19 Resources Canada,” 2021), not including those from official government sources, indicating the popularity and perceived usefulness of such tools by the public. Of the most popular COVID-19 dashboards, the Johns Hopkins COVID-19 dashboard received a billion views per day at the peak of its popularity (Center for System Science and Engineering (CSSE) at Johns Hopkins University (JHU), 2021; Dempsey, 2020). Dashboards such as these provide a level of autonomy and allow for the user to monitor data pertinent to them and make informed decisions accordingly.

In this project, the *HIVE* dashboard, an online tool for monitoring *Varroa* mite epidemiology in Ontario, was developed and proposed following key criteria found to be common amongst highly actionable COVID-19 dashboards, as identified by Ivanković *et al.*, 2021. Included in the *HIVE* dashboard are interactive visuals depicting the distribution of *Varroa* across both space and time, in addition to publishing filterable tabular data and general information regarding *Varroa* mites specific to OMAFRA recommendations. When applying a third-party actionability assessment, modified slightly from that proposed initially by Bos *et al.*

(2021), the *HIVE* dashboard scored highly in all of the 7 criteria. This assessment identified several areas of the dashboard that could then be subsequently improved. The dashboard was scored lowest in the following 3 criteria: (#3) clear reporting of data sources and methods, (#4) linking time trends to policy decisions, and (#6) disaggregating the information into relevant subgroups. There was a noticeable contrast between the reviewer scores in several of the criteria where some would state that the criterion was “clearly met”, and some would state that it was “not met”. This disagreement between the reviewers could have been mitigated with an in-person survey or a live demonstration of the dashboard rather than a self-guided online survey.

A clear presentation of the data, the methods used to develop indicators, and an explanation of the limitations of the data used are an important aspect in transparency and trust of an actionable dashboard (Ivanković *et al.*, 2021). The *HIVE* dashboard was developed with this criterion in mind and presented information related to it in the supplementary information section, however, through the third-party assessment it was revealed that the information was neither easy to find nor sufficiently detailed in the eyes of some evaluators. Following feedback from members of the beekeeping community, this information was enhanced, and made more easily accessible by including a more obvious link on the homepage to the data information section. A more detailed description of how the present data was collected, as well as plans for how data will be collected in the future, through a combination of apiary inspections and self-reported observations, was also included.

Linking of time trends to policy decisions was another criterion that was not ranked exceptional by third-party assessment. Ideally, this criterion would be achieved through annotations regarding the date of policy changes, and intervention implementations included on the time series plots of the *Varroa* data. Inclusion of such information can be easily realized,

however, annotations to the time series plots were not included at this stage of dashboard development due to the lack of sufficient data. It was believed that without sufficient data coverage of the province and at a fine enough temporal resolution, that including annotations such as “implementation of a *Varroa* awareness campaign” accompanying the time series data could be misinterpreted and not show the true influence of these strategies. A function such as this could be introduced later to provide encouragement to beekeepers that policy interventions are successful in reducing mite populations. For now, a figure depicting the theoretical population dynamics of *Varroa* mites across the beekeeping season was developed and included. This figure illustrates the timeline of recommended sampling and treatment policy and how such policy aids in maintaining low mite levels. Although this figure does not provide empirical evidence of the impact of *Varroa* management in Ontario, it provides evidence-based information and can serve until enough data are collected and time series plots can be appropriately annotated.

Likewise, limitations of data quantity hindered the ability to disaggregate the data further without resulting in instances of either no data or very little data in some breakdowns, leading to the low score achieved for the sixth criterion identified by Ivanković *et al.* (2021). Third-party reviewers of the dashboard noted that, in some instances, when the information being presented was broken down by region and/or year, there were very few data being presented. Furthermore, some expressed interest in seeing the data further subdivided by management factors (i.e., commercial vs. hobbyist, or *Varroa* treatment protocol), but this is not currently possible with the data available. Although many data points exist in the dataset, achieving sufficient and representative coverage for all regions, and management factors is difficult to achieve. A long-term solution to this bottleneck is the implementation of a citizen science data collection system

(discussed in the following). In the short term, a coarser spatial region structure could allow for additional disaggregation factors. However, too coarse of a structure could begin to lose biological relevance. Future versions of the dashboard would benefit from further levels of data disaggregation, provided that enough data are present to represent each category sufficiently. The current iteration of the *HIVE* dashboard, although actionable, will primarily serve to generate interest in this new *Varroa* monitoring system by providing a tangible tool for beekeepers and encourage more self-submitted mite count data by offering information in return.

Previous attempts have been made at producing an actionable dashboard for monitoring *Varroa* mite epidemiology, but they lacked several key components when compared to the *HIVE* dashboard. *MiteCheck*, developed by the North American Pollinator Partnership, is perhaps the most similar of these systems to the *HIVE* dashboard proposed in this work, but has only been moderately successful in attracting and retaining users; only having 500 users across North America in 2020 (Bee Informed Partnership, 2021b; Pollinator Partnership, 2020). *MiteCheck* is a volunteer-oriented program to support the monitoring of *Varroa* mites across North America and provides large-scale maps and preliminary plots to visualize *Varroa* distributions. *MiteCheck* parallels the proposed tool in a number of ways. As an education tool, both *MiteCheck* and the *HIVE* dashboard aim to educate beekeepers on how to monitor and treat for mites, as well as provide a community awareness of mite distributions and motivation for beekeepers to survey for *Varroa* (Engelsma, Milbrath, Rennich, & Willkes, 2019). And as a surveillance tool, both projects aim to offer information regarding the distribution of mites across a population. Because *MiteCheck* is a volunteer driven project, official ministry reports are not included in the dataset and self-reported mite counts are the primary source of data.

The *MiteCheck* project mainly falls short in three areas as a surveillance system: limited local relevance to a targeted area of beekeeping, lack of ability to disaggregate data into smaller sub-groups, and lack of visuals depicting continuous temporal information, to monitor changes in *Varroa* mite prevalence over time. Providing information specific to a particular location is not easily accomplished in the *MiteCheck* application because of the large target study area.

Although the *MiteCheck* app services a broad geographical area, the uptake in any subregion is less than that of the *HIVE* dashboard, that benefits from using government inspection data. By utilizing government inspection results to provide an initial source of information for the *HIVE* dashboard, beekeepers can get a tangible feel for the tool and how their data will be used before submitting their own counts. In contrast, the reliance on only self-submitted data by projects such as *MiteCheck* can lead to issues of building initial momentum to grow a large user base.

Furthermore, dashboards without a specific target region may be able to broadcast general information but would struggle to provide locally specific information (e.g., reminders of an upcoming provincial *Varroa* campaign, or notification of a newly approved treatment for *Varroa*). Furthermore, although it is useful to see a broad geographic map, end users should be provided with “data close to home” in order for them to make informed decisions based on locally relevant information (Ivanković *et al.*, 2021). Presenting information across too large a study area does not allow for any one area to be comprehensively represented. Additionally, in the absence of reported time series data, previously proposed tools make it difficult to monitor short and long-term trends or compare data to previous seasons. By not presenting data on a linear plot over time, users are not able to make inferences about the trajectory of population-level mite infestations or recognize patterns to anticipate high mite levels in their area. Unlike

previous *Varroa* dashboards, the one proposed in this work clearly depicts mite levels over time both at a regional scale and a provincial scale.

The *HIVE* dashboard, developed in this project, and *MiteCheck* share a similar goal but differ in function and actionability to a substantial degree. The *MiteCheck* dashboard excels in providing data across a large geographic area and has established a self-reporting system to collect information directly from beekeepers. However, the proposed *HIVE* dashboard is better suited to provide more locally specific information with more surveillance functionality. Both dashboards present similar information and are not inherently competing tools as they can both fill different needs in the beekeeping community. Surveillance tools reporting information over both broad (e.g., national, continental, or global) and specific (e.g., regional, or provincial) regions can, and should, co-exist to allow for comparisons between inter and intra-provincial apiaries. Ideally, they would be interoperable, using data standards with data available through an API (application programming interface), so that data could be combined from multiple sources as needed.

The data used in the development and first iteration of the *HIVE* dashboard come from a secondary database of apiary inspections which were collected before the conception of this project. The benefits of using such data are cost-effectiveness and convenience, but this also brings about several limitations to the presented information. Apiary inspections occur largely because of inspection requests, and the OMAFRA dataset is not necessarily representative of the true status of *Varroa* in the province. Requests for inspection may have been initiated to confirm a *Varroa*-free colony before travel or to address a weakened colony. Both of these scenarios would bias the data in opposite extremes of the true population mite level. A third reason for inspection is to support regular *Varroa* mite surveillance efforts, which is expected to be more

representative of the target population. As such, these data must be interpreted with a level of caution. Furthermore, the use of secondary data limits the functionality of this dashboard in terms of variables and metrics available by which the data can be filtered and presented.

5.5.1 Future Opportunities

5.5.1.1 Citizen Science

Looking forward, there are several opportunities to improve the usability and functionality of this dashboard and other related tools. First and foremost, would be the introduction of a citizen science data collection model to enhance the data on *Varroa* in Ontario. Citizen science is a subset of crowdsourcing and a broad term for involving non-scientists in the scientific process with a long history in epidemiology (Berke, Sobkowich, & Bernardo, 2020). With regards to the developed dashboard and proposed surveillance system, citizen science could be employed in the data collection stage through self-submitted mite counts. The number of citizen science projects in ecology has rapidly multiplied in recent years and is considered to be a viable tool for improving the scalability of data collection (Lepczyk, Boyle, Vargo, Gould, & Jordan, 2008). Several beekeeping organizations including the Ontario Animal Health Network and the Pollinator Partnership currently utilize beekeeper submitted mite counts in some capacity (Bee Informed Partnership, 2021a; OAHN Bee Network, 2021) with moderate success, meaning that sampling activity by citizen scientists is currently much lower than that through official inspections. The Pollinator Partnership's 'Mite-A-Thon' campaign (in partnership with the *MiteCheck* dashboard) relies on beekeepers self-submitting their mite counts but is conducted as a survey, open only for reporting during two distinct months every year (Pollinator Partnership, 2021) rather than a continuously open data submission portal over the beekeeping season. Therefore, it cannot be used to actively monitor trends between the reporting periods to track the

success of interventions or detect emerging outbreaks. In theory, deploying a comprehensive citizen science data collection tool in Ontario that allows continuous submission of mite counts during the season would enhance surveillance activities at no additional expense to beekeepers or ministry officials since self-conducted inspections are already part of responsible beekeeping practices. With sufficient daily submissions from beekeepers across a larger geographic area and at regular enough intervals, information on *Varroa* hotspots and emerging high-risk areas could be identified and communicated back to beekeepers; similar to how the ‘Outbreaks Near Me’ program tracked and mapped self-reported COVID-19 symptoms during the pandemic (Harvard Medical School, Boston Children’s Hospital, & University of Toronto, 2021). Crowdsourced data collection permits a greater quantity of data to be collected across large study areas. This method of data collection increases the potential for representation of those in remote areas which may otherwise be sampled infrequently. Citizen science can therefore improve the power to detect anomalies in areas typically under-surveyed and increase the number of repeated samples occurring within a single region over time to monitor changes in mite populations.

