The Impact of Neonicotinoid Pesticides on Wild Bees in an Intensive Agriculture System

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Abstract

Neonicotinoids are broad spectrum insecticides that are widely used to control many insect pests. In Ontario they are applied directly to the seeds of most corn and soy crops, after which they are incorporated into the tissues of the developing plant. While researchers have investigated how these insecticides impact honeybee health, much less attention has been given to the effects of neonicotinoids on wild pollinators. Ground nesting bees face exposure to neonicotinoids both in the soil, where they nest, as well as through other exposure routes (e.g, nectar and pollen of treated plants). I studied whether a higher concentration of neonicotinoid pesticides in soils within and near corn and soy crops is negatively associated with the abundance and diversity of ground nesting bees. To determine whether an association exists between soil neonicotinoid concentration and the abundance of associated ground nesting bee communities, I surveyed 16 eastern Ontario (mainly corn and soy) farms once per month from May to August, 2019, sampling the bee communities, soil pesticide levels, and floral resources. I found a significant interaction between the sampling period and neonicotinoid soil concentrations on ground nesting bee abundance. However, no similar relationship existed for an analysis with non ground nesting bees only. Specifically, I found that high concentrations of soil neonicotinoids were associated with lower expected bee abundances and low seasonal variation, a low concentration of soil neonicotinoids was associated with a high degree of seasonal variation, including spikes of relatively high expected abundances, and that sites with no neonicotinoids were associated with low seasonal variation and moderately high expected bee abundances. The number of floral units at a site was also positively associated with bee abundance, which is consistent with what has been reported in other studies. Diversity data are currently being processed off-site and unavailable at the time of publication. My results provide evidence that there exists the potential

for higher risks of neonicotinoid seed treatments to ground nesting bees compared to the non-ground nesting community.

Résumé

Les néonicotinoïdes sont des insecticides systématiques à large spectre qui sont largement utilisés pour lutter contre de nombreux insectes nuisibles. En Ontario, ils sont appliqués sur les semences de la plupart des cultures de maïs et de soja. Si les chercheurs ont étudié de manière approfondie l'impact de ces insecticides sur la santé des abeilles mellifères, peu d'attention a été accordée à leurs effets sur les pollinisateurs sauvages. Les abeilles qui nichent dans le sol (terricoles) ont été particulièrement peu étudiées, bien que ces espèces soient les plus communes dans le monde. Les abeilles terricoles sont exposées aux néonicotinoïdes, tant dans le sol que par d'autres voies d'exposition (par exemple, le nectar et le pollen des plantes traitées). J'ai étudié si la concentration de pesticides néonicotinoïdes dans le sol est associée de manière négative à l'abondance et à la diversité des abeilles terricoles. Pour cela, j'ai étudié 16 fermes de l'est de l'Ontario (principalement du maïs et du soja) une fois par mois de mai à août 2019, en échantillonnant les communautés d'abeilles, les niveaux de pesticides dans le sol et les ressources florales. J'ai constaté une interaction significative entre la période d'échantillonnage et les concentrations de néonicotinoïdes dans le sol sur l'abondance des abeilles terricoles, mais pas sur l'ensemble de la communauté d'abeilles. Plus précisément, une concentration élevée de néonicotinoïdes dans le sol était associée à des abondances d'abeilles attendues relativement faibles qui ne variaient pas selon les saisons ; une faible concentration était associée à un degré élevé de saisonnalité, avec des pics d'abondances attendues relativement élevés, tandis que les sites où aucun néonicotinoïde n'a été détecté étaient associés à une faible saisonnalité, à aucun pic dans les valeurs attendues, et à des abondances d'abeilles attendues modérément élevées. Les données sur la diversité sont actuellement traitées hors site et ne sont pas disponibles pour le moment. Le nombre d'unités florales sur un site a également été associé de manière positive à l'abondance des abeilles, ce qui est cohérent avec

d'autres études. Mes résultats prouvent qu'il existe un potentiel de risques plus élevés pour les abeilles nichant dans le sol que pour l'ensemble de la communauté d'abeilles en ce qui concerne les traitements de semences aux néonicotinoïdes.

Chapter 1

Literature review

Bees are experiencing worldwide declines, the causes of which are diverse. Habitat loss through land use change, pesticides, pathogens, and climate change are the most likely drivers (Cameron et al., 2020; Sanchez-Bayoa & Wyckhuys, 2019). These challenges are especially prominent in agricultural lands, which currently comprise ~40% of the earth's ice-free land (Burkle et al., 2017). There is an increasing demand for pollinator services in agriculture, especially in areas with declining wild pollinator populations (Koh et al., 2016). Improving the habitat for wild bees in these areas could benefit both the conservation of pollinator diversity and agricultural production (Burkle et al., 2017).

Neonicotinoid history and uses

The first neonicotinoid insecticide, imidacloprid, was introduced to the world market in 1991. Since then six other neonicotinoids have been invented: these insecticides now make up approximately 25% of the global insecticide market (Bass et al., 2015; Simon-Delso et al., 2015). Neonicotinoids work by selectively binding to the nicotinic acetylcholine receptors in insect neurons, causing over-excitation of the neuronal membranes, followed by paralysis and cell energy exhaustion (Bass et al., 2015; Simon-Delso et al., 2015). This makes them extremely toxic to insects, but much less toxic to mammals, especially compared to older classes of insecticides (Simon-Delso et al., 2015, see also Appendix 1). The three main neonicotinoids sold worldwide are imidacloprid, clothianidin, and thiamethoxam (Bass et al., 2015; Simon-Delso et al., 2015). While these insecticides can be useful for preventing insect damage to crops, there are major concerns about their impacts on non-target organisms, including bees (Bass et al., 2015; Main et al., 2020; Simon-Delso et al., 2015). There have also been concerns raised about the evolution of resistance to neonicotinoids in pest insects (especially to imidacloprid) (Bass et al., 2015). Neonicotinoids can be applied in a variety of ways, including prophylactically as seed coats, which is the focus of my thesis. Research shows that 2-20% of the seed coat application is systemically incorporated in the plant tissue and the remainder stays in the soil or is washed away in runoff water (Simon-Delso et al., 2015; Sur and Stork 2003). Seed coat application is considered outside of an integrated pest management (IPM) framework as its application is not based on the actual level of pest activity prior to application, nor does it consider the risks to beneficial insects (Tooker et al., 2017). Seed coat applications of neonicotinoids are suspected contributors to the worldwide declines in wild and managed pollinators, as well as aquatic and predatory insects (Douglas et al 2015; Hladik et al. 2018; Sánchez-Bayoa, 2014). The potential for neonicotinoid insecticides to harm pollinators has resulted in changes to regulations in many jurisdictions including Ontario (MECP & OMAFRA 2015), Canada as a whole (Health Canada., 2020) and the European Union (Sgolastra et al., 2017).

Neonicotinoid contribution to crop yield

Neonicotinoids can kill insects at very low concentrations, yet, paradoxically, their ubiquitous use as seed coats does not consistently result in improved crop yield (Krupke et al., 2017; Mourtzinis et al., 2019; Sgolastra et al., 2017). For example, in a study of conventional corn crops in Italy, there was no significant change in crop yield in the eight years before and after a ban on thiamethoxam, imidacloprid, and clothianidin seed coats (Sgolastra et al., 2017). A study of 194 soy farms from 14 states in the United States found that neonicotinoid seed coats were associated with very small or no increase in soybean yields (Mourtzinis et al., 2019). Instead, site specific farming practices such as irrigation, crop spacing, and seeding rate were much more important for predicting crop yield (Mourtzinis et al., 2019). This marginal effect of seed coats on soybean yield is likely due to the typically low density of soybean pest insects in North America (Krupke et al., 2017; Mourtzinis et al., 2019). Using IPM to treat these pests with foliar insecticide sprays only when their densities reach an economically damaging threshold has been shown to be a more cost effective and environmentally friendly control method (Krupke et al., 2017).

Changing regulations in Canada

In Canada, there have been several government evaluations of the safety of neonicotinoids to non-target organisms. A 2016 re-evaluation of the neonicotinoid imidacloprid followed concerns regarding the health of aquatic insects (Health Canada 2020). In April of 2019, the federal government released new regulations on the three main neonicotinoids in response to concerns for pollinator health (Health Canada 2020). The resulting regulations restrict or prevent the use of neonicotinoids on pollinator attractive crops and added additional label information on cereal and legume crops to reduce planting dust (Health Canada 2020). These regulations are set to be placed on all products by 2021. There will also be a special evaluation of clothianidin, imidacloprid, and thiamethoxam on cucurbit crops to determine their risk to squash bees. Another review found that the detected levels of neonicotinoids in water samples are likely harmful to aquatic insects (Health Canada 2020). Currently, clothianidin and thiamethoxam are among the top ten insecticides sold (by weight) in Canada (Health Canada 2018). However, there are concerns that their phase-out could result in shifts to older insecticides that have similar or even more dire environmental effects, or to new insecticides with unknown consequences to the environment and beneficial insect health. As part of my thesis research I investigated what is known about these alternative insecticides with regards to bee health (Appendix 1).

Changing regulations in Ontario

Between 2009 and 2012, the area of crop land in Ontario treated with neonicotinoids increased by ~30%, mainly in the form of seed coats on soy and corn crops (Hladik et al 2018). Corn and soy are wind- and self-pollinated respectively and are therefore not usually considered to provide much in the way of foraging resources for bees. However, recent research has shown that honeybees and wild bees will forage on soy flowers and that bee pollination can increase the seed set of soybeans by 18% (honeybees) (Sholahuddin et al., 2019) and 23% (wild bees) (Cunninham-Minnick et al., 2019). In 2012, there were 104 reports of honeybee pesticide ‘incidents’ (defined as: reported incidents of an abnormal proportion of bees in a hive dying or exhibiting abnormal behaviour) near corn and soy farms in Ontario (Cutler et al., 2014). This was a substantial increase relative to the six reports made between 2007 and 2011 (Cutler et al., 2014). It was determined that many of these incidents were likely due to the dust from seed coats generated through the process of vacuum planting seeds (Cutler et al., 2014). After the impacts of the dust on honeybee colony survival was recognized, dust reducing lubricants were made available, after which honeybee pesticide incident reports declined by 70-92% (Health Canada 2020). Unfortunately, concerns about the impacts of these pesticides on bees remain. In 2015, provincial regulations in Ontario were put in place to reduce the quantities of the three main neonicotinoids applied in Ontario. These new regulations required farmers to obtain a pesticide licence, integrated pest management (IPM) certification, and provide pest assessment and end of year reports in order to qualify for neonicotinoid coated seed purchases (MECP & OMAFRA 2015). The Ontario Ministry of Environment Conservation and Parks (MECP & OMAFRA 2015) estimated that approximately 2 467 255 acres of Ontario farmland were planted with neonicotinoid- (imidacloprid, thiamethoxam and/or clothianidin) treated soy and corn seeds in

2018 (MECP & OMAFRA 2015), a reduction compared to pre-regulation estimates (L. Gue, *personal communication*).

Toxicity to bees

Neonicotinoids are known to be extremely toxic to bees, with a honeybee contact LD50 of 40.0 ng/bee for imidacloprid, 35.88 ng/bee for clothianidin, and 25.64 ng/bee for thiamethoxam (Chan et al., 2019). Bees can be exposed to systemic pesticides (including neonicotinoids) in a variety of ways, including: dust from vacuum planting (Cutler et al., 2014; Samson-Robert et al., 2014), consumption of pollen and nectar from treated crops or nearby wildflowers (Blacquiere al., 2012; Botías et al., 2015), exposure through contaminated soil (for ground nesting bees) (Chan et al., 2019), and by drinking contaminated water (Schaafsma et al., 2015).

Assessing the impacts of agriculturally applied neonicotinoid insecticides on bees is difficult, and the results presented so far are heavily debated. Field-realistic doses of neonicotinoid exposure in laboratory experiments with honeybees show a range of sub-lethal effects on behaviour, reproduction, longevity, and immune function (Fairbrother et al., 2014; Van Der Sluijs et al., 2015). However, the results from field studies (on honeybees) have been less consistent, and there are often difficulties in preventing contamination of control groups due to the large flight range of honeybees (Blacquiere et al., 2012; Cutler et al., 2014; Fairbrother et al., 2014; Van der Sluijs et al., 2015; Woodcock et al., 2017). Synergistic effects between neonicotinoids and fungicides have also been observed in honeybees, which could further reduce the LD50 of neonicotinoid applications as these two types of pesticides are commonly found together in the same commercial products (Sanchez-Bayo & Goka 2014; Tsvetkov et al., 2017; Van der Sluijs et al., 2015).

Research into the effects of neonicotinoid exposure on bumble bees and cavity nesting bees has highlighted the differences in neonicotinoid impacts among species. While bumble bees appear to have lower individual sensitivity than honeybees, some studies on bumblebees have shown that neonicotinoids can have colony wide impacts on reproduction and colony growth (Arena & Sgolastra, 2014; Van der Sluijs et al., 2015). Exposure to sub-lethal levels of neonicotinoids has also been shown to reduce the pollination services provided by bumble bees to apple orchards (Stanley et al., 2015). Field experiments with *Bombus impatiens* colonies have found seasonal increases in acetylcholinesterase levels in worker's brains associated with neonicotinoid coated corn exposure (Samson-Robert et al., 2015). A large field experiment found a reduction in *Bombus terrestris* colony growth and *Osmia bicornis* nesting success in clothianidin and beta-cyfluthrin treated oilseed rape fields, even in the absence of detectable differences in honeybee colony health in the same fields (Rundlof et al., 2015). Research on cavity nesting bees, including the orchard mason bee (*Osmia lignaria*) and the alfalfa leafcutter bee (*Megachile rotundata*) showed higher sensitivity to neonicotinoid exposure than honeybees or bumblebees, possibly due to their greater surface area to volume ratio (Scott-Dupree et al., 2009). One lab study used immature *O. lignaria* and *Megachile rotundata* as a proxy for ground nesting bees to determine the risk of imidacloprid contact exposure (Anderson et al., 2019). This study found decreased longevity of female *O. lignaria* but increased longevity and accelerated development time in male *Megachile rotundata* compared to the control group (Anderson et al., 2019).