Channeling self-submitted mite counts directly into an analysis tool such as a dashboard allows for a near real-time information system without reliance on further human interaction. With rapidly updated information, beekeepers could be warned of increasing mite levels surrounding their yards and receive recommended intervention strategies to limit the spread. Government officials could also benefit from continuously updated reports on population mite loads to determine if control thresholds are being met or if further interventions are required.

A major concern in citizen science projects is the retention of contributors. Several motivating factors have been identified from “usefulness of the project” to “recognition of their contributions”, and thus consideration of how to optimize retention must be given in the planning

stages (Asingizwe *et al.*, 2020). Participants' awareness that they are contributing to a tool that is beneficial to both themselves as well as the community can increase retention on the basis of social motivations and personal empowerment, as identified by West & Pateman (2016).

5.5.1.2 Big Data and Machine Learning

Increased data volume derived from citizen scientist beekeepers will allow for more sophisticated analysis, higher degrees of disaggregation in dashboard figures, and greater power to detect irregularities, but will be accompanied by the challenges that come with big data. Big data are defined as data possessing five key characteristics: velocity, volume, value, variety, and veracity (Jain, 2016) all of which can be present in crowdsourced data for a *Varroa* surveillance dashboard. With beekeepers' varied levels of experience in sampling for *Varroa*, variations in sampling techniques and potential dishonesty about true mite counts, comes an inherent "messiness" in these data. Sampling for *Varroa* mites will inevitably incur some amount of error and therefore there is an increase in the expected levels of measurement error and bias in the data which will need to be addressed. However, there should not be a reliance exclusively on "perfect" data when analyzing population levels (Mayer-Schönberger & Cukier, 2013). Big data allows for the luxury of large sample sizes to outweigh some inexact measurements rather than removing them, reducing random error, and arriving at the same correct value on average through the benefit of "collective intelligence". Collective intelligence in principle enables information to be generated by a group that could not exist at an individual level (Suran, Pattanaik, & Draheim, 2021). In other words, although no single observation is guaranteed to be correct, on average the data collected through citizen science will be correct as a combination of over and underreporting balance each other out. Although collective intelligence is able to reduce the impact of random error, it cannot account for systematic error. Regardless of if a dataset is

collected by professionally trained inspectors or citizen scientists, statistical methods are available to control for their impacts (Bird *et al.*, 2014; Kosmala, Wiggins, Swanson, & Simmons, 2016).

Programs such as the ‘BlueDot Outbreak Intelligence System’ have shown that by combining big data with machine learning, outbreaks can be detected much earlier as demonstrated by their recognition of the COVID-19 outbreak in Wuhan, China nine days before it was announced by the World Health Organization (Stieg, 2020). The success of BlueDot’s Outbreak Intelligence System was the result of synergistically using multiple streams of data, including web-scraping techniques to gather unofficial information from sources such as Twitter and Google. The use of Google search trends alone for disease surveillance has also been previously successful in smaller-scale projects (Kutera, Berke, & Sobkowich, 2021) and could be implemented to supplement *Varroa* surveillance. Monitoring web search results regarding *Varroa*, and *Varroa*-related symptoms could be used to recognize higher than normal queries in a given region, suggesting that mite levels could be rising. Automated systems could be integrated within disease surveillance dashboards, such as the *HIVE*, and utilize machine learning, or other methods for aberration detection, to provide beekeepers with an early warning of elevated mite risk specific to their region so that targeted measures can be implemented.

5.5.1.3 Sensors

Sensors and smartphone applications provide another opportunity for enhancement of dashboards. Early-stage applications such as *Bee Health Guru* (Firth, 2020) and *ApiZoom* (Bugnon, Thiran, & Chevassus, 2021) offer rapid *Varroa* mite sampling using either the camera or microphone of a smartphone, which could allow for more observations by reducing the workload of beekeepers and also provide a standardized level of accuracy across inspections.

However, further testing is needed to confirm the validity of these technologies before widespread adoption. A review of colony sensors (Meikle & Holst, 2015) recognized the promise of sensor technology and states that the use of a combination of sensors, monitoring a variety of colony variables (e.g., weight, colony temperature, and sound) continuously, will become more common place in the future of beekeeping. This technology could therefore lead to an abundance of data and a great need for information dashboards such as the *HIVE* project to manage and consolidate basic information into a digestible format for a broad audience. This level of sensor uptake likely will not exist for several years, however in the meantime, small amounts of strategically placed sensors in sentinel colonies (Bee Informed Partnership, 2016) across the province could achieve a level of additional information to supplement current data, and offer the ability to cross-reference against beekeeper submitted data.

5.5.2 Privacy Considerations

When designing data dashboards such as these, special consideration must be given to protect the privacy of the data being collected, in this case primarily the location and *Varroa* status of individual beekeeping operations. Censorship of exact apiary locations is necessary for the protection of property from vandalism and theft as well as the encouragement of beekeepers to submit accurate *Varroa* counts without fear of being blamed for elevated regional mite levels. Although spatial epidemiological analyses typically perform better with exact point data, privacy considerations are an important aspect of public and animal health and must be a priority (Olson, Grannis, & Mandl, 2006). Spatial epidemiological methods can still achieve locally relevant information for stakeholders while maintaining individual privacy rights through methods such as interpolation and masking (Armstrong, Rushton, & Zimmerman, 1999). Aggregation of spatial data can impact the interpretation of the results depending on which regional structures

are selected (i.e., the modifiable areal unit problem (Openshaw, 1984)) but this is less of a concern for descriptive mapping applications commonly found in dashboards. Furthermore, data privacy must be considered when implementing high degrees of data filtering and disaggregation, since higher levels of specificity in filtering criteria can result in smaller possible populations and potential exposure of individual operation identities. Although the collection and analysis of data could be conducted using data of detailed spatial resolution and then communicated as aggregated data to censor private information, hesitancy of the beekeepers to submit identifying information could hinder participation and thus the quantity of data collected leading to a lower quality of information. These privacy considerations remain the most important factor when designing and implementing citizen science supported data dashboards.

5.6 Conclusion

Interactive data dashboards played a large role in mobilizing epidemiologic information throughout the SARS-CoV2 (COVID-19) pandemic, and their widespread uptake indicates an appreciation and perceived value by the general public. This project demonstrates that these same knowledge mobilization tools can be adapted for use in other diseases and animal species to aid in surveillance and to support integrated pest management systems. Although the presented dashboard is limited by a relatively low quantity of data, a high degree of actionability was subjectively awarded by third-party reviewers, and opportunities for future improvements were identified. This project provides a framework for epidemiologists and ministries to produce similar tools to support surveillance of other diseases across all species and can even be beneficial in monitoring plant disease states, such as the spread of leaf rust (*Puccinia triticina*) in wheat crops. As big data becomes the norm, there will be a greater need for automated surveillance systems to manage databases and conduct primary epidemiologic analyses to

monitor disease, generate hypotheses, and reallocate skilled researchers to more intensive studies.

5.7 References

- Aldea, P., Rodriguez, R., Olivares, A., Farffin, M., Riveros, D., Nfifiez, F., & Trivelli, L. (2013). Effect of ambient temperature and humidity conditions on the efficacy of organic treatments against *Varroa destructor* in different climatic zones of Chile. *Journal of Agricultural Science and Technology*, 3(6), 474–483.
- Armstrong, M. P., Rushton, G., & Zimmerman, D. L. (1999). Geographically masking health data to preserve confidentiality. *Statistics in Medicine*, 18(5), 497–525.
[https://doi.org/https://doi.org/10.1002/\(SICI\)1097-0258\(19990315\)18:5<497::AID-SIM45>3.0.CO;2-%23](https://doi.org/https://doi.org/10.1002/(SICI)1097-0258(19990315)18:5<497::AID-SIM45>3.0.CO;2-%23)
- Asingizwe, D., Poortvliet, P. M., Koenraadt, C. J. M., van Vliet, A. J. H., Ingabire, C. M., Mutesa, L., & Leeuwis, C. (2020). Why (not) participate in citizen science? Motivational factors and barriers to participate in a citizen science program for malaria control in Rwanda. *PLOS ONE*, 15(8), e0237396. <https://doi.org/10.1371/journal.pone.0237396>
- Barbazza, E., Ivanković, D., Wang, S., Gilmore, K. J., Poldrugovac, M., Willmington, C., ... Kringos, D. (2021). Exploring changes to the actionability of COVID-19 dashboards over the course of 2020 in the Canadian context: descriptive assessment and expert appraisal study. *Journal of Medical Internet Research*, 23(8), e30200. <https://doi.org/10.2196/30200>
- Bee Informed Partnership. (2016). Sentinel apiary project. Retrieved December 7, 2021, from <https://beeinformed.org/2016/03/21/sentinel-apiary-project/>
- Bee Informed Partnership. (2021a). Citizen science. Retrieved December 7, 2021, from <https://beeinformed.org/citizen-science/>
- Bee Informed Partnership. (2021b). MiteCheck. Retrieved December 7, 2021, from <https://research.beeinformed.org/mitecheck/>
- Berke, O., Sobkowich, K. E., & Bernardo, T. M. (2020). Celebration day: 400th birthday of John Graunt, citizen scientist of London. *Environmental Health Review*, 63(3), 67–69. <https://doi.org/10.5864/d2020-018>
- Bernardo, T. M., Sobkowich, K. E., Forrest, R. O., Stewart, L. S., D'Agostino, M., Perez Gutierrez, E., & Gillis, D. (2021). Collaborating in the time of COVID-19: the scope and scale of innovative responses to a global pandemic. *JMIR Public Health and Surveillance*, 7(2), e25935. <https://doi.org/10.2196/25935>
- Bird, T. J., Bates, A. E., Lefcheck, J. S., Hill, N. A., Thomson, R. J., Edgar, G. .., ... Frusher, S. (2014). Statistical solutions for error and bias in global citizen science datasets. *Biological Conservation*, 173, 144–154. <https://doi.org/10.1016/j.biocon.2013.07.037>
- Bos, V., Jansen, T., Klazinga, N. S., & Kringos, D. S. (2021). Development and actionability of the Dutch COVID-19 dashboard: descriptive dsessment and expert appraisal study. *JMIR Public Health and Surveillance*, 7(10), e31161. <https://doi.org/10.2196/31161>
- Bugnon, A., Thiran, J. P., & Chevassus, G. (2021). Apizoom. Retrieved from <https://www.apizoom.app/>

- Center for System Science and Engineering (CSSE) at Johns Hopkins University (JHU). (2021). COVID-19 Dashboard. Retrieved December 7, 2021, from <https://coronavirus.jhu.edu/map.html>
- Cheng, J., Karambelkar, B., & Xie, Y. (2018). Leaflet: create interactive web maps with the JavaScript “Leaflet” library. R package version 2.0.2. Retrieved from <https://cran.r-project.org/package=leaflet>
- Claing, G., Kempers, M., Kennedy, K., Kozak, P., Lafrenière, R., Maund, C., ... Hoover, S. (2020). Statement on honey bee wintering losses in Canada (2020). *Canadian Association of Professional Apiculturists*. Retrieved from <http://capabees.org/shared/2015/07/2015-CAPA-Statement-on-Colony-Losses-July-16-Final-16-30.pdf>
- COVID-19 Resources Canada. (2021). Retrieved December 7, 2021, from <https://covid19resources.ca/public/other-initiatives/>
- Dahle, B. (2010). The role of *Varroa destructor* for honey bee colony losses in Norway. *Journal of Apicultural Research*, 49(1), 124–125. <https://doi.org/10.3896/IBRA.1.49.1.26>
- Dempsey, C. (2020). The Johns Hopkins coronavirus map dashboard receives a billion hits a day. Retrieved January 15, 2022, from <https://www.gislounge.com/the-johns-hopkins-coronavirus-map-dashboard-receives-a-billion-hits-a-day/>
- Engelsma, J., Milbrath, M., Rennich, K., & Willkes, J. (2019). The MiteCheck app. Retrieved January 13, 2022, from <https://www.beeculture.com/the-mitecheck-app/>
- Firth, D. (2020). Bee Health Guru - a smartphone app for beekeepers. Retrieved January 15, 2022, from <https://www.kickstarter.com/projects/beehealthguru/bee-health-guru-a-smartphone-app-for-beekeepers>
- Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443–450. <https://doi.org/10.1051/apido/2009076>
- Harvard Medical School, Boston Children’s Hospital, & University of Toronto. (2021). Outbreaks Near Me. Retrieved December 7, 2021, from <https://outbreaksnearme.org/ca/en-CA/>
- Ivanković, D., Barbazza, E., Bos, V., Brito Fernandes, Ó., Jamieson Gilmore, K., Jansen, T., ... Kringsos, D. (2021). Features constituting actionable COVID-19 dashboards: descriptive assessment and expert appraisal of 158 public web-based COVID-19 dashboards. *Journal of Medical Internet Research*, 23(2), e25682. <https://doi.org/10.2196/25682>
- Jain, A. (2016, September). The 5 V's of big data. *IBM: Watson Health Perspectives*. Retrieved from <https://www.ibm.com/blogs/watson-health/the-5-vs-of-big-data/>
- Kankanhalli, A. (2020). Artificial intelligence and the role of researchers: can it replace us? *Drying Technology*, 38(12), 1539–1541. <https://doi.org/10.1080/07373937.2020.1801562>
- Kosmala, M., Wiggins, A., Swanson, A., & Simmons, B. (2016). Assessing data quality in citizen science. *Frontiers in Ecology and the Environment*, 14(10), 551–560.

<https://doi.org/10.1002/fee.1436>

- Kozak, P., Eccles, L., Kempers, M., Rawn, D., Lacey, B., & Guzmán-Novoa, E. (2021). Ontario treatment recommendations for honey bee disease and mite control. Retrieved June 24, 2021, from <http://www.omafra.gov.on.ca/english/food/inspection/bees/2017-treatment.htm#VM>
- Kralj, J., Brockmann, A., Fuchs, S., & Tautz, J. (2007). The parasitic mite *Varroa destructor* affects non-associative learning in honey bee foragers, *Apis mellifera* L. *Journal of Comparative Physiology A*, 193(3), 363–370. <https://doi.org/10.1007/s00359-006-0192-8>
- Kralj, J., & Fuchs, S. (2006). Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. *Apidologie*, 37(5), 577–587. <https://doi.org/10.1051/apido:2006040>
- Kutera, M., Berke, O., & Sobkowich, K. E. (2021). Spatial epidemiological analysis of Lyme disease in southern Ontario utilizing Google Trends searches. *Environmental Health Review*, 64(2), 1–6. <https://doi.org/10.5864/d2021-025>
- Le Conte, Y., Ellis, M., & Ritter, W. (2010). *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie*, 41(3), 353–363. <https://doi.org/10.1051/apido/2010017>
- Lepczyk, C. A., Boyle, O. D., Vargo, T. L., Gould, P., & Jordan, R. (2008). Citizen science in ecology: the intersection of research and education. In *Citizen Science in Ecology: the Intersection of Research and Education*. Bulletin of the Ecological Society of America. Retrieved from <https://esajournals.onlinelibrary.wiley.com/doi/pdf/10.1890/0012-9623-90.3.308>
- Mayer-Schönberger, V., & Cukier, K. (2013). *Big Data: a Revolution That Will Transform How We Live, Work and Think*. London: John Murray.
- Meikle, W. G., & Holst, N. (2015). Application of continuous monitoring of honeybee colonies. *Apidologie*, 46(1), 10–22. <https://doi.org/10.1007/s13592-014-0298-x>
- Neuwirth, E. (2014). RColorBrewer: ColorBrewer palettes. Retrieved from <https://cran.r-project.org/web/packages/RColorBrewer/index.html>
- OAHN Bee Network. (2021). Submit *Varroa* mite counts. Retrieved December 7, 2021, from <https://www.oahn.ca/network/bee/>
- OIE: World Organization for Animal Health. (2021). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2021. Retrieved December 7, 2021, from <https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>
- Olson, K. L., Grannis, S. J., & Mandl, K. D. (2006). Privacy protection versus cluster detection in spatial epidemiology. *American Journal of Public Health*, 96(11), 2002–2008. <https://doi.org/10.2105/AJPH.2005.069526>
- OMAFRA. (2021). *Varroa* mite - sampling and monitoring infestation levels. Retrieved December 7, 2021, from <http://www.omafra.gov.on.ca/english/food/inspection/bees/varroa->

sampling.htm

- Openshaw, S. (1984). *The Modifiable Areal Unit Problem*. Norwich, England: Geo Books.
- Peck, D. T., & Seeley, T. D. (2019). Mite bombs or robber lures? The roles of drifting and robbing in *Varroa destructor* transmission from collapsing honey bee colonies to their neighbors. *PLoS ONE*, 14(6), 1–14. <https://doi.org/10.1371/journal.pone.0218392>
- Peck, D. T., Smith, M. L., & Seeley, T. D. (2016). *Varroa destructor* mites can nimbly climb from flowers onto foraging honey bees. *PLoS ONE*, 11(12), e0167798. <https://doi.org/https://doi.org/10.1371/journal.pone.0167798>
- Pollinator Partnership. (2020). *2020 Mite-A-Thon final report*. Retrieved from <https://www.pollinator.org/pollinator.org/assets/globals/2020-Mite-A-Thon-Report.pdf>
- Pollinator Partnership. (2021). North American Mite-A-Thon: May 1st-16th & August 14th-29th. Retrieved December 7, 2021, from <https://www.pollinator.org/miteathon>
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103(SUPPL. 1), S96–S119. <https://doi.org/10.1016/j.jip.2009.07.016>
- Sievert, C. (2020). *Interactive Web-Based Data Visualization with R, Plotly, and Shiny*. Chapman and Hall/CRC. Retrieved from <https://plotly-r.com>
- Sobkowich, K. E., Berke, O., Bernardo, T. M., Pearl, D. L., & Kozak, P. (2021). Mapping the population density of managed honey bee (*Apis mellifera*) colonies in Ontario, Canada: 2018. *Journal of Apicultural Science*, 65(2), 303–314. <https://doi.org/10.2478/jas-2021-0023>
- Statistics Canada. (2018). Census consolidated subdivisions (CCS). Retrieved December 7, 2021, from <https://www150.statcan.gc.ca/n1/pub/92-195-x/2011001/geo/ccs-sru/ccs-sru-eng.htm>
- Stieg, C. (2020, March 6). How this Canadian start-up spotted coronavirus before everyone else knew about it. *CNBC*. Retrieved from <https://www.cnbc.com/2020/03/03/bluedot-used-artificial-intelligence-to-predict-coronavirus-spread.html>
- Suran, S., Pattanaik, V., & Draheim, D. (2021). Frameworks for collective intelligence. *ACM Computing Surveys*, 53(1), 1–36. <https://doi.org/10.1145/3368986>
- Talbot, J., Setlur, V., & Anand, A. (2014). Four experiments on the perception of bar charts. *IEEE Transactions on Visualization and Computer Graphics*, 20(12), 2152–2160. <https://doi.org/10.1109/TVCG.2014.2346320>
- Underwood, R. M., & Currie, R. W. (2003). The effects of temperature and dose of formic acid on treatment efficacy against *Varroa destructor* (Acar: *Varroidae*), a parasite of *Apis mellifera* (Hymenoptera: *Apidae*). *Experimental and Applied Acarology*, 29.
- University of Guelph. (2021). University of Guelph Honey Bee Research Centre. Retrieved December 7, 2021, from University of Guelph Honey Bee Research Centre
- Vidal-Naquet, N. (2018). *Honeybee Veterinary Medicine*: Apis mellifera L. Sheffield, UK: 5m

Publishing.