To my knowledge, there are only two studies to date that report on the impacts of agricultural neonicotinoid pesticides on ground nesting bees, in spite of the fact that these pollinators are abundant in agricultural ecosystems and make up approximately 70% of all described wild bee species globally (Agriculture and Agri-foods Canada, 2014; Chan et al., 2019; Main et al., 2020). The first study found that squash bees (*Peponapis pruinosa*) nesting in *Cucurbita*-crop farms in Ontario are exposed to soil containing clothianidin and imidacloprid levels that exceed the 5% acceptable risk threshold in acute exposure scenarios, particularly during burrow construction (Chan et al., 2019). A second study found that wild bee communities sampled near neonicotinoid seed coat treated farms were less diverse than those collected in control fields (Main et al., 2020), although the latter was compromised by the fact that the control fields were all untreated hay fields, while the treatment fields were all neonicotinoid treated corn or soy, confounding the crop type with the presence of the insecticide.

Ground nesting bee biology

The lack of research is especially troubling considering that the lifecycle of ground nesting bees could lead them to greater exposure to neonicotinoids. The ground nesting bee life cycle involves males emerging from the burrows in the spring or summer (depending on the species) followed by females, and then mating (Michener, 2000). The females are typically active for a few weeks, during this time they dig a burrow 15 - 30 cm deep with side chambers where they construct nest cells (Agriculture and Agri-foods Canada, 2014; Michener, 2000). Nest cells are coated with a waxy waterproof substance in most species (Michener, 2000). One egg is laid in each cell and the female collects pollen provisions for each cell before it is sealed off (Michener, 2000). The larvae develop in the cells until the weather gets cold then they go into a dormant state until it is time for them to emerge the following year (Agriculture and Agri-foods Canada, 2014). Previous research on squash bees has shown that the burrow constructions could expose female ground nesting bees to hazardous levels of neonicotinoids through acute or chronic contact exposure (Chan et al., 2019). Less is known about the exposure risk to the developing bees, but it is possible that the waxy lining may provide some protection from pesticides in the soil (although this has not been tested).

Summary

Wild bees are facing unprecedented challenges to their survival and reproduction, especially in agro-ecosystems. The widespread use of neonicotinoids does not always correspond with actual levels of pest damage, and therefore doesn’t always improve crop yield (Krupke et al., 2017; Mourtzinis et al., 2019; Sgolastra et al., 2017). Concerns about the impacts of these insecticides on beneficial insects, especially honeybees, have led to changes in their regulations in Europe and North America (Health Canada, 2020; Sgolastra et al., 2017). While a large body of research has examined the impacts of neonicotinoid insecticides on honeybees, much less is known about the impacts on other bee species, especially ground nesting bees which could be uniquely susceptible to neonicotinoids due to contact exposure during nest construction (Chan et al., 2019; Lundin et al., 2015). My thesis examines the impact of commonly applied neonicotinoid seed coat insecticides on wild bee populations near corn and soy farms in Ontario.

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Chapter 2

Introduction

Until recently, the pollination services of wild bees have been largely underappreciated. We now know that, in addition to their role in the pollination of wild plants, wild bees are involved in the pollination of upwards of 63 agriculturally important species (Klein et al., 2007), and can improve crop fruit set, even in the presence of managed honeybee colonies (Garibaldi et al., 2013). The effects of agricultural intensification, including habitat loss and pesticide use, have been shown to reduce the services of wild pollinators (Kremen et al., 2002; Pindar et al., 2017). The lack of research on wild bees is troubling, particularly in the realm of pesticides, where almost all bee-related research has been performed on honeybees and a handful of other (mostly managed) bee species (Lundin et al., 2015). This lack of research on wild bees is especially worrying given the worldwide growth in neonicotinoid insecticide application.

Neonicotinoid insecticides are widely used, broad-spectrum insecticides that currently make up ~25% of the global insecticide market (Bass et al., 2015). Neonicotinoids can be applied in several ways, including as a prophylactic application to seeds before planting (MECP & OMAFRA 2015). When applied as seed coats, 2-20% of the insecticide becomes systemically incorporated in the plant tissue, including bee food resources such as pollen and nectar, as the plant undergoes development (Simon-Delso et al., 2015). The remainder stays in the soil and eventually leaches into the ground water where it finally breaks down (Sur and Stork 2003).

Research on honeybees has shown that field-realistic doses of neonicotinoid exposure in lab experiments are associated with a range of sub-lethal effects on the behaviour, reproduction, longevity, and immune function of bees (Fairbrother et al., 2014; Van Der Sluijs et al., 2015). Field studies on honeybees have been less consistent in their findings, possibly due to a lack statistical power in many studies, and difficulties preventing contamination of control groups (Blacquiere et al., 2012; Cutler et al., 2014b; Fairbrother et al., 2014; Van der Sluijs et al., 2015; Woodcock et al., 2017). Research on bumble bees has found that while they appear to be individually less sensitive to neonicotinoids than honeybees, field realistic exposure levels can reduce their cognitive function, foraging efficiency, and colony success (Arena & Sgolastra, 2014; Cameron et al., 2020).

Almost no research has been done on the impacts of neonicotinoids on wild bee species other than bumble bees, especially ground nesting bees, which make up ~70% of bee species globally (Agriculture and Agri-foods Canada, 2014; Chan et al., 2019; Main et al., 2020). In addition to the routes of pesticide exposure shared with other bees (i.e., nectar, pollen, crop dust), ground nesting bees are more likely to be exposed directly to pesticides in the soil, where 80-98% of the neonicotinoids from seed coats remains until it breaks down (Sur and Stork 2003). Clothianidin has a half-life of 148 - 6,931 days (typically about 182 days) and thiamethoxam has a half-life of 7 - 353 days, largely dependent on soil type (Ritchie et al., 2019; Schaafsm et al., 2016). Pesticide residues in soil could potentially expose bees nesting in the ground near crops to higher pesticide concentrations than non-ground nesting bees (Jones et al., 2014; Mogren & Lundgren 2016). Unfortunately, because bee nests are very difficult to locate and the reproductive success of these species is tricky to study, we have very little information about the impacts of neonicotinoid applications on the nesting and reproductive success of ground nesting bees. The two existing studies on the impacts of neonicotinoids on ground nesting bees report that neonicotinoid exposure during nest construction could be a major hazard for (ground nesting) squash bees (Chan et al., 2019), and that surveys near neonicotinoid-treated soy farms show lower wild bee species diversity than untreated hay fields (Main et al., 2020).

The goal of my thesis was to study the effects of field-realistic neonicotinoid exposures on bee communities in agro-ecosystems (Lundin et al., 2015; Van der Sluijs et al., 2015). Specifically, I investigated the diversity and abundance of wild bee communities located within, and on the margins of, soy and corn fields in southeastern Ontario that varied in terms of their soil neonicotinoid exposure. I hypothesized that because their life history involves direct contact with soil, ground nesting bees would have lower reproductive success and survival in agricultural settings with high neonicotinoid seed coat use, compared to no/lower use areas, resulting in a lower abundance and diversity at sites with higher neonicotinoid soil concentrations. I also hypothesised that soil neonicotinoids would have a lower impact on non ground nesting bee survival and reproduction as they are mainly exposed through pollen and nectar which typically have lower neonicotinoid concentrations than soil.

Methods

2018 and 2019 Data Collection

Fieldwork was conducted over two years with a pilot study of 16 soy and corn farms in 2018 and a full field season on 18 (mainly) soy and corn farms in 2019. For the 2018 pilot season, I collected late summer data on bees, floral resource availability, soil pesticide concentrations (including neonicotinoids), soil hardness, air temperature, wind speed, and crop type of the field and the adjacent field. The 2019 study followed the same basic methodology as the 2018 study, except that sampling was extended to collect survey data from each farm once a month from May-August. The 2019 fieldwork was also expanded by additional bee collection methods (pan traps), and expanded soil pesticide testing.

2018 Site selection

In collaboration with Dr. Ilona Naujokaitis-Lewis from Environment and Climate Change Canada and Drs. David Lapen and Sophie Cardinal from Agriculture and Agri-foods Canada (AAFC), I identified fields of corn (N = 9) and soy (N = 7) in the eastern Ontario region with hedgerows that, based on prior surveys (Fahrig et al., 2015), were deemed likely to vary in terms of their historical and current level of neonicotinoid seed use. In order to ensure the independence of replicate site bee communities, I selected sites that were at a minimum distance of three km apart (Greenleaf et al., 2007), for a total of 16 sites (1 organic soy, 1 organic corn, 6 conventional soy, and 7 conventional corn; Appendix 2). The 2018 data is not presented in this chapter because of limited early season bee survey data (in 2018 surveys were performed only in July and August), soil pesticide data (only 2 organic and 2 conventional fields were tested for pesticides in 2018), and bee survey data methods (no pan traps were set in 2018). The 2018 data can be found in Appendix 2.

2019 site selection

My 2019 surveys included eight of the 16 sites surveyed in the 2018 pilot season (Appendix 2). I also selected five additional conventional farm fields from a larger pool of sites used in previous ECCC studies and four organic fields (described in a previous study by Put et al., (2018)), for a total of 18 fields. In order to control for the effect of field size on site biodiversity (see Fahrig et al 2015), I chose fields of a similar size. I also narrowed down fields by choosing conventional sites where previous insecticide testing had demonstrated detectable neonicotinoid levels ranging from 0 - 24 ppb in hedgerow plants (I. Naujokaitis-Lewis & S. Robinson, unpublished data). Two of the selected and surveyed conventional fields were later removed from the dataset due to a lack of permission to collect soil, leaving 16 sites in total (5 organic and 13 conventional) for the 2019 analyses. In 2019, five site operators planted corn (all conventional) and nine planted soy (four organic operations and five conventional). One site surveyed in 2019 was planted with soy in 2018 but switched to alfalfa hay in 2019 and a second site surveyed grew organic corn in 2018 and organic hay and oats in 2019 (Table 2.1; Appendix 2). Neither of these alternate crops were likely providing significant floral resources as they were both were regularly mowed short to prevent flowering. All site environmental data is provided in Table 2.1.

Surveys

In both 2018 and 2019, at each site survey, my field assistant and I set up a 100 m transect at one edge of the field, starting at a randomly chosen point along the hedgerow. Flags or pan traps were set out every 10 m along the transect (Figure 2.1). This transect was then sampled by sweep netting, I also surveyed available floral resources and ground nesting bee habitat and took soil samples as described below. In 2018, I visited each site once in July and once in August on not rainy days between 9 am and 5 pm on days when the average temperature was at least 13˚C. In 2019 I followed the same protocol but visited each site once per month from May - August. The first sampling period was timed to occur during the pre-planting period (May 22 - June 10), the second post-planting (June 18 - July 3), the third mid-season (July 16 - 20), and the final visit late in the growing season (Aug 15 - 28), but before harvesting. The timing of planting period was established through monitoring of field activities and on-site observations.

Bee collection

At the start of each survey I recorded wind speed and air temperature using a Kestrel 2000 pocket weather meter® at the start of the survey. I then walked up and down the transect for 10 min (at approximately 20 m/min, not counting time collecting bees from net), collecting bees with a 38 cm diameter sweep net (90 cm handle). I collected bees on the vegetation in the hedgerow, sweeping all flowers. Collected bees were then transferred into small plastic vials which were placed into a cooler packed with dry ice. To prevent unnecessary damage to local bumblebee populations, queen bumblebees were placed into a vial, photographed for later identification, and then released. At each site visit, I set up nine bee bowls alternating blue, yellow and white UV reflective paint along the hedgerow transect line (Figure 2.1). Pan traps were filled with water and a few drops of blue Dawn® dish soap and placed 10 m apart along the transect. The traps were hitched to PVC pipes one metre above the ground so that they would be taller than the surrounding vegetation of the hedgerow (Martin et al., 2020). Pan trap contents were collected after 24 hours using a strainer to drain the water, and then samples were transferred to ethanol filled WhirlPak™ bags and stored in a -6 ℃ freezer until they could be pinned and identified.

Bee identification and site nesting characteristics

At the end of the field season I pinned the bees, identified them to genus or species using Discover Life (Ascher & Pickering, 2020), and stored them in collection boxes. Bees that I could not confidently identify to species are currently being DNA barcoded at Guelph Centre for Biodiversity Genomics. Pictures were taken of each of these bees and they were uploaded to BOLD (http://www.boldsystems.org/). I identified each bee's nesting habitat as ground nesting (bees that excavate burrows in soil), cavity nesting (bees that nest in premade cavities of various material including plant stems, wood, snail shells, and manmade structures), pith nesting (bees that excavate the pith from plant stems to make nests), wood nesting (bees that excavate nests in wood), and parasitic (bees that lay their eggs in other bees nests) using a variety of literature sources (Ascher et al., 2014; Mattenson et al., 2008; Normandin et al., 2017; Richards et al., 2011). I only considered a genus as ground-nesting if the majority of species in that genus found in the area were ground nesting (Appendix 3). I did not count parasitic species as ground nesting even if they were parasites of ground nesting genera (except for potentially parasitic *Lasioglossum*, due to the uncertainty of its nesting behaviour, (Onuferko et al., 2015)). Species that occupy pre-made cavities in the ground (i.e., bumblebees) were not considered to be ground nesting in this study. This follows the assumption that for ground nesting bees, pesticide exposure would mainly occur through nest construction (Chan et al. 2019).