West, S., & Pateman, R. (2016). Recruiting and retaining participants in citizen science: what can be learned from the volunteering literature? *Citizen Science: Theory and Practice*, 1(2), 15. <https://doi.org/10.5334/cstp.8>

Xie, Y., Cheng, J., & Tan, X. (2021). DT: a wrapper of the JavaScript library “DataTables.” Retrieved from <https://cran.r-project.org/package=DT>

5.8 Tables

Table 5.1: Seven criteria for an actionable data dashboard.

Criteria	Description
1 Knowing the audience and their information needs	Dashboards with a known audience and explicit aim had focus and continuity in their content, analysis, and delivery. Techniques such as guiding key questions or overall composite scores clearly communicated the decision they intended to support. Multilanguage functionality and exact timing of updating signaled an awareness and intent to encourage their regular use by the intended decision maker.
2 Managing the type, volume, and flow of displayed information	The selection of a concise number of indicators brought focus and importance to the information and the possibility to view indicators together at a glance. The use of indicators in moderation, although still spanning varied types of information, was especially effective. The ordering of information, from general to specific or in sections based on theme, made the flow of information intuitive.
3 Reporting data sources and methods clearly	A clear source of data and explanation of an indicator's construction, including potential limitations, was found to be an important component of trust in the dashboard and clarity in its reporting. This information can be provided in short narratives that support users to understand what is in fact being presented.
4 Linking time trends to policy decisions	Reporting data over time together with the introduction of key infection control measures facilitated an understanding of their effect (or lack thereof). This was found to be conducive to generating public support for infection control measures.
5 Providing data "close to home"	To inform individuals of risks in their immediate surroundings, granular geographic breakdowns are needed. Data that are highly aggregated are difficult to understand. Maps (over tables and charts) were most effective to provide geographic information.
6 Disaggregating the information into relevant subgroups	Providing data with the possibility to explore varied population characteristics made indicators relatable to individual users. It enables understanding of risks and trends based on one's own demographics. It can also facilitate equity-driven decision-making by exposing differences among the population.
7 Using storytelling and visual cues	A concise narrative explaining the significance of a trend supports users to understand the importance of the information. Bare statistics without a narrated analysis leave the burden of interpretation solely to the user. Brief explanations on the meaning of trends used in combination with visual techniques, such as intuitive color schemes and icons, supported ease of interpretation.

Retrieved from Ivanković *et al.* (2021)

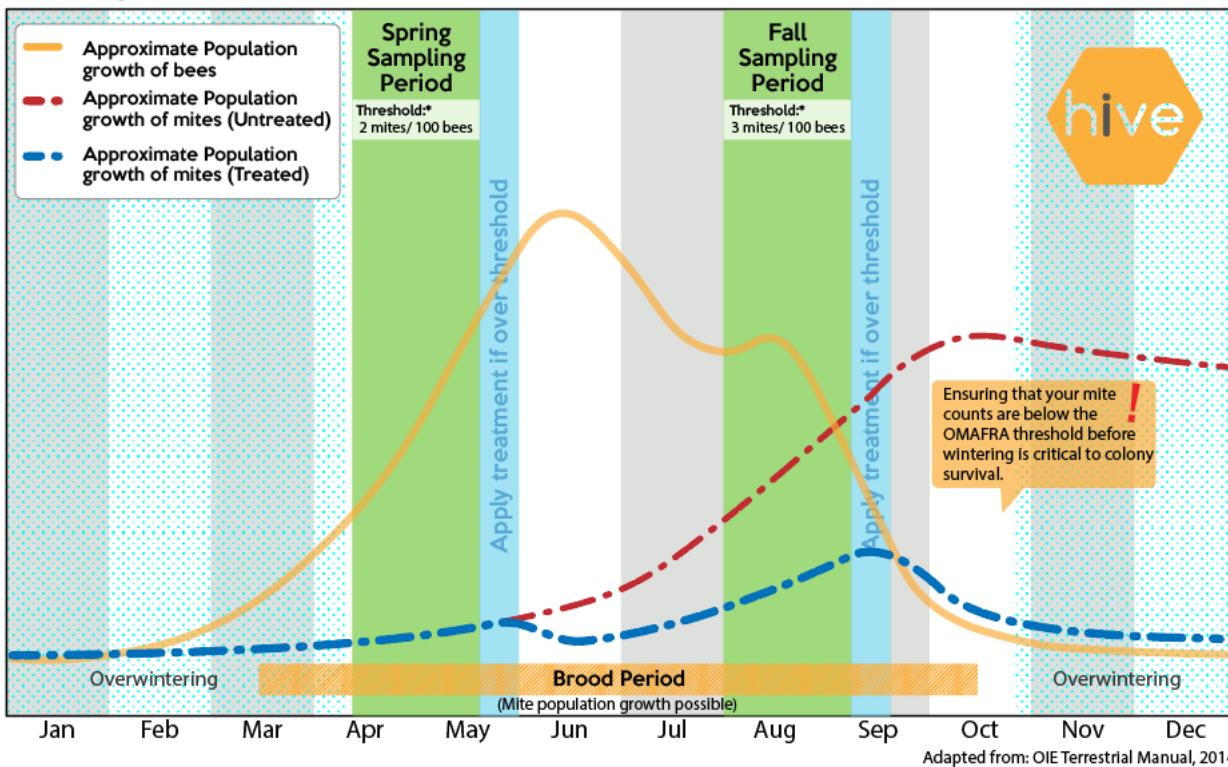
Table 5.2: Descriptive statistics of *Varroa* sampling inspections in Ontario between 2015-2019 at the CCS level.

Year	Total Inspections	CCS Regions Sampled (max. 273)	Percentage of Regions Sampled	Average Regional <i>Varroa</i> Count per 100 Bees Sampled			
				Mean	Median	Minimum	Maximum
2015	1101	133	48.7%	1.31	0.40	0.00	15.67
2016	1040	142	52.0%	0.98	0.30	0.00	23.00
2017	794	120	44.0%	1.39	0.23	0.00	51.00
2018	586	115	42.1%	0.39	0.11	0.00	7.56
2019	537	107	39.2%	0.85	0.17	0.00	7.55
All	4058	208	76.2%	1.21	0.57	0.00	23.50

Table 5.3: Results of third-party assessment of the actionability of the *HIVE* dashboard based on seven criteria developed by Ivanković *et al.* (2021).

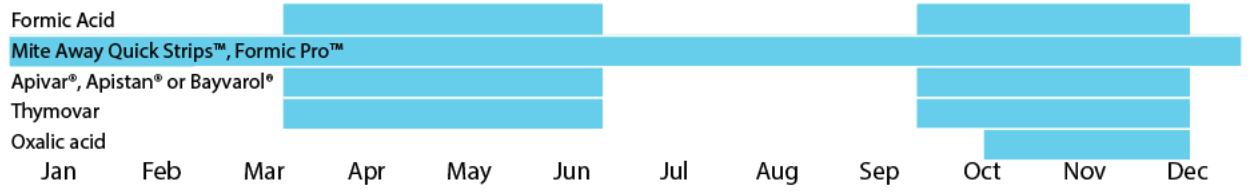
	Survey Responses (n=14)			Total Points (Max. 28)
	2 Criterion Clearly Met	1 Criterion Somewhat Met	0 Criterion Not Met	
Criterion #1: Knowledge of the audience and their information needs	10 (71%)	4 (29%)	0	24
Criterion #2: Appropriate management of type, volume and flow of displayed information	12 (86%)	2 (14%)	0	26
Criterion #3: Clear reporting of data sources and methods	6 (43%)	6 (43%)	2 (14%)	18
Criterion #4: Linking of time trends to policy decisions	8 (57%)	3 (21%)	3 (21%)	19
Criterion #5: Providing data "close to home"	11 (79%)	1 (7%)	2 (14%)	23
Criterion #6: Dividing information into relevant subgroups	7 (50%)	6 (43%)	1 (7%)	20
Criterion #7: Use of storytelling and visual cues	9 (64%)	4 (29%)	1 (7%)	22

5.9 Figures



Treatments Available

(bars indicate suitable months for each treatment)



^{*}Threshold values based on alcohol wash method.
Ether Roll: (Spring) 1m/b, (Fall) 2m/b
Sticky Board: (Spring) 9 m/24hrs, (Fall) 12 m/24hrs

Figure 5.1: *Varroa* population dynamics and treatment recommendations specific to Ontario, illustrated and included in the *HIVE* dashboard.

5.10 Appendix A. Access to the *HIVE* Dashboard

The *HIVE* dashboard is currently hosted on a free server through ShinyApps.io. To access the current version please follow the link provided:

<https://kurtissobkowich.shinyapps.io/20211025VarroaOntarioV3/>

In the event that the link is no longer active, please contact the corresponding author.

5.11 Appendix B. Screen Captures of the *HIVE* Dashboard

The screenshot shows the homepage of the *HIVE* dashboard. At the top left is a yellow hexagonal logo with the word "hive" in white. To its right is a red button with white text that says "Submit your mite counts". Below the logo is a navigation bar with five tabs: "Overview" (selected), "Maps", "Trends", "Historical Data", and "Varroa Timeline". To the right of the tabs is another tab labeled "Information".