Floral resource surveys

On the days when I conducted sweep net transects, I also conducted a floral resource availability survey. I estimated floral resource availability by setting up 1 m^2 quadrats every 10 m along the 100 m transect at the edge of the field (Figure 2.1) and recording all animal pollinated flowers that were in bloom at the time. I also took pictures of each quadrat and high-resolution photographs of any flowering plants that I could not identify in the field. Following Guezen (2017), I identified and counted flowers, estimating flowers per inflorescence, which were counted as individual floral units (except for Asteraceae, and *Trifolium* where heads/umbels were considered one flower) (Appendix 4). I later identified the photographs of unknown species using local wild plant guides (Native Plant Trust, 2020; Royer & Dickinson, 1999).

Soil collection

At each site I collected ten soil samples from the hedgerow along the same transect described above. I also collected ten soil samples from a parallel transect line set ten metres into the crop, using a 25 mm diameter Oakfield Apparatus® 30 cm soil corer (Figure 2.1). Hedgerow soil samples were collected every month from May-August in 2019 (and in July in 2018), and crop soil samples were collected in May and June in 2019 and in July in 2018. Soil samples from the top 15 cm of soil were separated using a stainless-steel scraper; the 10 samples from each site were then mixed together to make one composite sample per site visit (Schaafsm et al., 2016).

The soil corer and the scraper were washed with laboratory detergent and distilled water, then acetone, followed by hexane, and finally rinsed again with distilled water between samples to prevent cross contamination (S. Baker, *personal communication*). The samples were then stored in Ziploc® bags in a -20˚C freezer until they were sent to laboratories for pesticide analysis (see below) (Schaafsm et al., 2016). I also took four soil hardness measurements in each quadrat using a penetrometer and recorded the crop and adjacent crop type. I also dried a sample of each soil and used sieves to measure the percentage of sand in the soil from each site.

The top 15 cm soil samples from the crop and hedgerow, collected between June 18 and July 3 2019, were sent for pesticide testing at the University of Guelph Agriculture and Food Laboratory Service in August of 2019 (https://afl.uoguelph.ca/). These samples were chosen for testing as they were collected soon after planting and were therefore expected to exhibit the highest seasonal neonicotinoid concentrations (De Perre et al., 2015). The lab tested for a broad range of insecticides, herbicides, and fungicides using the LC-MS/MS multi-residue screen and a Multiresidue Pesticide Analysis by modified QuEChERS extraction with LC-MS/MS detection method (https://afl.uoguelph.ca/pesticide-residue-analysis). The detection limits of these tests were not sensitive enough to give concentration estimates (limit of detection: clothianidin = 7ppb, thiamethoxam = 5ppb) , so we had them re-tested at SGS AXYS Analytical services labs in Feb 2020, which was able to provide lower detection limits (limit of detection: clothianidin = 0.511 ppb, thiamethoxam = 0.511 ppb) (https://www.sgsaxys.com/). The pesticide testing from SGS AXYS Analytical services labs are more precise than the AFL tests and were therefore used for the statistical analyses. The AFL results can be found in Appendix 5. Samples collected at other sampling periods were not analyzed due to limited funding.

Statistical analyses

The sweep net and pan trap bees from each subsample were pooled together to obtain site-wide bee abundance estimates for each (monthly) site visit. Ultimately, the soil from the crop was used for pesticide testing as opposed to the hedgerow because crop pesticide detections were expected to correspond more closely with neonicotinoid seed coat use compared to hedgerow pesticide levels, and I lacked funding to test both. Based on the distribution of the neonicotinoid concentrations from these results, I binned the sites based on the distribution of neonicotinoid detections into five sites exhibiting no detection of neonicotinoids, five sites with < 4 ppb, or low concentration, and six sites with ≥ 4 ppb, or high concentration (Figure 2.2). This binning was done to allow the interpretation of non-linear effects for any interaction between neonicotinoid concentration and sampling month, which was not expected to have a linear relationship. Using R statistical software, version 3.6.2 (R Core team., 2017), and the package lme4 (Bates et al., 2020), I used generalized linear mixed-effects models with a log link (Poisson family) to examine two statistical models, one with ground nesting bees alone as a response variable, and the other with only non-ground nesting bees. The fixed effects for both models included soil neonicotinoid category (no detection, low concentration, high concentration), number of floral units (a continuous variable), sampling period (May 22 - June 10, June 18 - July 3, July 16 - 20, and Aug 15 - 28) and the interaction between pesticide concentration and sampling period. Site was included as a random effect. I tested for overdispersion using a ratio of sum of squared Pearson residuals divided by residual degrees of freedom (Bolker, 2018). Based on the results of this test, I added an individual ID variable to each table row and included this as a random effect to correct for overdispersion (Elston et al., 2001). I plotted the effects from the GLMMs using the effects package in R (Fox et al., 2019).

Exploratory analysis

After I analyzed the main effects from the models described above, I explored how including crop type (soy, corn, hay), adjacent crop type (soy, corn, hay, wheat), and temperature as fixed effects impacted the model results. I added the three variables one at a time into three ground nesting bee models and non-ground nesting bee abundance models.

Results

Soil pesticide levels

The soil neonicotinoid analysis detected clothianidin at 11 sites. Clothianidin levels ranged from 1.59 - 22.9 ppb. Thiamethoxam was also detected at three sites with concentrations ranging from 0.658 - 0.743 ppb. Imidacloprid was not detected at any of my sites. Five sites had no soil neonicotinoids detected. Neonicotinoid concentrations for each site can be found in Table 2.1. Non-neonicotinoid insecticides were not included in the analysis but were tested for and their concentrations/detection can be found in Appendix 5.

Bee community surveys categories

Over the course of the summer of 2019 I collected 1,081 bees from 26 genera. Consistent with literature reports, 67.5% of surveyed bees were ground nesting. The six highest concentration sites (≥ 4ppb) had a total of 414 bees collected (with 278, or 67.1% ground nesting); the most abundant bee genera at these sites were *Lasioglossum* (135), *Melissodes* (88), and *Hylaeus* (59) (Appendix3). There were 405 bees collected at the five low concentration sites (< 4ppb) (with 254, or 62.7% ground nesting); the most abundant bee genera at the low concentration sites were *Lasioglossum* (145), *Bombus* (55), and *Andrena* (53) (Appendix 3). There were 262 bees collected at the five no detection sites (with 194, or 74% ground nesting); the most abundant bee genera at these sites were *Lasioglossum* (120), *Melissodes* (29), and *Apis melifera* (23) (Appendix 3). Ground nesting bees exhibited the highest mean abundance and variability at low neonicotinoid concentration sites (Figure 2.3). Over the season the third sampling period had the highest mean ground nesting bee abundance and the first sampling period had highest variation (Figure 2.4).

Floral resources

I recorded a total of 76 species of plants across the 16 sites. The six ‘high’ concentration sites had a total of 30 plant species with a mean of 32,336 floral units per site. The most abundant animal-pollinated species flowering there were *Daucus carota*, *Pastinaca sativa*, and *Solidago* spp. There were 44 species of flowering plants across the five low concentration sites with a mean of 11,339 floral units per site; the most abundant animal-pollinated flowering plant species at ‘low’ concentration sites were *D. carota*, *Solidago spp.*, and *Pastinaca sativa* (Appendix 4). There were 40 species of flowering plants with a mean of 8,116 floral units per site at the five non detection sites; the most abundant plant species at the no detection sites were *Solidago spp.*, *Viburnum nudum*, and *Viburum* spp. (Appendix 4). The full list of plant species identified across the 16 sites is listed in Appendix 4.

Soil neonicotinoid levels and bee abundance

There was no significant interaction between soil neonicotinoid category and sampling month for non-ground nesting bee abundance, so the interaction was removed from the model. The final model found no significant effect of neonicotinoid category on non-ground nesting bee abundance; floral resource availability was the only significant predictor of non-ground nesting bee abundance (Table 2.2; Table 2.3; Figure 2.5A).

In contrast, the analysis of ground nesting bee abundance indicated a significant interaction between neonicotinoid category and sampling period, and the interaction was retained for the final model. In the final model, I also detected a significant effect of neonicotinoid category and floral resource availability on ground nesting bee abundance (Table 2.4). The significant interaction between neonicotinoid category and sampling period indicates that ground nesting bee abundance changed over the course of the season, with fields in different neonicotinoid categories following different seasonal patterns. Specifically, at the ‘high’ concentration sites, the predicted ground nesting bee abundance was consistently low and relatively invariable among sampling periods (Figure 2.6). On the other hand, at the ‘low’ concentration sites, initially high predicted abundances of ground nesting bees in May/early June were followed by a steep decline in the June sampling period, higher predicted abundances again in July, with the lowest levels predicted at the final sampling period in August (Figure 2.6). ‘No detection’ sites were characterized by relatively low predicted abundances in the pre-planting sampling period, followed by relatively high predicted abundances in the following three periods (Figure 2.6). Floral resource availability was also a significant factor in this model, with higher floral availability associated with higher predicted abundances of ground nesting bees (Figure 2.5B).

Exploratory analysis: ground nesting bees

My exploratory analysis found that adjacent crop was a significant predictor of ground nesting bee abundance. Sites adjacent to hay fields had significantly lower ground nesting bee abundance compared to sites adjacent to corn and soy fields with soy having the highest predicted abundance (Table 2.4). Crop and temperature did not have a significant effect on ground nesting bee abundance (Table 2.4).

Exploratory analysis: non ground nesting bee abundance

The interaction between soil neonicotinoid category and sampling period was not significant in any of the three non ground nesting bee abundance exploratory models so it was removed from these models (Table 2.5). Crop was a significant predictor of non-ground nesting bee abundance, but temperature and adjacent crop were not (Table 2.5). Hay and soy crops both had significantly lower predicted non ground nesting bee abundances than corn. The number of floral units had a significant positive correlation in all models except for the model with crop added.

Discussion

My thesis provides evidence that agricultural sites with different soil neonicotinoid concentrations are associated with different overall and seasonal patterns of ground nesting bee abundance. Interestingly, there was no impact of soil neonicotinoid concentration or sampling period on non-ground nesting bee abundance. These results are consistent with my hypothesis that ground nesting bees are more vulnerable to seed coat applications of neonicotinoid pesticides than bees with other nesting habits (e.g., cavity, parasitic, pith, wood or stem nesting). The higher overall predicted abundance of ground nesting bees at the low concentration sites (Figure 2.6) was at least partly driven by a much higher predicted abundance of ground nesting bees collected there during the pre-planting and mid-season (July) visits. Pre-planting is the time of year when soil neonicotinoid levels are expected to be the lowest because the insecticide has not yet been applied to the field (De Perre et al., 2015). Sites in different neonicotinoid categories (no detection, low concentration, high concentration) exhibited markedly different patterns of ground nesting bee abundance throughout the season, and, although a consistent negative impact of high soil neonicotinoid concentration across sampling periods was not observed, these sites had relatively low predicted ground nesting bee abundance throughout the season (Figure 2.6).

At the low concentration sites, the earliest (mid-May to early June) sampling period had significantly higher predicted ground nesting bee abundance relative to the high concentration and no detection sites (Figure 2.6). Also, at the low concentration sites, approximately two weeks after planting (sampling period 2), there was a clear drop in the predicted ground nesting bee abundance. This drop corresponds to the time of year soil neonicotinoid concentration is expected to be highest, due to the planting of coated seeds at these sites (Figure 2.6; De Perre et al., 2015). However, this same drop was not observed at the high neonicotinoid concentration sites, making it difficult to conclude that the decline is related to the planting period. It's not clear why the three soil neonicotinoid categories had such different predicted abundances of bees in the pre-planting period, but it could be at least partly due to differences in the bee communities of the fields in the three site types that are unrelated to soil neonicotinoids. Ground nesting bees in southern Ontario have been shown to have peak abundance in late April, mid-May to early June, and mid-late July (Richards et al., 2011). Since soil neonicotinoid concentrations are expected to be at their lowest levels before planting, early season bees should be the least impacted by neonicotinoids released during seeding, as burrow construction would largely be completed by that time (Chan et al., 2019; Xu et al., 2016). The lower abundance of ground nesting bees following planting at the low detection sites could be due to chronic exposure during nest construction when soil neonicotinoid concentrations are at their highest levels, which likely impacts the survival, longevity, and reproduction of bees active at this time of year (Chan et al., 2019). Thus, the detected pattern could at least partly be the result of years of consecutive neonicotinoid use, reducing the abundance of ground nesting bees that are active soon after planting. Surprisingly, the high concentration sites did not exhibit a post planting drop in ground nesting bee abundance. The seasonal pattern of ground nesting bee abundance at the no detection sites falls between the low and high detection sites, with fewer bees expected in the pre-planting period, followed by sustained, relatively high expected abundances in the following three sampling periods. Overall, while the three neonicotinoid categories clearly show distinct patterns, they are not clearly associated with a hypothesized graded depression of abundance with increasing soil neonicotinoid concentration.

Little is known about the sensitivity of most bee species (especially ground nesting bees) to neonicotinoids (Arena & Sgolastra, 2014). Across all sites, the most common bee genera detected at the final (August) sampling period were *Melissodes* and *Lasioglossum;* however, the low concentration sites had very few individuals from either genus (*Melissodes* (9), *Lasioglossum* (4)) compared to both the no detection (*Melissodes* (20), *Lasioglossum* (20)) and high concentration sites (*Melissodes* (75), *Lasioglossum* (20))(Appendix 3). Confounding factors such as the presence of nearby sunflower farms might explain the differences in *Melissodes* abundance among sites, however, due to limited power, I was not able to include these landscape variables in my final models. *Melissodes* are known to be particularly attracted to sunflower crops and are relatively large bees, which corresponds to a longer flight range (Mallinger et al., 2019; Portlas et al 2018; Zurbuchen et al., 2010). Nearly two thirds of all the *Melissodes* collected in August were collected at a single high concentration site (Table 2.1; Site 9), which may have been particularly attractive to them as it also had the second highest August floral resource availability of all the sites. The high concentration sites generally had high floral resources, which is one of the most consistent predictors of wild bee abundance in both agricultural and natural systems (Isbell et al., 2017). These factors highlight the difficulty of assigning causality to patterns of association in correlational studies, especially those performed in nature (where many potentially confounding variables are uncontrolled and difficult to measure).

Non-ground nesting bee abundance

Soil neonicotinoid category was not a significant predictor of non-ground nesting bee abundance, unlike the ground nesting bee abundance model. This could be because other bees are not generally in contact with neonicotinoids applied as seed coats, except for the dust generated during early season planting and very low concentrations in pollen and nectar translocated into wildflowers in the hedgerow (Botias et al., 2015; Cutler et al., 2014a). The most common genera of non-ground nesting bees in my samples were *Hylaeus*, *Bombus*, *Apis*, and *Ceratina* (Appendix 3). Although most *Bombus* species in the area are known to nest in underground cavities, they typically form their colonies in pre-existing cavities such as abandoned rodent burrows, which may limit their exposure to neonicotinoids in soil. However, the gynes do dig a small hibernaculum for themselves in the fall, which may increase their exposure through soil, although soil neonicotinoid levels should be low at this time of year (Liczner & Colla 2019; Purvis et al., 2019). Their large flight range also means that they can nest in the wild/semi-wild areas surrounding crops, which could limit their exposure (Agricultue and Agri-foods Canada.,2014; Zurbuchen et al., 2010). *Apis mellifera* are a managed bee species so their density depends on the presence of managed hives. *Hylaeus* are most active later in the season, likely lessening their exposure to neonicotinoids associated with planting dust. *Ceratina* were most active during planting time and are relatively small bees, meaning that of all the common non-ground nesting bees, they would be most likely to be exposed to dust during planting, due to their activity period coinciding with planting, and their small body size limiting their flight range, and higher surface area to volume ratio possibly reducing their exposure tolerance (Scott-Dupree et al., 2009). It should also be noted that neonicotinoids are more toxic to insects exposed orally than by contact exposure, so higher concentrations of neonicotinoids in the soil would be needed to cause the same impact as those in pollen or nectar (Chan et al., 2019). However, since soil concentrations are much higher, they could still have a much greater impact then pollen or nectar exposure (Chan et al., 2019).

Consistent with previous studies (Blaauw &Isaacs 2014a; Blaauw & Isaacs 2014b), floral resources were a highly significant predictor of wild bees in models describing the abundance of both total and ground nesting bees alone. In fact, floral resources appear to be a stronger predictor of wild bee abundance in agro-ecosystems than soil pesticides, within the range of concentrations at the sites in my study. Neonicotinoid exposure from pollen and nectar is typically much lower than soil concentrations, and the neonicotinoid levels in the soil of these farms was already lower than in most similar studies (De Perre et al., 2015; Main et al., 2020; Xu et al., 2016). These lower levels may be a result of farmers using less treated seeds in response to the 2016 regulations (MECP & OMAFRA 2015), less densely planted crops, or it could be due to the uncertainty of the timing of soil sampling relative to planting at my sites. While soil samples were collected less than two weeks after planting it is difficult to tell if this was the maximum seasonal concentration since we did not test soil pre-planting or later in the season due to limited funding. The dispersal of neonicotinoids can also vary a lot depending on soil type and weather (Ritchie et al., 2019; Schaafsma et al., 2016). It is possible that some quantity of neonicotinoids were washed away in spring rains before sampling. Alternatively, it may have taken more than two weeks for the neonicotinoids to disperse in the soil. Unfortunately, without more precision in the timing of soil sampling, we are unable to pinpoint the exact concentration of soil neonicotinoids at the time of planting.

While none of the fields contained crops that provided significant floral resources throughout the season (with the possible exception of soy during flowering), adjacent hay fields likely provide high levels of floral resources throughout the season as these crops typically include flowering alfalfa, clover, and other wildflowers, which are important foraging resources for bees (Cunningham-Minnick et al., 2019). Mowing of hay fields could disrupt the bee communities but overall, they are more like natural bee foraging habitat than are soy or corn fields (Buri et al., 2014). Consistent with this, the conversion of hay fields and semi-natural area to monoculture crops, such as corn, has been linked to wild bee declines (Koh et al., 2016). Sites with adjacent flowering hay fields in this study might show lower than expected bee abundance since bees could be attracted to the hay field over the relatively few floral resources in the hedgerows where the pan traps and net samples were taken.

The hedgerows adjacent to the study fields also varied widely in terms of the floral resources that they provided. Some hedgerows contained an abundance of trees, which resulted in shadier hedgerows with fewer floral resources below. The sites with no neonicotinoids detected had the fewest floral units overall with an average of 8,116 ± 923 (SE) floral units/site over the season. In contrast, the high concentration fields had an average of 32,336 ± 2,937 (SE) floral units/site, while low concentration sites had 11,339 ± 1,205 (SE) floral units/site (Appendix 4). The diversity and temporal availability of flowers are also important factors for predicting bee abundance in hedgerows, as some bees are specialists on specific plant genera or families, and the nutritional value/chemical defence of pollen and nectar from different species varies. Finally, not all species of plants provide suitable pollen or nectar resources for bees (Sutter et al., 2017).

Organic vs. conventional farms

In general, soil neonicotinoid level categories were consistent with a farm's designation as conventional or certified organic (Table 2.1). However, one study field, which has been in organic production since prior to 2017 (Put et al., 2018) had detectable levels of clothianidin in its crop soil sample (Tables 2.1). I can only speculate, but is possible that the source of this clothianidin was from run-off water or pesticide drift from a neighbouring conventional farm, or some other source of contamination. There was also one conventional farm with no detectable clothianidin levels in the soil (Table 2.1). It is possible that this farm stopped using neonicotinoid treated seeds in response to the Ontario neonicotinoid regulations put in place in 2016, as did roughly 25% of Ontario corn and soy producers between 2016 and 2020 (L. Gue, *personal communication*). Overall, while most organic and conventional farms fit the expectations of neonicotinoid soil concentrations, soil testing is clearly an important step in confirming the presence and concentration of neonicotinoids. Although I tried to minimize the inter site variability of size and floral resources between conventional and organic farms used in this study, they may also differ in other ways that are important predictors of bee abundance, such as herbicide and fungicide use, and landscape level factors that weaken our ability to pinpoint pesticides as a driver (Kennedy et al., 2013). Testing the soil also allows testing of the impacts of different soil concentrations of neonicotinoids on bee richness and abundance.

Study limitations and future directions

This was a short-term correlational study. Although I was able to include some of the covariates known to impact on wild bee abundance in my statistical models (e.g., floral resource availability, sampling period), other factors, including larger scale land use patterns, soil type, and availability of nesting habitat had to be left out due to lack of power. A study with at least ten sites for each of these variables would be necessary to test all of these factors. In addition, the samples in this study only represent a limited range of soil neonicotinoid concentrations, which tended to be lower than those reported in other similar studies (De Perre et al., 2015; Main et al., 2020; Xu et al., 2016). For example, while I found an average clothianidin concentration of 6.4

ppb (maximum, 22.9 ppb) and average thiamethoxam concentration of 0.7 ppb (maximum, 0.7 ppb) at sites where neonicotinoids were detected, Main et al., (2020), in a similarly designed study, found average post-seeding clothianidin concentrations of 8.04 ppb and (maximum of 55.7 ppb), and average imidacloprid concentrations of 1.22 ppb (maximum 11.6 ppb) in neonicotinoid treated fields (2020). A long-term study is necessary to determine if the patterns I detected are consistent from year to year. While I was able to do a pilot study in 2018, I am unable to compare the data between years because of changes to study fields, methodology, and a lack of early season bee survey data between the two years (Appendix 2). Future research on wild bee species in controlled lab, experimental farm, and semi-field experiments would be useful in determining both the consistency and the mechanisms driving these patterns. I performed a small-scale semi-field experiment trying to determine which species would be suitable for future experiments (Appendix 6).

Due to the high costs associated with soil pesticide testing, I was only able to test the soil once at each site during the field season, so I decided to test soil samples collected soon after planting (sampling period 2), when soil neonicotinoids released from seed coats are expected to be at their highest levels (De Perre et al., 2015). I also only tested soil from within the crop field not the hedgerow for the purposes of dividing the farms into neonicotinoid use categories. Previous testing indicated that when clothianidin was detected in the field it was usually also detected in the corresponding hedgerow (Appendix 5). Testing soil neonicotinoids every month during the summer would be helpful in determining the breakdown of neonicotinoids on these farms which can vary depending on soil composition and weather (Ritchie et al., 2019; Schaafsma et al., 2016).

Soil texture and precipitation can also have a major impact on neonicotinoid retention in the soil, and how likely it is to bind to insects (Ritchie et al., 2019). Every site had sandy soil (with more then 60% sand determined by soil texture analysis) which does not bind well to neonicotinoids, potentially leaving more available to bind to insects living in the soil (Ritchie et al., 2019). Sandy soil also results in higher leaching with rain, reducing retention (Schaafsma et al., 2016). Annual variability in precipitations can also impact soil neonicotinoid retention, so long-term studies are important to disentangle this natural variation.

Little is known about where ground nesting bees preferentially nest in agricultural settings. However, some studies have found bees nesting in soy fields (Cunningham-Minnick et al., 2019). Ground nesting bees are also likely to nest in field margins and have been found to nest more in agricultural areas with more wildflowers (Cope et al., 2019). It is likely that ground nesting bees nest mainly in the hedgerows and in the edges of the crop next to the hedgerows so that they can access nearby floral resources. In contrast, non-ground nesting bees would not be nesting in the crop , as there is no suitable vegetation for stem nesters to nest in in the crop, and there would be more nesting cavities for bumble bees in the hedgerow although, bumble bee gynes might overwinter there (Liczner & Colla 2019; Purvis et al., 2019). However, it is difficult to know for sure where bees were nesting as my sampling methods did not give information on specific nesting sites. In general, more research is needed on the nesting habits of ground nesting bees in agro-ecosystems.

Exploratory analysis

Non of the variables added in the exploratory analysis were significant for both ground nesting and non-ground nesting bees. Ground nesting bees exhibited a significantly lower abundance in sites with adjacent hay fields. This could indicate that bees are preferentially foraging in the resource rich hay fields instead of the hedgerow at these sites, which could result in fewer bees in hedgerows next to hay fields (artificially lowering the estimated bee abundance and richness at those sites).

Crop had a significant effect on non-ground nesting bee abundance but not ground nesting bee abundance, and it's not clear why. Temperature was likely not a significant factor because I only sampled bees when the temperature was warm enough for them to be active and any temperature increase above that did not significantly increase their activity.

When the exploratory variables were added to the ground nesting bee models, the other described factors remained significant in the models, except for the overall neonicotinoid effect which was not significant in the model that included adjacent crop (Table 2.4). In the non-ground nesting bee models, the floral resource metric was not significant in the model containing crop type. This could indicate a greater role of crop type in determining the habitat preference of certain non-ground nesting bees. More research is needed on why specific bee taxa might be better suited to living near different crop types, especially crops that are not considered pollinator attractive.

When crop was added to the non-ground nesting bee abundance model, the overall neonicotinoid effect was significant, with the low concentration and no detection sites having higher non-ground nesting bee abundance than the high concentration sites. However, when either adjacent crop or temperature were added to the model, the overall neonicotinoid effect was not significant. Together, these exploratory studies highlight the fact that many complex factors contribute to bee abundance on these farms. A larger number of fields and a longer-term study would be needed to disentangle these key drivers of the bee community composition.

Diversity.

I am currently having my bee collection DNA barcoded and will be assessing if bee diversity is impacted by soil neonicotinoid concentration, using similar analyses to those already described. Based on my current findings, I expect that higher neonicotinoid concentrations may predict a lower ground nesting bee diversity and total bee diversity. I also expect floral resources to be positively associated with bee species diversity, following previous studies (Isbell et al., 2017).

Implications

I found that soil levels of neonicotinoid pesticides were associated with differences in the seasonal abundance of bees, particularly species that nest in the ground. Perhaps not surprisingly, given a relatively low final sample size (16 fields), and large environmental variation among fields, I did not find a clear negative relationship between ground nesting bee abundance and soil neonicotinoid concentration. I did find evidence for a seasonal pattern of ground nesting bee abundance at sites, and that pattern varied according to different soil neonicotinoid concentrations. Fields with low concentrations of neonicotinoids exhibited a particularly prominent pattern of seasonal variation in bee abundance. The pattern in these fields could indicate that ground nesting bees whose seasonal activity levels coincide with planting are more impacted by neonicotinoids than earlier emerging species. Floral resource availability in adjacent hedgerows showed a consistent, positive impact on bee abundance in all models. Consistent with previous studies, this suggests that intensively managed soy and corn farms have the potential to provide valuable habitat for wild bees through hedgerow plantings. My results support the idea that the ecological needs of ground nesting bees should be specifically addressed when determining the environmental impacts of pesticides. The use of non-ground nesting bees as surrogates for regulations regarding pollinator health is insufficient.