Welcome to the *hive* dashboard

This project is designed to help inform beekeepers in Ontario about the landscape of Varroa mites across the province and the methods available to protect your hives. In order to accurately represent the prevalence of mites in the province, more samples are required. Please consider supporting this project and beekeeping in Ontario by submitting your mite counts regularly throughout the season.

To learn more about monitoring your mite counts refer to the information tab.

For more information about this dashboard, including the origins of the data presented, please click the Information tab

The main content area features several sections with icons and descriptions:

- Map**: Easily view the distribution of observed varroa.
- Trends**: Monitor the trends of varroa prevalence over time.
- Data**: View provincial varroa data and subset by your region.
- General Varroa Timeline**: See a general overview of how varroa mite populations progress throughout a year.
- Information**: Get more information on varroa sampling, treatment and management.
- Resources**: Helpful resources on varroa mites and best practices.

A large, blurred background image at the bottom shows a person's hands working with bees.

Figure 5.2: Screen capture of the *HIVE* dashboard landing page.

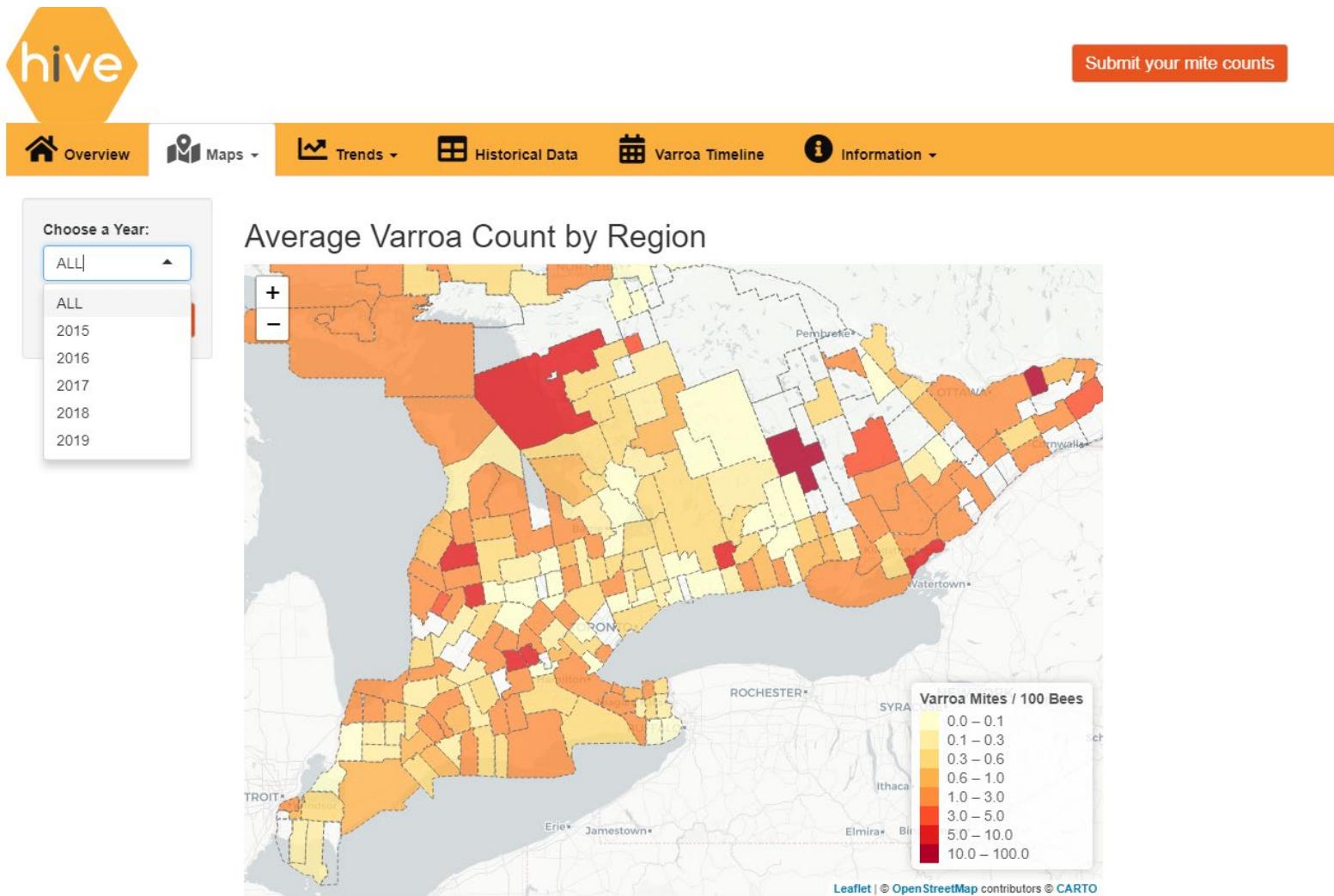


Figure 5.3: Screen capture of the *HIVE* dashboard ‘Map’ section.

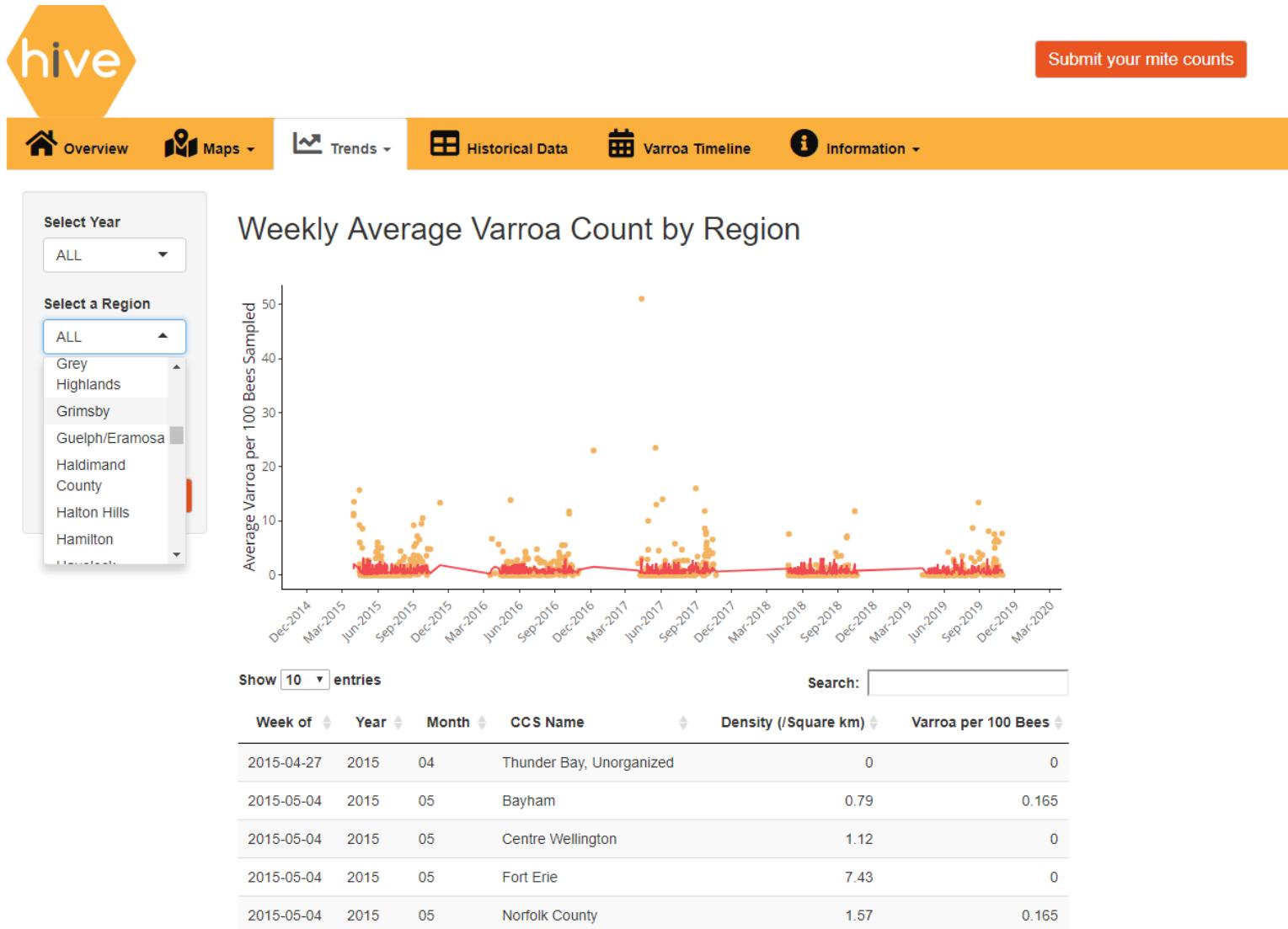


Figure 5.4: Screen capture of the *HIVE* dashboard ‘Trends’ section.

The screenshot shows the HIVE dashboard interface. At the top left is the HIVE logo. To its right is a red button labeled "Submit your mite counts". Below the logo is a navigation bar with six items: "Overview", "Maps", "Trends", "Historical Data" (which is highlighted in yellow), "Varroa Timeline", and "Information".