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Chapter 3: Conclusions and Future Directions

Bees are extremely important both ecologically, as pollinators of wild plants, and agriculturally, as pollinators of many crop species. However, as the demand for food production grows, there is a need to balance agricultural intensification with the maintenance of insect populations that provide critical ecosystem services, including pollination and biological control (Burkle et al., 2017). Unfortunately, the importance of wild beneficial insects including pollinators is often overlooked when regulating insecticides (Lundin et al., 2015). One prominent example of this is the use of neonicotinoids applied as seed coats. Research on the impacts of these insecticides on bees has mainly focused on honeybees under the assumption that the same research is applicable to other wild bee species (Lundin et al., 2015). However, since most wild bees nest in the ground, this assumption overlooks exposure to neonicotinoids in the soil where most seed coat neonicotinoids end up (Sur and Stork 2003). In order to determine if neonicotinoids in the soil impact wild ground nesting bee communities, I surveyed bee communities from soy and corn farms in southern Ontario with different levels of soil neonicotinoids and compared their abundance. My research is consistent with a negative impact of high concentrations of soil neonicotinoids on wild bee abundance. However, as a single year study with a relatively small sample size, my study lacked statistical power and inter-field controls that could be alleviated with a larger, longer term study.

Future studies should focus on controlled experiments, possibly by randomly assigning ground nesting bee nests to sites with differing (known) levels of neonicotinoid use. Longer term, multi-year field studies with more control over floral resources, adjacent crop management, and other confounding variables would also be beneficial in determining how soil pesticides impact ground nesting bees. Despite its limitations, my study does provide evidence that ground nesting bees have a stronger community level response to soil neonicotinoids than the non-ground nesting bee community, and that this should be considered when testing the ecological impacts of insecticides.

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Table 2.1 Site environmental data (2019 only). Non ground nesting bee abundance, total ground nesting bee abundance, and number of floral units are summed across all four samples during the field season. Clothianidin and thiamethoxam limit of detection = 0.511ppb.

Site ID	O* or C	Crop	Adjacent Crop	Nearest town (all Ontario)	Soil clothianidin concentration ppb	Soil thiamethoxam concentration ppb	Neonicotinoid Category*	ground nesting bee abundance	Non ground nesting bee abundance	Number of floral units
1	C	soy	wheat	Winchester North	0	0	ND	33	18	17576
2	C	corn	corn	Dundas	9.22	0.743	high	60	34	84139
3	C	soy	corn	Sarsfield North	22.9	0	high	18	1	265
4	C	corn	soy	Stormont Munster	5.4	0.719	high	59	17	18488
5	C	corn	soy	Hamlet South	3.13	0	low	59	54	32205.2
6	C	soy	soy	Dundas	2.81	0	low	75	38	9389
7	C	hay	hay	Ottawa North	5.87	0	high	5	7	13886
8	C	soy	soy	Grenville North	3.59	0	low	81	34	13152.2
9	C	corn	corn	Grenville North	6.21	0	high	86	28	63378.4
10	C	corn	corn	Grenville North	5.82	0.658	high	50	49	13860.2
11	C	soy	hay	Dundas	1.59	0	low	17	9	68
12	O	hay	hay	South Glengarry North	0	0	ND	62	11	2338.6
13	O	soy	hay	Glengarry	0	0	ND	23	8	6383

14	O	soy	corn	North Dundas	0	0	ND	36	14	752.5
15	O	soy	wheat	Woodlawn	0	0	ND	40	17	13530
16	O	soy	corn	Woodlawn	3.67	0	low	26	12	1880

Asterisk indicates C=conventional farm and O= organic farm. Neonicotinoid category ND=no detection, low= < 4ppb, high= ≥ 4ppb

Table 2.2 Type 3 ANOVA of a GLMM for non-ground nesting bee abundance with the predictor variables: soil neonicotinoid category, sampling period, number of floral units and the interaction between sampling period and neonicotinoid category. Estimates and standard error from the GLMM summary included for each level underneath each variable in italics. Site was included as a random effect. Four visits took place approximately one-month apart from May- August. The three neonicotinoid levels are: low concentration (< 4 ppb), high concentration (≥ 4 ppb), and not detected.

Response: Non-ground nesting bee abundance	Estimate	Standard error	χ^2	Degrees of freedom	Pr(>χ^2)
Intercept			6.4089	1	0.01135
Neonicotinoid level			2.5797	2	0.27532
High	Reference	Reference			
Low	0.507347	0.398814			
ND	-0.098765	0.411057			
Sampling period			4.2933	3	0.23149
Visit 1	Reference	Reference			
Visit 2	0.142461	0.325357			
Visit 3	-0.263637	0.356991			
Visit 4	0.368210	0.336010			
Number of floral units			6.3676	1	0.01162
Number of floral units	0.019688	0.007802			

Table 2.3 Type 3 ANOVA of a GLMM for ground nesting bee abundance with the predictor variables: soil neonicotinoid category, sampling period, number of floral units and the interaction between sampling period and neonicotinoid category. Estimates and standard error from the GLMM summary included for each level underneath each variable in italics. Site was included as a random effect. Four visits took place approximately one-month apart from May- August. The three neonicotinoid levels are: low concentration (< 4 ppb), high concentration (≥ 4 ppb), and not detected.

Response: Ground nesting bee abundance	Estimate	Standard error	χ^2	Degrees of freedom	Pr(>χ^2)
Intercept			12.208	1	0.000476
Neonicotinoid level			9.54	2	0.00849
High	Reference	Reference			
Low	1.424032	0.557101			
ND	-0.179702	0.595457			
Sampling period			0.512	3	0.917
Visit 1	Reference	Reference			
Visit 2	0.258495	0.538302			
Visit 3	-0.147246	0.612130			
Visit 4	0.011129	0.567023			
Number of floral units			10.9	1	0.000952
Number of floral units	0.027545	0.008336			
Neonicotinoid level: Sampling period			14.6	6	0.0234
High: visit 1	Reference	Reference			
Low: visit 2	-1.590699	0.775196			
ND: visit 2	0.562578	0.792396			
Low: visit 3	-0.142728	0.794912			
ND: visit 3	1.123053	0.843583			
Low: visit 4	-1.883759	0.783944			
ND: visit 4	0.630294	0.803924			

Table 2.4 Exploratory analyses of crop, adjacent crop, and temperature of ground nesting bee abundance. Summary table of a type 3 ANOVA of the GLMM for bee abundance with the predictor variables: soil neonicotinoid category, sampling period, number of floral units and the interaction between sampling period and neonicotinoid category. Estimates and standard error from the GLMM summary included for each level underneath each variable in italics. Site was included as a random effect.

Exploratory variable	Fixed Factors	Estimate	Standard error	χ^2	df	p-value
Crop	Intercept			18.1	1	2.14e-05
	Crop			5.27	2	0.0717
	Corn	Reference	Reverence			
	Hay	-0.986	0.445			
	Soy	-0.619	0.351			
	Neonicotinoid Category			9.89	2	0.00710
	High	Reference	Reverence			
	Low	1.697	0.399			
	ND	0.259	0.633			
	Sampling period			0.179	3	0.981
	Visit 1	Reference	Reverence			
	Visit 2	0.225	0.536			
	Visit 3	0.0957	0.615			
	Visit 4	0.116	0.568			
	Number of floral units			5.41	1	0.0201
	Number of floral units	0.0199	0.00856			
	Neonicotinoid category: sampling period			13.5	6	0.0361
	High: visit 1	Reference	Reverence			
	Low: visit 2	-1.536	0.770			
	ND: visit 2	0.572	0.789			
	Low: visit 3	-0.346	0.792			
	ND: visit 3	0.850	0.844			
	Low: visit 4	-1.878	0.779			
	ND: visit 4	0.590	0.804			
Adjacent crop	Intercept			14.0	1	0.000181
	Adjacent crop			15.2	3	0.00162
	Corn	Reference	Reference			
	Hay	-0.818	0.323			
	Soy	0.616	0.310			
	Wheat	-0.681	0.432			
	Neonicotinoid Category			4.94	2	0.0846
	High	Reference	Reference			
	Low	1.157	0.524			
	ND	0.421	0.595			

	Sampling period			0.466	3	0.926
	Visit 1	Reference	Reference			
	Visit 2	0.261	0.507			
	Visit 3	-0.0729	0.571			
	Visit 4	0.104	0.532			
	Number of floral units	0.0249	0.00748	11.1	1	0.000873
	Neonicotinoid category: sampling period			16.9	6	0.00961
	High: visit 1	Reference	Reference			
	Low: visit 2	-1.602	0.729			
	ND: visit 2	0.565	0.746			
	Low: visit 3	-0.181	0.739			
	ND: visit 3	1.035	0.787			
	Low: visit 4	-1.990	0.738			
	ND: visit 4	0.520	0.755			
Temperature	Intercept			0.654	1	0.419
	Temperature	0.0299	0.0414	0.522	1	0.470
	Neonicotinoid Category			9.09	2	0.0106
	High	Reference	Reference			
	Low	1.432	0.560			
	ND	-0.112	0.606			
	Sampling period			0.383	3	0.944
	Visit 1	Reference	Reference			
	Visit 2	0.0488	0.608			
	Visit 3	-0.300	0.646			
	Visit 4	-0.0768	0.577			
	Number of floral units			8.24	1	0.00410
	Neonicotinoid category: sampling period			15.0	6	0.0203
	High: visit 1	Reference	Reference			
	Low: visit 2	-1.586	0.768			
	ND: visit 2	0.597	0.788			
	Low: visit 3	-0.206	0.795			
	ND: visit 3	1.0421	0.846			
	Low: visit 4	-1.958	0.785			
	ND: visit 4	0.569	0.802			

Table 2.5 Exploratory analyses of models that include the additional predictors of either crop, adjacent crop, or temperature on non ground nesting bee abundance. The table reports Type 3 ANOVA for GLMM's for ground nesting bee abundance with the predictor variables: soil neonicotinoid category, sampling period, number of floral units and the interaction between sampling period and neonicotinoid category. Estimates and standard error from the GLMM summary included for each level underneath each variable in italics. Site was included as a random effect.

Exploratory variable	Response variable	Fixed Factors	Estimate	Standard error	χ^2	df	p-value
Crop	Non ground nesting bee abundance	Intercept			17.736	1	2.538e-05
		Crop			11.616	2	0.00300
		Corn	Reference	Reference			
		Hay	-1.200	0.481			
		Soy	-1.334	0.407			
		Neonicotinoid Category			8.383	2	0.0151
		High	Reference	Reference			
		Low	1.138	0.394			
		ND	0.772	0.434			
		Sampling period			4.394	3	0.222
		Visit 1	Reference	Reference			
		Visit 2	0.118	0.319			
		Visit 3	-0.201	0.350			
		Visit 4	0.420	0.329			
		Number of floral units			3.637	1	0.0565
		Number of floral units	0.0139	0.00730			
Adjacent crop	Non ground nesting bee abundance	Intercept			8.148	1	0.00431
		Adjacent Crop			6.861	3	0.0765
		Corn	Reference	Reference			
		Hay	-0.661	0.403			
		Soy	0.510	0.399			
		Wheat	-0.124	0.560			
		Neonicotinoid Category			0.407	2	0.816
		High	Reference	Reference			
		Low	0.240	0.382			
		ND	0.139	0.445			
		Sampling period			4.195	3	0.241
		Visit 1	Reference	Reference			

		Visit 2	0.149	0.324			
		Visit 3	-0.246	0.353			
		Visit 4	0.372	0.333			
		Number of floral units			6.655	1	0.00989
		Number of floral units	0.0190	0.00737			
Temperature	Non ground nesting bee abundance	Intercept			0.211	1	0.646
		Temperature			2.684	1	0.101
		Temperature	0.0644	0.0393			
		Neonicotinoid Category			1.5552	2	0.460
		High	Reference	Reference			
		Low	0.472	0.420			
		ND	0.0197	0.436			
		Sampling period			6.439	3	0.0921
		Visit 1	Reference	Reference			
		Visit 2	-0.301	0.402			
		Visit 3	-0.708	0.429			
		Visit 4	0.0556	0.366			
		Number of floral units			4.563	1	0.0327
		Number of floral units	0.0161	0.00752			

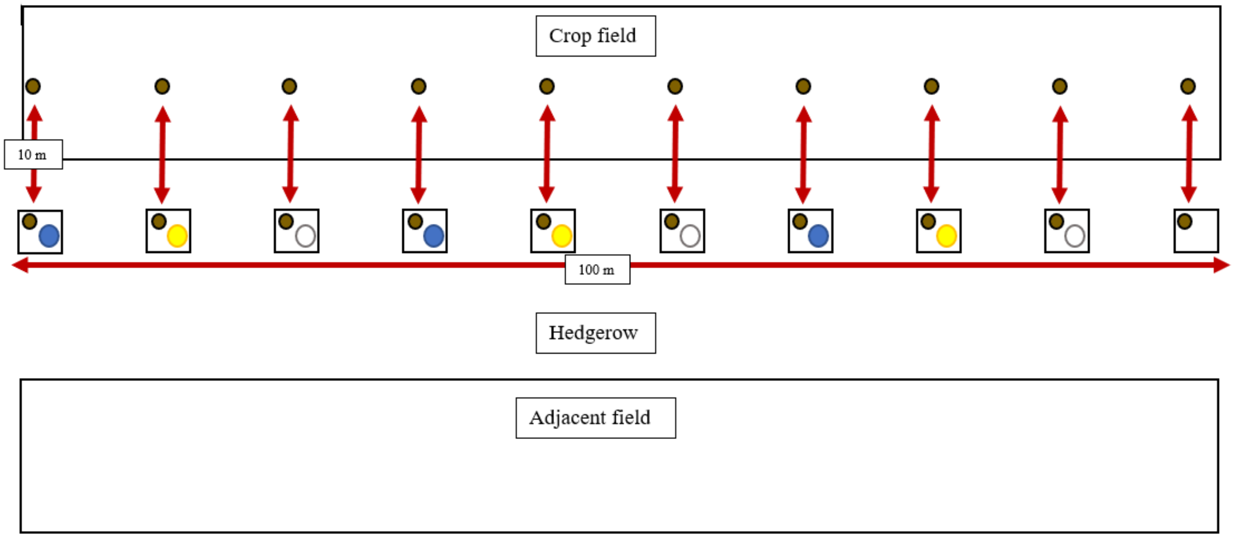


Figure 2.1 Transect set up. Squares represent quadrats, multicolored circles represent pan traps of differing colours, black circles represent where soil samples were taken.

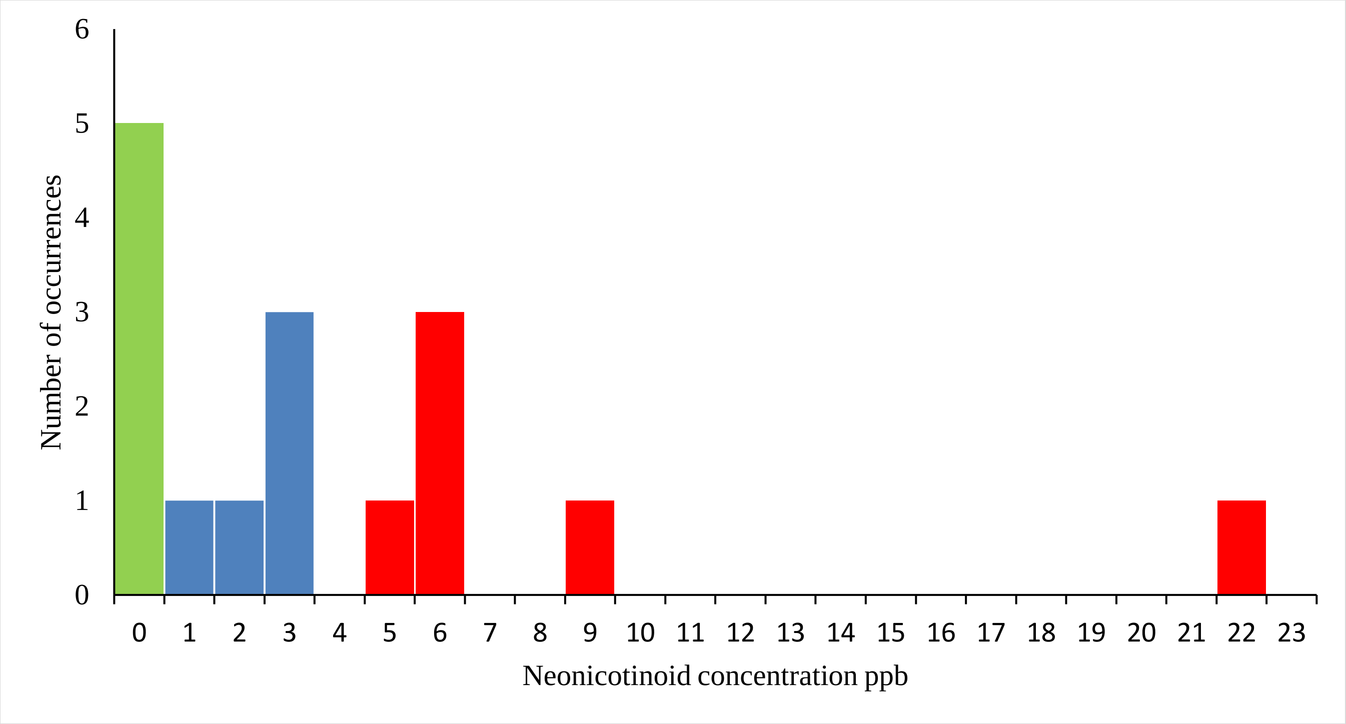


Figure 2.2 Histogram of total soil neonicotinoid concentrations. Green = no neonicotinoids detected; Blue = low concentration (< 4 ppb); Red = high concentration (≥ 4ppb).

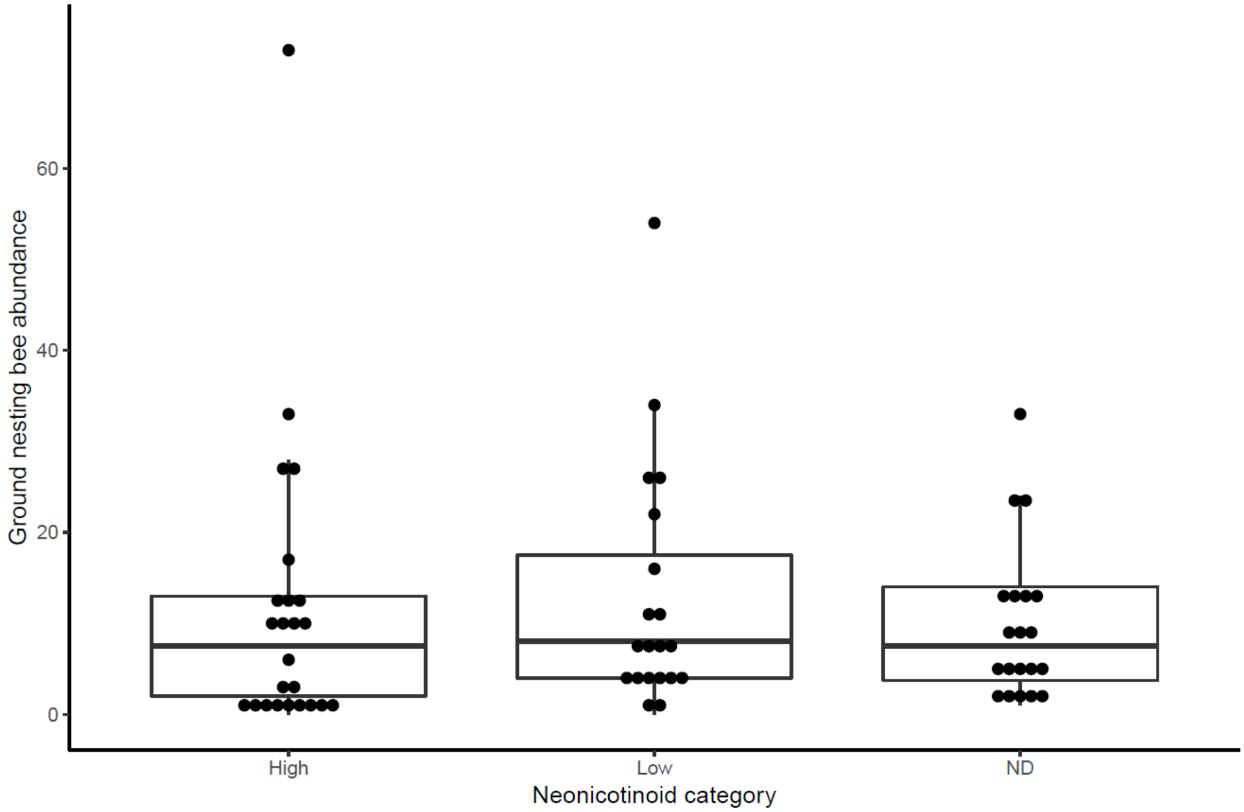


Figure 2.3 Boxplots of total ground nesting bee abundance for the three neonicotinoid categories: high concentration (≥ 4ppb), low concentration (< 4 ppb), ND, no neonicotinoid detected. The box represents the interquartile range, the horizontal line is the median.

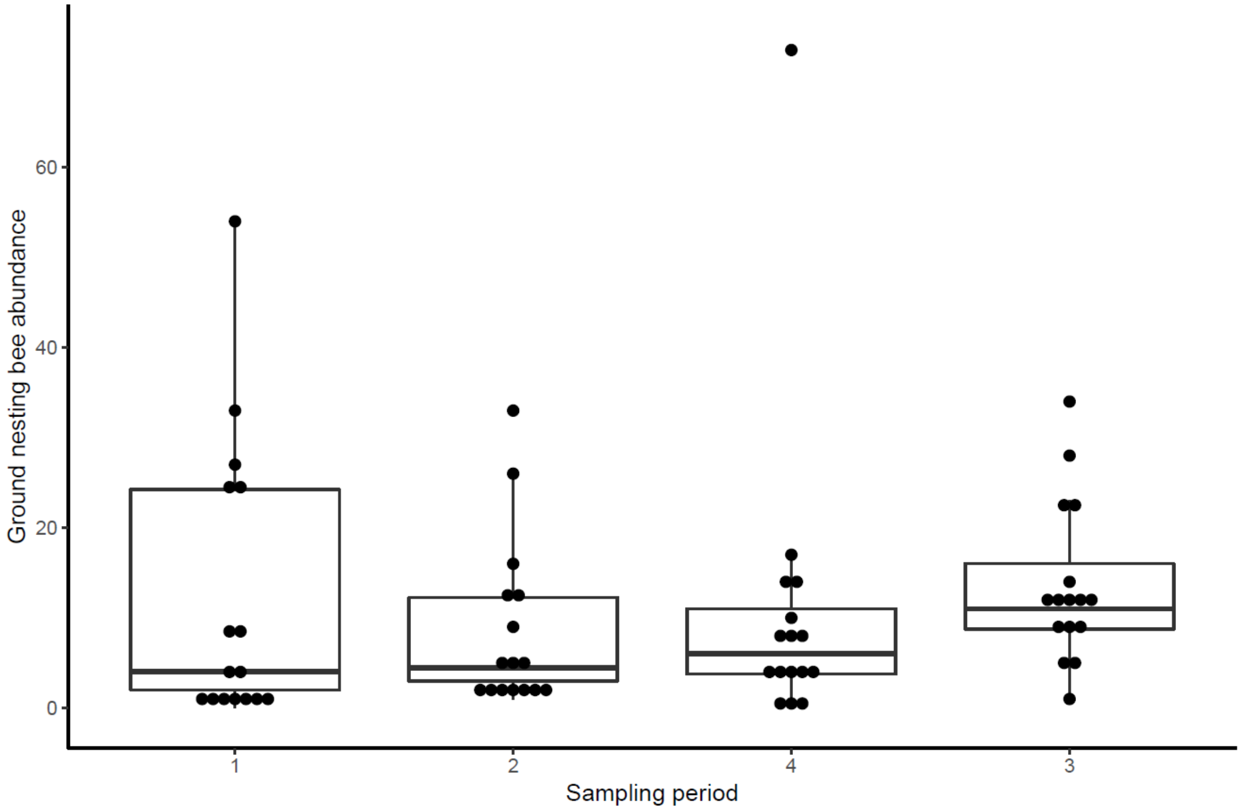
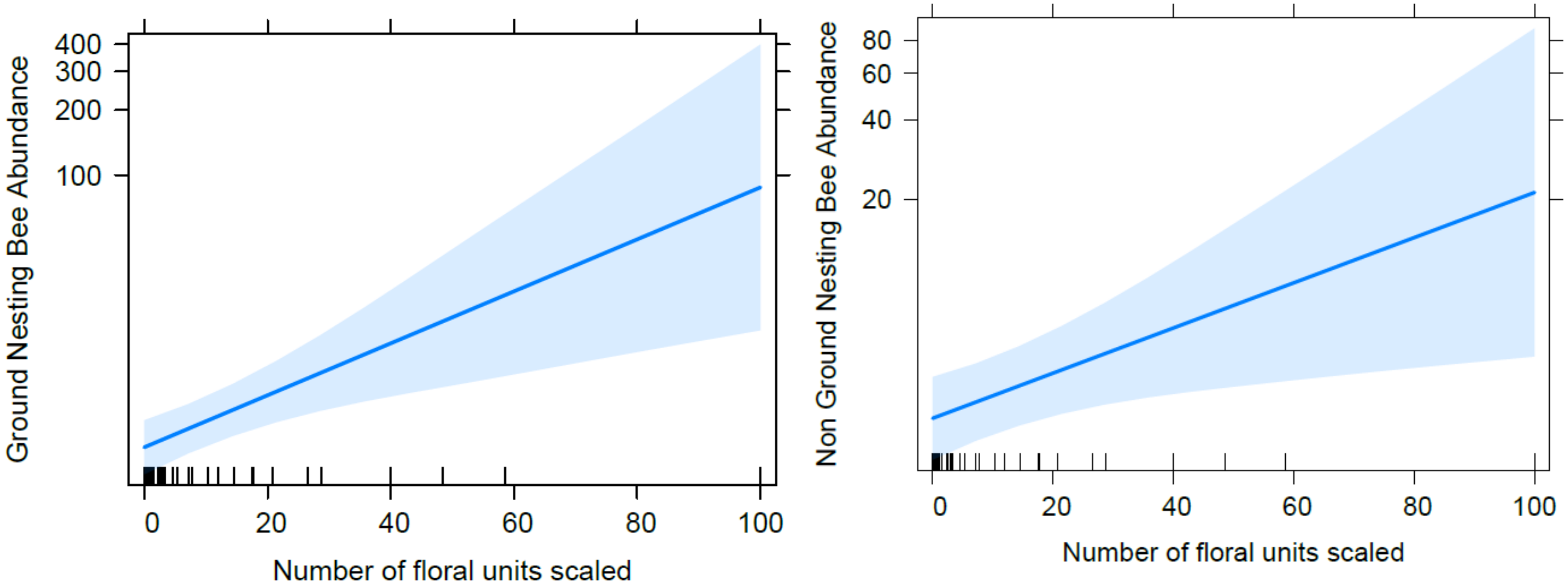


Figure 2.4 Boxplots of total abundance of ground nesting bees over the four sampling periods during 2019. Sampling period 1 = May 22-June 10, sampling period 2 = June 18-July 3, sampling period 3 = July 16-20, sampling period 4 = Aug 15-28. The box represents the interquartile range, the horizontal line is the median.