Ontario Varroa Data

Show 10 entries

Search:

Year	Month	Region	Samples	Density	Varroa
All	All	Guelph	All	All	All
2015	June	Guelph/Eramosa	1	2.31	0
2015	August	Guelph/Eramosa	1	2.31	0
2016	May	Guelph/Eramosa	4	2.31	0
2017	April	Guelph/Eramosa	2	2.31	4
2017	June	Guelph/Eramosa	13	2.31	0.23
2017	July	Guelph/Eramosa	2	2.31	1.5
2017	August	Guelph/Eramosa	1	2.31	0
2017	September	Guelph/Eramosa	2	2.31	1
2018	June	Guelph/Eramosa	4	2.31	0
2018	August	Guelph/Eramosa	2	2.31	0.5

Showing 1 to 10 of 15 entries (filtered from 1,127 total entries)

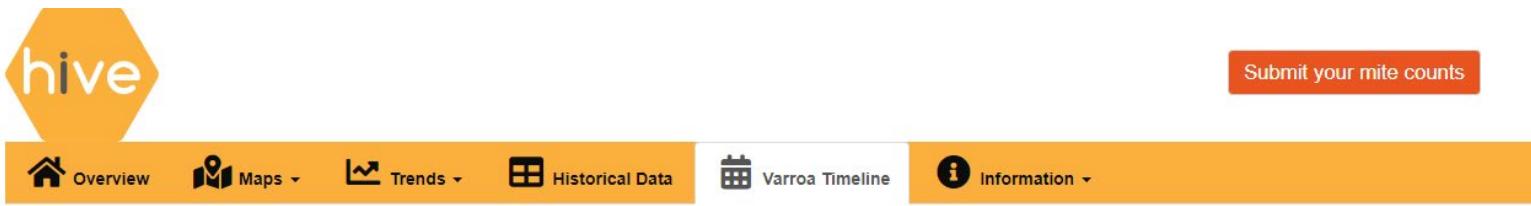
Previous 1 2 Next

*Samples = the total number of colonies inspected in the specified month.

*Density = the number of registered colonies in a region divided by the landmass of the region in kilometers. (Colonies per Square Km)

*Varroa = the average number of varroa mites sampled. Measured as the number of mites per 100 bees.

Figure 5.5: Screen capture of the HIVE dashboard ‘Historical Data’ section.



Typical Timeline of Varroa Mite Growth

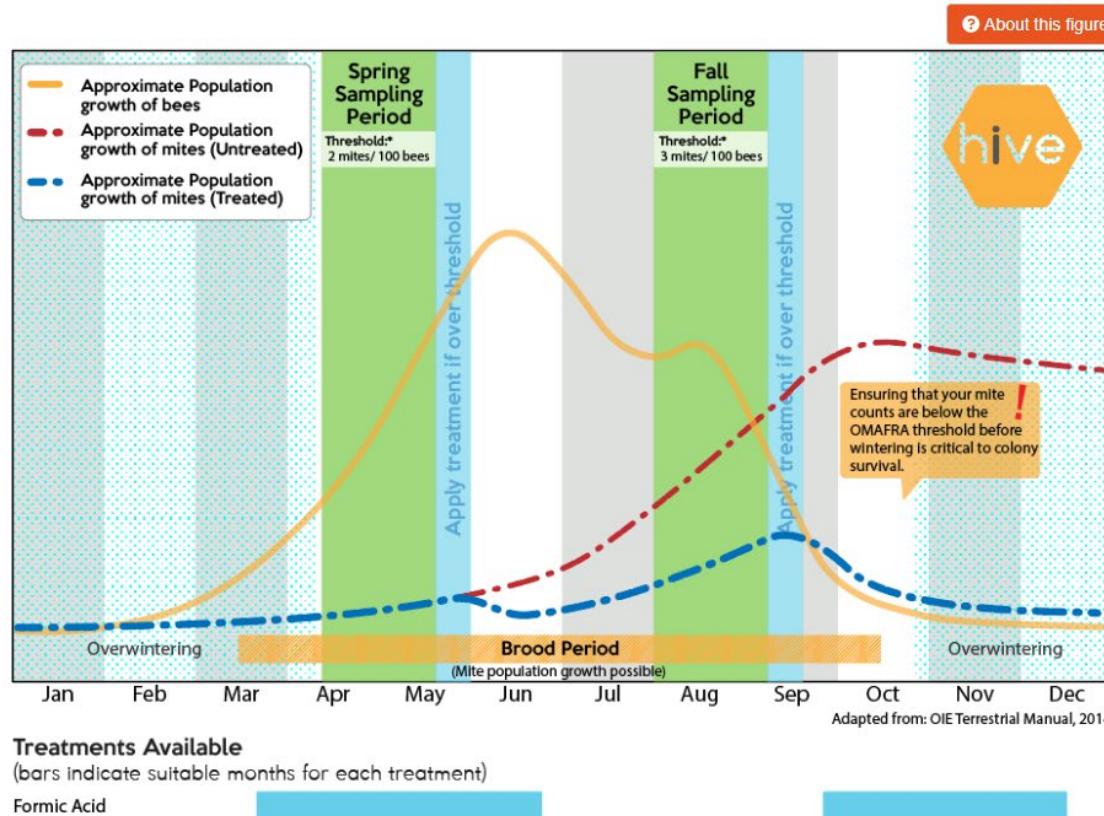


Figure 5.6: Screen capture of the HIVE dashboard ‘Varroa Timeline’ section.



Varroa Population Dynamics



Varroa Population Dynamics

:

VARROA POPULATION DYNAMICS

Brief Overview

The varroa mite (*Varroa destructor*) is a parasite of honey bees, and a global issue within the beekeeping community. These mites are classified as an external parasite, meaning they remain on the surface of the bee, like how a tick remains on the surface of a dog or human but embed their heads beneath the surface to feed. Varroa mites are widely regarded as the greatest threat to colony health and can decimate a colony if left untreated.

What do they look like?

Mature varroa mites are roughly 1 to 2mm in size and a dark red-brown in colour. They have a flat oval body shape with 8 protruding legs. These mites are visible with the naked-eye but may be tricky to spot given their preferred feeding location on the underside of the abdomen of the bee and their dark colour. A visual check of your bees and the brood is not sufficient to determine your mite load and a proper sampling method should be implemented. See the 'Varroa Sampling' tab for more information.

Figure 5.7: Screen capture of the HIVE dashboard 'General Information' section.

The screenshot shows the HIVE dashboard with a yellow hexagonal logo on the left. The top navigation bar includes links for Overview, Maps, Trends, Historical Data, Varroa Timeline (which is highlighted in orange), and Information. A red button on the right says "Submit your mite counts". Below the navigation, there are two sections: "Alcohol Wash Method" and "Sticky Board Method".

Alcohol Wash Method

Varroa Alcohol Wash

VARROA ALCOHOL WASH

Steps

1. Collect half a cup of worker bees (approximately 300 bees) from the brood chamber of the colony. Place the bees inside a well sealed container and add alcohol (at 70%).
2. Ensure the alcohol completely covers the honey bees in the container, level approximately 2 cm above the surface of the bees.
3. Vigorously shake the sample in the container for two minutes to dislodge the varroa from the bodies of the worker bees.
4. Pour the mixture of dead bees, mites and alcohol onto a 1/8 inch hardware cloth, mesh wire screen over a receiving container or pan to filter out the honey bees from the smaller varroa. The container or pan should be light coloured or clear so the varroa can be easily seen.
5. Count the varroa mites in the container or pan. Divide by three to obtain the percentage of infestation. For example, if you have 3 varroa in a sample of 300 bees then $3/300 = 1/100$ or 1% infestation.
6. Dispose of the dead bees and rinse the container with water to remove the mites between samples.

Sticky Board Method

Varroa Sticky Boards

Steps

1. Mark a piece of heavy paper (40 x 29.5 cm) with a grid to facilitate the counting process. A letter size folder is an excellent sticky board. Open and flatten the file, then cut approximately 5 cm off one end. Mark the colony number on the tab at the other end.
2. Coat the paper evenly with Tangle-Trap Insect Trap Coating (paste formula). A combination of Crisco and vegetable oil or petroleum jelly can

Figure 5.8: Screen capture of the *HIVE* dashboard ‘Varroa Sampling’ section.



Submit your mite counts

Overview

Maps

Trends

Historical Data

Varroa Timeline

Information

Further Resources for Ontario Beekeepers

Ontario Ministry of Agriculture, Food and Rural Affairs



OMAFRA is the governing body of beekeeping in Ontario. This page contains official reports on varroa mites along with detailed information on sampling, treatment, and management practices.

University of Guelph - Honey Bee Research Centre



The University of Guelph Honey Bee Research Centre YouTube channel provides educational content regarding all aspects of beekeeping, from setting up your first hive to advanced management strategies. Check back regularly for new uploads.

Ontario Animal Health Network



The Ontario Animal Health Network is a collaborative project regarding the health of all species in Ontario. The bee network of OAHN is your source for more information on honey bee disease surveillance and allows you to submit your varroa mite counts to support projects such as this one.

Ontario Beekeepers' Association



The Ontario Beekeepers' Association is a not-for-profit organization concerned with all things beekeeping in the province. The OBA undertakes its own honey bee health research, but also reviews and incorporates research and recommendations from other reputable sources into programs.

Figure 5.9: Screen capture of the HIVE dashboard ‘Resources’ section.

5.12 Appendix C. Third-party actionability assessment survey

Introduction

The *HIVE* Dashboard was developed with the intention of providing information on the status of *Varroa* destructor mites to members of the Ontario beekeeping community through interactive and intuitive visuals. The dashboard is divided into several sections, each depicting the distribution of mites in a different way: maps, plots, and data tables. Supplementary to these sections is further information on basic *Varroa* reproduction, biology and management.

We kindly ask you to provide feedback on the actionability of this dashboard as a tool for beekeepers and policy stakeholders. To be actionable, means fit for purpose and fit for use in a manner that can be understood.

This survey is collecting your feedback in the form of multiple choice questions regarding seven (7) criteria of the dashboard's actionability. Each question will have three (3) options for a response:

- 0 - Criterion is not met;
- 1 - Criteria is somewhat met;
- 2 - Criteria is clearly met.

Additional space is provided for you to provide further comments if you would like. You are welcome to refer back to the dashboard during the completion of this survey to help determine if a specific criterion was met.

All responses to this survey will be kept anonymous, and participation is optional and can be stopped at any time.

Thanks for your support! Continue to the next page to provide your feedback. There are seven pages - one page per criterion.

Actionability Criteria

Criterion #1: Knowledge of the audience and their information needs

The dashboard has a clear audience and focus. It clearly communicates the intent to support surveillance and decision making, and encourages regular usage.