A)

B)

Figure 2.5 A) GLMM model predictions of non ground nesting bee abundance with number of floral units scaled; **B)** GLMM model predictions of non ground nesting bee abundance and number of floral units scaled. Shaded area is the 95% confidence interval dashes along x axis indicate data points.

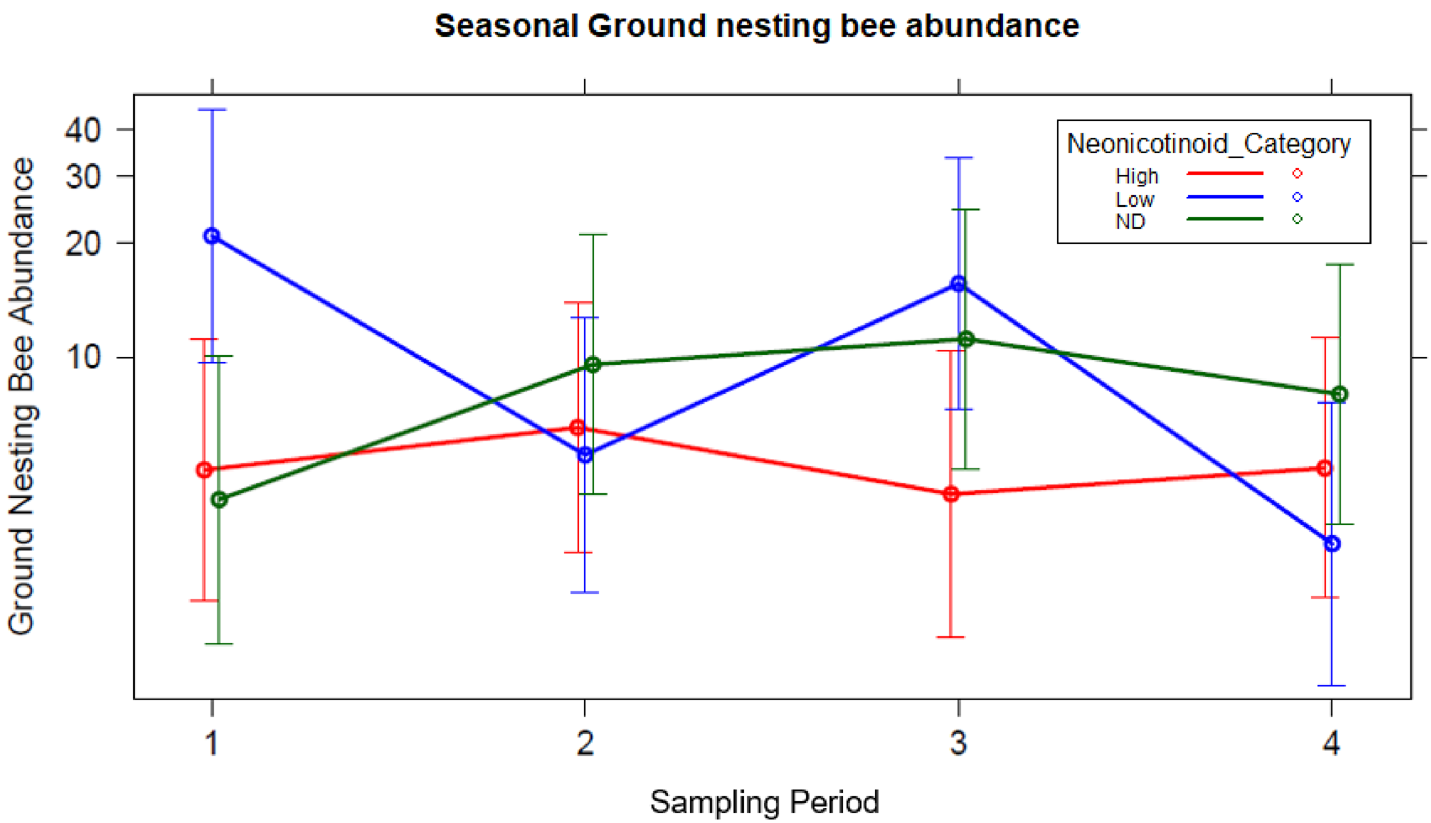


Figure 2.6 GLMM predicted effects of the interaction between sampling period and soil neonicotinoid concentration (i.e., High, ≥ 4ppb, Low, ≤4 ppb, ND, no neonicotinoid detected) on ground nesting bee abundance. Sampling period 1 = May 22-June 10, sampling period 2 = June 18-July 3, sampling period 3 = July 16-20, sampling period 4 = Aug 15-28. Error bars are 95% confidence intervals.

Appendix 1 A review of the knowledge gaps in bee research on non-neonicotinoid insecticides in Canada.

Abstract

There is increasing concern about the role insecticides may be playing in global bee declines. Most of the focus has been on the impacts of neonicotinoids on bees over the past decade. However, other insecticides known to be toxic to bees have not received the same level of attention. Here I report the results of a literature review of 28 insecticides registered in Canada known to be toxic to bees to determine their impacts, including relative toxicity and synergistic effects. In this report, I synthesize the state of the literature, including sales data, and identify and describe knowledge gaps. Many active ingredients in insecticide applications were reported to have synergistic effects with other insecticides and/or fungicides, and sensitivity varied by bee species. I found no relevant research for seven of the insecticides included in my review. Many insecticides had very few relevant studies and there was almost no research of the impacts of any of the insecticides on wild bee species. This highlights the need to pay attention to insecticides other than neonicotinoids that are toxic to bees.

Introduction

Pollinator populations are experiencing declines worldwide, often with corresponding declines in pollination services (Pindar et al. 2017). In Canada, the overall number of honeybee (*Apis mellifera*) colonies has increased since 1924. However, in Ontario, colony numbers have recently been on the decline (Pindar et al. 2017). Overwinter declines in Canada and the US have been much higher than the acceptable 15% loss rate indicated by beekeepers for the last eight years; Ontario's overwinter loss rate has averaged 35% (Pindar et al. 2017). This trend of overall pollinator loss as well as European honeybee colony loss is especially alarming considering that

76% of food crops worldwide depend on insect pollination, as do most wild flowering plant species (Klein et al., 2007). There have been worldwide reports of wild bee declines (Biesmeijer et al., 2006; Goulson et al., 2015). Bumblebee (*Bombus spp.*) population health in Canada varies considerably between species with some species exhibiting rapid recent declines, while others continue to do well (Colla et al., 2012). There have been no studies assessing population declines of non-*Bombus* native bees in eastern Canada (Pindar et al. 2017). Computer models predict that 23% of the United States (by area) experienced a decline in wild bee populations between 2008 and 2013 (Koh et al. 2016). Most of these areas correspond to regions with increasing agricultural intensity (Koh et al. 2016). Agricultural intensification typically corresponds with many factors linked to bee declines, including land-use changes, pests and pathogens, changes in agricultural management practice, climate change, and pesticide use (Pindar et al., 2017). The combination of these factors is likely the cause of the decline. The degree to which each factor contributes is currently unknown. This review will focus on the "pesticide stressor", the impacts of specific insecticides on pollinator survival, reproduction, and behaviour.

The recent literature on the impacts of pesticides on bees is mainly focused on neonicotinoids. A Web of Science search of the most commonly applied pesticides in Canada resulted in hundreds of results for each of the three main neonicotinoids (imidacloprid 447, and thiamethoxam 221, clothianidin 217). Conversely, the same search for other insecticides registered in Canada resulted in many fewer hits, with the highest being 105 (Deltamethrin) and some having no results (Table 1).

In 2016, Ontario introduced provincial regulations to reduce the amount of the three main neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) applied to corn and soy seed crops (Ontario Ministry of Agriculture, Food, and Rural affairs 2015). According to the pest management regulatory agency of Canada (PMRA) records, before the 2014 regulations were put in place, >50 000 kg of imidacloprid, >50 000 kg of thiamethoxam, and >100 000 kg of clothianidin were sold in Canada (Health Canada 2014). One year after the start of Ontario's regulatory restrictions, imidacloprid and clothianidin were reduced to <50 000 kg while thiamethoxam sales increased to >100 000 kg as thiamethoxam based seed coat formulas became more popular (Health Canada 2017) (N.B., PMRA records preclude more precise estimates). New federal pollinator risk assessments completed in 2019 led to the cancelation and restrictions to the use of imidacloprid, thiamethoxam, and clothianidin on some crops after it was determined that these uses are harmful to aquatic insects (Health Canada., 2019) (Health Canada., 2017). These recent assessments of neonicotinoids and effects on non-target insects raise questions about the effects of other agricultural insecticides on pollinators. Unfortunately, comparatively little research has focused on the impacts of other insecticides known to be toxic to bees, which include older pesticides that may have higher mammalian toxicity and newer pesticides for where there has been little published research.

Goals

The goals of this literature review are:

1. Review literature on commonly used insecticides known to be toxic to bees in Canada (excluding imidacloprid, thiamethoxam, and clothianidin).
2. Identify knowledge gaps and discuss the potential for lesser-known insecticides to harm bees.

Methods

I searched the PMRA (Pest Management Regulatory Agency) pesticide e-label database in October 2018 using the search term "insecticides" and then selected all that showed up in the categories of “Registered” and “Commercial”. This search resulted in 616 compounds (Health Canada 2016). I then reviewed each label and recorded the active ingredients, area of use, and environmental toxicity of each pesticide. I eliminated any non-insecticides (i.e., fungicides, surfactants, herbicides), as well as any products that are not used in agricultural field crops. I also eliminated the three neonicotinoids that are being considered (as of October 2018) for regulatory evaluation in Canada (imidacloprid, thiamethoxam, and clothianidin). I then made a summary table of all active ingredients and included a list of how many registered pesticides contain each active ingredient (Table 1). In the table, I included only insecticides that were marked as toxic to bees on the PMRA environmental toxicity warning label. This resulted in a list of 55 active ingredient combinations with 45 active ingredients. Next, I looked at the 2016 PMRA pesticide sales report (the most recent available at the date of writing, October 2018) and categorized the 45 active compounds into 20 chemical groups (Health Canada 2016). Fourteen of the active ingredients were fungicides that are used in combination with insecticides; these were eliminated, resulting in 31 active compounds from 14 chemical groups. For each of these chemical groups I recorded how many products are registered on the PMRA database, how much was sold in 2016, and the mechanisms of action (Table 1) (Health Canada 2018, Health Canada 2016).

I conducted a database search on Web of Science (Clarivate 2019) for each of the 31 active ingredients and the following search terms: (Bee OR Bees OR Apis mellifera OR Honeybees OR Bumblebee OR Bumblebees OR Stenotritidae OR Colletidae OR Andrenidae OR Halictidae OR Melittidae OR Megachilidae OR Apidae OR Apis OR Bombus). This resulted in 1,701 citations. I reviewed a maximum of 20 of the most recently published search results for each active compound and made a second spreadsheet where I recorded the bee species, insecticide(s) used in the study, the type of study (i.e., field, lab, semi-field etc.), the effect(s) measured (i.e., survival, reproduction, LD 50, colony weight, etc.), and the impacts on the effects measured (Pesticide paper reference summary). Lab studies were defined as studies where bees were confined in a laboratory space and were unable to forage naturally; semi-field studies were those carried out on bees that were placed in large enclosures with the ability to forage naturally (including those in greenhouse settings), and field studies were those where bees were not confined in any type of enclosure. Studies on tropical stingless bees and other non-North American bee species were left in, as there are very few studies on non-*Apis* species, and I did not want to limit my information on the interspecies difference in sensitivity. I did eliminate studies that did not directly measure bee health, including studies that were not on bees, those solely focused on chemical detection and methodology papers. I also eliminated papers that were not published in English. Papers that only give information on which insecticides were detected in an area were eliminated because they did not provide useful information on the impact of the insecticides on bees. After I eliminated papers that did not match my criteria, some compounds had no relevant research papers (but will be noted in the results section), resulting in a final list of 22 active ingredients.

Results

Abamectin

Abamectin is in the organic acids chemical group. There are seven commercial products registered in Canada containing Abamectin. Three are used on crops, one is used in greenhouses, and two are used for pest control in and around buildings. There were <50 000 kg of abamectin sold in Canada in 2016 (Health Canada 2017). I found six papers on abamectin. These papers included five experiments on honeybees, two were on *Bombus impatiens,* one on *B. terrestris* and one on *Melipona quadrifasciata*. One of the lab experiments found that oral exposure to Abamectin was less toxic to honeybees than emamectin, benzoate, spintoram, or spinosad (Abdu-Allah & Bittendrigh 2018). However, oral exposure to abamectin was also associated with a greater reduction in lifespan than deltamethrin in a different lab study (Aljedani 2017). Finally, a third lab experiment found it to be extremely toxic to honeybees exposed orally, topically, or by indirect contact (Costa et al. 2014). Another lab study found that abamectin was highly toxic to *M. quadrifasciata* and honeybees when applied orally but had low toxicity to *M. quadrifasciata* and moderate toxicity to *A. mellifera* when applied topically and *M. quadrifasciata* was more sensitive to abamectin than *A. mellifera* with exposure to dried insecticide residue (Del Sarto et al. 2014). Abamectin caused 100% mortality in *B. terrestris* at field-realistic doses in the lab (Besard et al. 2010). Abamectin was also found to have detrimental impacts on *B. impatiens* (Gradish et al. 2010).