- 0 - Criterion is not met
- 1 - Criterion is somewhat met
- 2 - Criterion is clearly met

Would you like to further elaborate? (Optional)

Actionability Criteria

Criterion #2: Appropriate management of the type, volume, and flow of displayed information

The dashboard contains a concise number of figures and metrics, and allows for important information to be viewed at a glance. The dashboard uses a variety of data visualization approaches, and the information is presented in a logical sequence.

0 - Criterion is not met

1 - Criterion is somewhat met

2 - Criterion is clearly met

Would you like to further elaborate? (Optional)

Actionability Criteria

Criterion #3: Clear reporting of data sources and methods

The dashboard explains the source of the data clearly, and also presents potential limitations to enhance trust in the information provided.

0 - Criterion is not met

1 - Criterion is somewhat met

2 - Criterion is clearly met

Would you like to further elaborate? (Optional)

Actionability Criteria

Criterion #4: Linking of time trends to policy decisions

The dashboard presents data regarding the the number of mites over time in combination with information on the timing of control measures. The combined information gives users an understanding of how the applied control measures impact mite counts over time.

- 0 - Criterion is not met
- 1 - Criterion is somewhat met
- 2 - Criterion is clearly met

Would you like to further elaborate? (Optional)

Actionability Criteria

Criterion #5: Providing data “close to home”

The dashboard provides relevant information to individuals, regarding their immediate surroundings by providing geographic information through maps, tables, and charts.

- 0 - Criterion is not met
- 1 - Criterion is somewhat met
- 2 - Criterion is clearly met

Would you like to further elaborate? (Optional)

Actionability Criteria

Criterion #6: Dividing the information into relevant subgroups

The dashboard makes information relatable to individual users by providing the ability to explore varied population demographics. Furthermore, the dashboard helps to expose differences among the population.

0 - Criterion is not met

1 - Criterion is somewhat met

2 - Criterion is clearly met

Would you like to further elaborate? (Optional)

Actionability Criteria

Criterion #7: Use of storytelling and visual cues

The dashboard provides brief explanations of the meaning of trends in combination with visual techniques, such as intuitive colour schemes and icons to support the interpretation of the information being presented.

0 - Criterion is not met

1 - Criterion is somewhat met

2 - Criterion is clearly met

Would you like to further elaborate? (Optional)

Block 8

Thank you for your feedback on the perceived actionability of the *HIVE* dashboard. This survey is now complete. If you would like to provide further comments, please feel free to do so in the text box below. (Optional)

To submit your responses please continue to the next page.

6 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Thesis Summary

Since the inception of the Canadian Association of Professional Apiculturists' report on annual colony losses in 2007, the amount of loss experienced by beekeepers has regularly surpassed typical expectations of 15% (Canadian Association of Professional Apiculturalists, n.d.). In Ontario specifically, losses as high as 58% have been reported (Kozak *et al.*, 2014) and represent a major cause for concern for beekeeping, as well as for the downstream impacts on the domestic and global food supply chain. Although no study has been able to identify a single cause for the elevated levels of colony loss, the parasitic mite *Varroa destructor* has consistently been identified as a key factor and researchers have labeled the *Varroa* mite as the most important risk factor in determining the overwinter survivability of a colony (Guzmán-Novoa *et al.*, 2010; Ramsey *et al.*, 2019; Rosenkranz, Aumeier, & Ziegelmann, 2010; Traynor *et al.*, 2020). *Varroa* mites exist in Ontario at hypoendemic levels and exist in virtually all colonies. Treatments exist to control mite infestations, but concerns of toxicity impacting the environment, bees, and the safety of human-consumed bee products, along with the potential for acaricide resistance have led jurisdictions, including Ontario, towards adopting integrated pest management (IPM) approaches as the best means for limiting mite levels in the population. An IPM approach aims to control mites below a critical threshold through a combination of surveillance, cultural control measures, mite resistant breeding, and chemical treatments. A critical component of a successful IPM strategy is the comprehensive knowledge of risk factors for infestations as well surveillance of the pest throughout the population to better design and tailor IPM strategies in a specific region (Ferguson *et al.*, 2003; Hughes, 1996; Perry, 1994). However, few studies globally have looked at the distribution of *Varroa* mites at the population

level over space and time, and until now, none have been conducted in Canada specifically (to the author's best knowledge), representing a challenge in deriving the information needed for a successful IPM program.

In Chapter 2 of the presented work, the spatial distribution of managed honey bee colonies was assessed to provide context about the population-at-risk for further provincial-level honey bee health studies. Without such information, disease metrics can be difficult to interpret. Epidemiologic or population health studies frequently require information on the population-at-risk to provide a denominator to standardize health measures (Dohoo, Martin, & Stryhn, 2014). Before this thesis, a spatially continuous estimation of the density of managed colonies was missing from the literature for Ontario, despite increased concern from beekeepers and ministry officials regarding the potential influence of neighboring bee yards on the health of one's colonies (Claing *et al.*, 2020). This study identified a geographically heterogeneous distribution of the locations to which colonies are registered, with a considerable discrepancy in colony density between the least and most dense regions (0 to over 14 colonies per square kilometer). The location where a colony is registered can differ from where the colony is situated throughout the beekeeping season, but nevertheless these results expose a substantial concentration of colonies in the southern Ontario region and specifically in the Niagara Peninsula for at least a portion of the year. While the results of this study are not surprising, they fill a basic need for honey bee research in Ontario and are a novel application of the methods used. In future works, these results will provide a means of standardizing disease counts, or investigating the potential influence of colony density as a risk-factor for the occurrence of disease.

In Chapter 3, the spatial distribution of *Varroa* mite infestations across Ontario was investigated and a continuous risk map for the province was developed. Spatial scan statistics

identified several high-risk clusters over a 5-year study period (2015 to 2019) that should be further investigated at a more localized scale to identify causes. Specifically, the northwestern quadrant of Southern Ontario showed evidence of temporally stable high-risk *Varroa* clusters and should therefore be targeted by future investigations to determine the cause. In the meantime, it may be beneficial for government officials to implement further intervention measures specific to this area of the province. Spatial regression modelling provided evidence for sporadic high-risk areas throughout the province that could not be fully explained by the surrounding landscape classification or colony density. No evidence was found to suggest that land-use surrounding the colony influenced *Varroa* infestation in Ontario, and regional colony density was only found to show marginal evidence of a negative association with *Varroa* infestation intensity. These results do not fully agree with previous studies which found evidence for an association between *Varroa* infestations and the surrounding land use (Giacobino *et al.*, 2017), suggesting that a different classification system of land type could be beneficial for further research into the topic, or that the data used in this study were not suitable to detect these relationships (further explained in the following section). The negative association found between density and colony-level *Varroa* infestation does not agree with the *a priori* beliefs of beekeepers in the province or findings of previous studies (Stevenson, Benard, Bolger, & Morris, 2005). The lack of evidence for an association between colony density and *Varroa* observed in Chapter 3 of this thesis is thought to be the result of limitations of the data regarding where colonies are registered in comparison to where they reside throughout the season. Nonetheless, although the spatial distributions of *Varroa* mites across the province could not be explained, the exposure of clusters and high-risk areas from this study present valuable information upon which to build.

Chapter 4 presents an assessment of the temporal distribution of *Varroa* mite infestation intensity over five consecutive beekeeping seasons. Descriptive time series assessment of apiary inspection data identified a consistent annual seasonal pattern of *Varroa* mite infestation intensity in line with previous literature modelling *Varroa* population dynamics (Fries, Camazine, & Sneyd, 1994; OIE: World Organisation for Animal Health, 2021). Mite counts were found to be initially high, upon hive opening following the overwintering period, then drop off sharply, remaining low throughout the summer months before rising rapidly in the early fall. This pattern is consistent with what would be expected based on the biology and reproduction of the *Varroa* mite (Rosenkranz, Aumeier, & Ziegelmann, 2010) in combination with the control measures implemented in Ontario. Time series regression modelling demonstrated that the average weekly temperature and average weekly dew point appear to explain some of the variations in mite infestation intensity at a 7-week lag interval. The results from this study offer a benchmark for expected *Varroa* counts at various times throughout the beekeeping season and support evidence put forward by previous research, validating its applicability to Ontario.

To consolidate and mobilize the information generated in the three initial research chapters of this thesis, Chapter 5 describes the production of an actionable tool for beekeepers, researchers, and government officials to continue to monitor the spatiotemporal distributions of *Varroa* mites in the future. As stated in Chapter 1, a key component of disease surveillance is the dissemination of the data and analysis to stakeholders and those who can practically apply the findings. The HIVE, an interactive online dashboard, was successfully developed to allow for the dissemination of *Varroa* infestation information while maintaining the anonymity of apiaries and inspection details. Interactive choropleth maps of honey bee colony density and *Varroa* mite infestation intensity allow for self-guided exploration of data on mite levels in a given region and

allow for the comparison of that region to the province. Additionally, interactive time series plots, able to be filtered by region and year, allow for the monitoring of developing mite population levels over time, which can be used to gauge an estimate of the provincial mite load and success of IPM strategies. By monitoring time series data continuously, as new intervention measures or initiatives are put forward by OMAFRA, their success can be visualized. Supplementary information regarding mite testing and control measures is included in the *HIVE* dashboard to aid in educating the broad beekeeping community.

6.2 Key Points

In summary, the research conducted within this thesis found:

- (1) managed honey bee colonies in Ontario are not spread equally across the province, where, Southern Ontario is notably more densely populated by managed honey bee colonies than Northern Ontario, and the Niagara Peninsula in particular is considerably more densely populated than the remainder of the province (Chapter 2);
- (2) high-risk spatial clusters of *Varroa* mite infestations exist and were identified throughout the province, with some appearing to be stable over multiple years (Chapter 3);
- (3) the landscape surrounding an inspected colony was not found to be associated with the intensity of the *Varroa* load experienced (Chapter 3);
- (4) at a population level, *Varroa* mite infestation intensity in Ontario shows moderate signs of improvement over the five years studied (Chapter 4);
- (5) previous population dynamics models and their findings of seasonal patterns for *Varroa* mites appear to hold in Ontario based on apiary inspection reports (Chapter 4); and

(6) evidence was found to suggest that weather variables, specifically ambient average weekly temperature, and dew point are associated (negatively and positively, respectively) with *Varroa* mite infestations at a 7-week lag interval (Chapter 4).

In addition to these research findings, this thesis also demonstrates that online interactive dashboards can be adapted for practical use in *Varroa* IPM strategies, to disseminate key findings and surveillance metrics to an audience of diverse knowledge backgrounds. The application of these tools can be further extended to other diseases and hosts, either by inclusion in existing dashboards or the development of new ones.

6.3 Study Limitations and Advantages

The primary advantage of the work presented in this thesis in comparison to previously published literature is the scale at which the analysis was conducted. Epidemiological and population-level analyses have been scarce in honey bee research, and few studies have previously focused on scales larger than a single beekeeping region. Several advantages exist for population-level research. Arguably the most important advantage in this context is the ability to compare colony health status across the province to identify areas of concern to equitably allocate resources for intervention and generate further hypotheses. Additionally, large geographic scale studies allow for the assessment of risk factors that likewise occur at a large scale, such as diverse geographical features and surrounding environments. Because beekeeping is unlike other forms of animal husbandry, and honey bees forage at a range of multiple kilometers, studies into their relationship with the surrounding environment must be conducted at a suitable scale. Province-level spatial epidemiology, therefore, provides an appropriate means for examining these interactions and should become more commonplace in honey bee research. Smaller-scale studies can then be used to further investigate and explain population-level

findings more specifically. Furthermore, utilizing apiary inspection data derived from multiple yards, under various management practices and environmental conditions improves the relevance to a broader population of beekeepers.

Using secondary epidemiologic data, however, is also the greatest limitation of this thesis. The routine apiary inspection data used in this study from OMAFRA arose from convenience and cost considerations, and were influenced by *a priori* beliefs regarding *Varroa* status. Apiaries closer together geographically may often be sampled closer together in time as inspectors choose to group yards in the same region to save travel time and resources. Because of the association between time and *Varroa* mite counts described in Chapter 4, grouping inspections by space and time can bias the results of spatial analyses. Furthermore, requested inspections are performed often to either diagnose a colony that is already failing (biasing the data towards samples with higher *Varroa* counts) or to ensure that a colony is clean before travel or trade (biasing the data towards samples with lower, or zero, *Varroa* counts). Although these data were the best available at the time of this thesis, all of these considerations impose a sampling bias which can blur the true associations being assessed and the models being developed. Therefore, a purpose built, probability sampling strategy could be beneficial. Such a strategy could be designed with the spatially continuous *Varroa*-risk in mind, something that was not possible before this thesis and the development of the provincial risk map (Chapter 3). However, implementing this type of sampling strategy would forgo the previous conveniences of the current apiary inspection protocols, and may not allow for as many inspection requests to be filled.

A second, and more practical, option for improving the data being collected on *Varroa* in Ontario would be the implementation of a citizen science approach, as suggested in the

discussion of Chapter 5. Citizen science-supported sampling and surveillance would allow for broad geographical coverage of data, and repeated observations from the same apiary multiple times in a season. Furthermore, trained inspectors would be made available to perform inspections required by the ministry, while greater quantities of data could be collected by the beekeepers for use in research and surveillance. While citizen science may not provide perfect data, and can be contaminated by misclassification bias, “it matters not to our purposes” (as stated by John Graunt, a pioneer of citizen science) as under- or overreporting can be remedied with adjustment factors and possess the same veracity as other sources of data (Berke, Sobkowich, & Bernardo, 2020). Dashboards, including the *HIVE* presented in Chapter 5, provide an avenue for citizen science data collection to occur, while simultaneously incentivizing beekeepers to do so by providing pertinent information in return.

The inherent drawbacks of utilizing non-random apiary inspection data should be considered when interpreting the results of this thesis. Despite the limitations of this thesis, the results offer a framework for honey bee researchers to perform similar analyses in other jurisdictions and for other bee diseases. Furthermore, this thesis concludes with a proposed solution for improved data collection through the implementation of citizen science.

6.4 Recommendations for Future Work

Based on the findings of this thesis, it is of urgent interest to identify the cause of the high-risk *Varroa* clusters across the province of Ontario, and especially those that appear to be temporally stable. Both large- and small-scale studies could prove useful in understanding these patterns depending on the scale of the risk-factors being investigated, but would benefit from enhanced surveillance data or primary data. A comparative study between high and low-risk regions is recommended to evaluate the differences in exposures that could explain the

discrepancies in *Varroa* counts. If funding permits, a cohort study of multiple apiaries across diverse landscapes and climates could prove most useful in reducing the biases experienced in this thesis and understanding which environmental risk-factors are most important. Such cohort studies should be conducted over multiple beekeeping seasons to assist in inferring causality and reduce the likelihood of identifying chance associations.

As citizen science becomes more popular, and OMAFRA is able to accumulate greater amounts of data (enabled by the work presented in Chapter 5), there will be increased opportunity for the application of health science data analytics methodologies such as machine learning, and network analysis. The application of these methods can allow for the development of early detection systems, automated risk analyses, and predictive modelling for outbreak preparedness and should be pursued. Further opportunities will emerge for big data analysis as sensors and automated detection and reporting systems are inevitably implemented, as has recently occurred in other animal husbandry professions.

6.5 Final Statement

The importance of honey bees for international and domestic food production has been well documented. However, meeting the agricultural needs for a growing global population may not be possible if beekeepers continue to experience the consistent levels of colony loss seen over the past 15 years. A collaborative interdisciplinary research approach is necessary to improve the negative health outcomes associated with colony infestations by *V. destructor*, while limiting undue harm caused by agrochemicals. The intertwinement of honey bees with their environment, and the human population calls for a true One Health approach powered by improved data collection, enhanced surveillance, and a community effort by researchers and

beekeepers globally. Exciting opportunities for bee health research emerge with rapid advancements occurring in beekeeping technology, and the rise of big data and health analytics.

6.6 References

- Berke, O., Sobkowich, K. E., & Bernardo, T. M. (2020). Celebration day: 400th birthday of John Graunt, citizen scientist of London. *Environmental Health Review*, 63(3), 67–69. <https://doi.org/10.5864/d2020-018>
- Canadian Association of Professional Apiculturalists. (n.d.). Annual colony loss reports. Retrieved December 4, 2020, from <https://capabees.com/capa-statement-on-honey-bees/>
- Claing, G., Kempers, M., Kennedy, K., Kozak, P., Lafrenière, R., Maund, C., ... Hoover, S. (2020). Statement on honey bee wintering losses in Canada (2020). *Canadian Association of Professional Apiculturists*. Retrieved from <http://capabees.org/shared/2015/07/2015-CAPA-Statement-on-Colony-Losses-July-16-Final-16-30.pdf>
- Dohoo, I., Martin, W., & Stryhn, H. (2014). *Veterinary Epidemiologic Research*. (S. M. McPike, Ed.) (2nd ed.). Charlottetown, PEI, Canada: VER Inc.
- Ferguson, A. W., Klukowski, Z., Walczak, B., Clark, S. J., Mugglestone, M. A., Perry, J. N., & Williams, I. H. (2003). Spatial distribution of pest insects in oilseed rape: implications for integrated pest management. *Agriculture, Ecosystems & Environment*, 95(2–3), 509–521. [https://doi.org/10.1016/S0167-8809\(02\)00200-1](https://doi.org/10.1016/S0167-8809(02)00200-1)
- Fries, I., Camazine, S., & Sneyd, J. (1994). Population dynamics of *Varroa jacobsoni*: a model and a review. *Bee World*, 75(1), 5–28. <https://doi.org/10.1080/0005772X.1994.11099190>
- Giacobino, A., Pacini, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., ... Signorini, M. (2017). Environment or beekeeping management: what explains better the prevalence of honey bee colonies with high levels of *Varroa destructor*? *Research in Veterinary Science*, 112, 1–6. <https://doi.org/10.1016/j.rvsc.2017.01.001>
- Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443–450. <https://doi.org/10.1051/apido/2009076>
- Hughes, G. (1996). Incorporating spatial pattern of harmful organisms into crop loss models. *Crop Protection*, 15(5), 407–421. [https://doi.org/10.1016/0261-2194\(96\)00003-8](https://doi.org/10.1016/0261-2194(96)00003-8)
- Kozak, P., Pernal, S. F., Kempers, M., Lafrenière, R., Leboeuf, A., Nasr, M., ... Ostermann, D. (2014). *Statement on honey bee wintering losses in Canada (2014)*. Retrieved from <https://capabees.com/shared/2013/07/2014-CAPA-Statement-on-Honey-Bee-Wintering-Losses-in-Canada.pdf>
- OIE: World Organisation for Animal Health. (2021). Manual of diagnostic tests and vaccines for terrestrial animals 2021. Retrieved December 7, 2021, from <https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>
- Perry, J. N. (1994). Sampling and applied statistics for pests and diseases. Sampling to make decisions. *Aspects of Applied Biology*, 37, 1–14.
- Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., ...

- VanEngelsdorp, D. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences*, 116(5), 1792–1801. <https://doi.org/10.1073/pnas.1818371116>
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103(SUPPL. 1), S96–S119. <https://doi.org/10.1016/j.jip.2009.07.016>
- Stevenson, M. A., Benard, H., Bolger, P., & Morris, R. S. (2005). Spatial epidemiology of the Asian honey bee mite (*Varroa destructor*) in the North Island of New Zealand. *Preventive Veterinary Medicine*, 71(3–4), 241–252. <https://doi.org/10.1016/j.prevetmed.2005.07.007>
- Traynor, K. S., Mondet, F., de Miranda, J. R., Techer, M., Kowallik, V., Oddie, M. A. Y., ... McAfee, A. (2020). *Varroa destructor*: a complex parasite, crippling honey bees worldwide. *Trends in Parasitology*, 36(7), 592–606. <https://doi.org/10.1016/j.pt.2020.04.004>