Acephate

Acephate is in the phosphoramidothioates chemical group. There are three commercial products registered in Canada that contain acephate. Two products are registered for use on crops, and one is registered for use in forestry and horticulture (Health Canada 2016). There were <50 000 kg of phosphoramidothioates sold in Canada in 2016 (Health Canada 2017). I found three studies on acephate including two lab studies and one field study, all were performed on honeybees. One study found that honeybees exposed to acephate in the lab exhibit reduced levels of esterase and AChE (Yao et al. 2018; Zhu et al. 2017b). The same study found that acephate was associated with a synergistic reduction in body weight when applied with the insecticides clothianidin, lambda-cyhalothrin, oxamyl, tetraconazole, and chlorpyrifos on body weight (Yao et al. 2018). In a separate field experiment, all acephate levels tested on honeybees caused severe reductions in reproduction, as well as food storage and honeycomb construction behaviors (Fiedler 1987).

Acetamiprid

Acetamiprid is in the pyridines chemical group. There are three commercial products registered in Canada containing acetamiprid, all are used on crops (Health Canada 2016). There were <50 000 kg of acetamiprid sold in Canada in 2016 (Health Canada 2017). I reviewed eight papers on acetamiprid including seven lab studies and one field study. Seven studies were on honeybees, and four were on other bee species. In a lab study where *A. mellifera* and *A. cerana* were exposed to pesticides topically or indirectly with filter paper, acetamiprid was found to be less toxic than chlorpyriphos, dichlorvos, malathion, profenofos, monocrotophos, methyl demeton,methyl deltamethrin, indozacarb, cartap hydrochloride, flubendiamide, spinosad, imidacloprid, or thiamethoxam to honeybees and *A. cerana* in the lab (Stanley et al. 2015). In a separate study, acetamiprid applied topically to honeybees was shown to be less toxic (LD50 7.07 ug/bee) to honeybees than imidacloprid (LD5 0.0179 ng/bee), clothianidin (LD50 0.0218 ng/bee), and thiamethoxam (LD50 0.0299 ng/bee) (Iwasa et al. 2004). In addition, lambda-cyhalothrin, dimethoate, and phosmet, had a similar result when applied to *Osmia cornifrons* except for phosmet was less toxic than acetamiprid (Biddinger et al. 2013). Acetamiprid has been shown to have synergistic interactions on mortality with several compounds including flusilazole,triflumizole and propiconazole, and piperonyl butoxide (Iwasa et al. 2004; Manning et al. 2017). The maximum field recommended dose of acetamiprid was shown to be much more toxic when sprayed directly on honeybees (100% mortality) compared to indirect topical (60%) or oral exposure (47.6% mortality) (Costa et al. 2014). *A. cerana* died with a lower dose of acetamiprid applied orally than honeybees (Yue et al. 2018). Two other lab studies on acetamiprid did not have significant impacts on honeybee mortality at field recommended dose applied topically or orally (Iwasa et al. 2004;Yue et al. 2018). However, another study found that chronic oral exposure to field-realistic levels of acetamiprid did not have any significant effects on bumblebee or honeybee survival but caused sublethal effects on proboscis extension and movement in *B. impatiens* and was toxic to *Megachile rotundata* (Baines et al. 2017). Acetamiprid applied to crops had lower levels of transference form the crops to honeybees hives than imidacloprid or thiamethoxam (Silvina et al. 2017).

Bifenthrin

Bifenthrin is in the pyrethroids, pyrethrins chemical group. There is one commercial product containing bifenthrin in Canada and it is used on crops (Health Canada 2016). There were <50 000 kg of acetamiprid sold in Canada in 2016 (Health Canada 2017). I reviewed eight papers on bifenthrin. Seven were performed in the lab and two were semi-field experiments. Seven of the studies looked at honeybees and one looked at *B. impatiens.* In honeybees, bifenthrin has been shown to cause 100% mortality at 35µl/ml after fifteen minutes (no mortality at 0.035µl/mL) (Zhou et al. 2011), cause reduced lifespan at low levels (Liao et al. 2017), and increase CYP902 transcripts, repress CYP902 transcripts (Mao et al. 2011), reduce neuron excitability which could cause changes in egg laying behavior of queen honeybees (Zhou et al. 2011), reduce fecundity and increase development time (Dia et al. 2010). In a greenhouse experiment on honeybees, bifenthrin was found to be more toxic than flonicamid, thiamethoxam, cartap, and lufenron (Thomazoni et al. 2009). Bifenthrin has synergistic effects with the miticide flavalinate in honeybees (Ellis et al. 1997) but had no synergistic effects with clothianidin on *B. impatiens* (Larson et al. 2014).

Carbaryl

Carbaryl is in the carbamates chemical group. There are eight commercial products registered in Canada that have carbaryl as an active ingredient. Four of these are used on field crops, one is intended for use in forestry and one is primarily applied to lawns and ornamentals (Health Canada 2016). There were <50 000 kg of carbaryl sold in Canada in 2016 (Health Canada 2017). I reviewed eight studies on carbaryl four of which were lab experiments and four of which were semi-field. These experiments included five studies on honeybees and six studies on other species of bees. A lab experiment reported that carbaryl and five other insecticides inhibited carbonic anhydrase enzyme (AmCA) activity in vitro at low micro molar levels with binding affinity from highest to lowest tebuconazole, carbaryl, cabofuran, atrazine, simazine, and propozur (Soydan et al. 2017). Carbaryl did not have a synergistic effect with the miticide fluvalinate (Ellis et al. 1997). When bacteria from *A. cerana* foragers were collected and grown in a lab where it was exposed to carbaryl and then fed to new foragers it increased their survival when exposed to carbaryl (Sharma & Nath 1996). In a second study, the toxicity of pesticides (including carbaryl) was tested on four bee species in the lab, found the general relative toxicity across species from highest to lowest was permethrin, mexacarbamate, aminocarb, fenitrothion, carbaryl, trichlorfon (Helson et al. 1994). A semi-field experiment with *B. impatiens* found that fields sprayed with chlorpyrifos, carbaryl, and cyfluthrin all had significantly lower colony growth compared to controls, with half of the *B. impatiens* colonies dying in the carbaryl and chlorpyrifos treatments (Gels et al. 2002). A field experiment of honeybees foraging near sweet corn sprayed with permethrin, carbofuran, or carbaryl and parathion found relatively low mortality in all treatments, with permethrin associated with significantly lower mortality than any of the other pesticides (Erickson et al. 1997). A lab study looking at the impacts of topical application of carbaryl, resmethrin, and diazinon on honeybees found that rasmethrin had the highest acute toxicity followed by diazinon and carbaryl (Mackenzie & Winston 1989). However, this same study found that topical application of carbaryl was associated with the highest level of sublethal effects on foraging, and longevity especially with newly emerged foragers (Mackenzie & Winston 1989). Finally, a semi-field study carbaryl and chlorpyfifos on *M. rotundata* found that spraying of chlorpyrifos was associated with higher mortality than carbaryl, and carbaryl bran baits had no significant impact on survival while spray treatments of both compounds did (Gregory et al. 1992).

Chlorantraniliprole

Chlorantraniliprole is in the benzamides chemical group. There are six commercial products registered in Canada with chlorantraniliprole as an active ingredient, four are used on crops one is for turfgrass, and one is for termite control (Health Canada 2016). There were <50 000 kg of chlorantraniliprole sold in Canada in 2016 (Health Canada 2017). I reviewed seven papers on chlorantraniliprole, including two experiments on honeybees and two on *B. terrestris,* three on *B. impatiens,* one of *Partamona helleri,* and one on *Scaptotrigona xanthotrica.* Six of these studies were performed in the lab, two were field, and one was semi-field. One of the lab studies reported that chlorantraniliprole caused changes in gene expression including changes in immune system-related genes in honeybees (Christen & Fent 2017). Another lab study exposed *B. terrestris* to low levels of chlorantraniliprole found acute and chronic impacts after drinking contaminated sugar water but no significant impact when bees were exposed topically to contaminated pollen (Smagghe et al. 2013). There was no significant mortality for *P. helleri, S. xanthotrica,* (after oral exposure) and *B. impatiens* (after topical exposure) exposure to chlorantraniliprole in a lab setting (Tome et al. 2015; Gradish et al. 2010). Field and semi-field studies of *Bombus* spp. showed no impact of chlorantraniliprole spray treatments on clover and bluegrass fields (Larson et al. 2014; Larson et al. 2013).

Chlorpyrifos

Chlorpyrifos is in the thiophosphates chemical group. There are seventeen commercial products in Canada containing chlorpyrifos, fourteen are used on crops two are used on turfgrass, one is used in forestry (Health Canada 2016). There were >100 000 kg of chlorpyrifos sold in Canada in 2016 (Health Canada 2017). I reviewed six papers on chlorpyrifos, including six lab experiments and two semi-field experiments. Five of the studies were on honeybees and four were on other bee species. In the lab, chlorpyrifos was associated with changes in honeybee gene expression for genes related to immune function and metabolism (Christen & Fent 2017) and was also associated with suppressed esterase activity (Yao & Abamczyk 2018). Chlorpyrifos (LD50 0.46 µg) was more toxic to honeybee larvae than fluvalinate (LD50 0.86 µg), coumaphos (LD50 2.70 µg), imidacloprid (LD50 4.17 µg), and amitraz (14.83) in a lab dietary exposure study (Dai et al. 2017). But a second study found that honeybee worker survival was not impacted by environmentally relevant concentrations of chlorpyrifos (Nagger et al. 2015). Chlorpyrifos was found to be toxic to two species of stingless bees when ingested (Dorneles et al. 2017). Chlorpyrifos was associated with significantly reduced survival of *B. impatiens* larvae and was more toxic than carbaryl or cyfluthrin in field and semi-field experiment (Gels et al. 2002). Chlorpyrifos was also shown to be more toxic to *M. rotundata* than carbaryl in a semi-field study (Gels et al. 2002).

Cyantraniliprole

Cyantraniliprole is in the benzamides chemical group. There are eleven products that have cyantraniliprole as an active ingredient all are used on (Health Canada 2016). The were <50

000 kg of cyantraniliprole sold in Canada in 2016 (Health Canada 2017). I only identified a single study of cyantraniliprole that met my criteria. This study reported no significant risk to mortality, reproduction or other sublethal effects of cyantraniliprole to *B. terrestris* exposed to flowers that were sprayed with cyantraniliprole and honeybees foraging in a semi-field experiment (Dinter et al. 2015)

Cypermethrin

Cypermethrin is in the pyrethroids, pyrethrins chemical group. There are five registered products containing cypermethrin in Canada, four are used on crops and one is applied directly to livestock (Health Canada 2016). There were <50 000 kg of cypermethrin sold in Canada in 2016 (Health Canada 2017). I reviewed ten papers on cypermethrin, including eight lab studies, one semi-field study, and one field study. Nine of the studies were performed on honeybees and one on *B. terrestris*. In honeybees, direct contact with cypermethrin was found to be less toxic than imidacloprid, fipronil, and more toxic than dimethoate (Pashte & Patil 2018). Overall, cypermethrin had the second-lowest time until half of the bees died after the application of insecticide of the compounds studied (Pashte &Patil 2018; Pashte & Patil 2017). Cypermethrin caused a reduction in proboscis extension and movement at 1, 10, and 15 µg/kg in honeybees (Alquisira-Ramirez et al. 2017), sublethal impacts at 0.0025µg (Charreton et al. 2015), slowed sodium channel deactivation in neurons (Kadala et al. 2014), and changes in immune system gene expression and enzyme transcription (Christen & Fent 2017). In the field, honeybees were found to forage on cypermethrin treated fields (Fagundez et al. 2016). Honeybees were also found to have lower mortality after cypermethrin treatments during the night and in summer compared to spring foraging seasons (Piechowicz et al. 2016). In *B. terrestris*, cypermethrin was shown to have synergistic effects on mortality with the fungicide imazalil (Raimets et al. 2018).

Deltamethrin

Deltamethrin is in the pyrethroids, pyrethrins chemical group. There are nine registered products containing deltamethrin eight that are used on crops, and one is used for mosquito control (Health Canada 2016). There were >50 000 kg of deltamethrin sold in Canada in 2016 (2016a). I reviewed fifteen papers on deltamethrin, including fifteen lab studies with one paper having a lab and field component. Twelve of the studies were on honeybees and ten were on other bee species. Deltamethrin is extremely toxic when sprayed directly on bees (Costa et al. 2014) but is less toxic than abamectin, and clothianidin and more toxic than novaluron (Aljedani 2017; Zhang et al. 2016). *P. helleri* and *M. quadrifasciata* died at a lower dose of deltamethrin than honeybees (Del Sarto et al. 2014; Tome et al. 2017). *M. rotundata* was found to have a similar LD 50 as honeybees for deltamethrin (Piccolomini et al. 2018). *M. rotundata* had a lower LD 50 value (0.13) to deltamethrin than *B. impatiens* (6.90) or *O. lignaria* (8.90) (Scott-Dupree et al. 2009). Honeybees topically treated with deltamethrin at night and in the spring were less susceptible to deltamethrin than those sprayed in the summer or day (Piechowicz et al. 2016). Honeybees topically treated with deltamethrin in the summer were also more susceptible to synergistic effects between deltamethrin and prochloraz compared to winter bees (Meled et al. 1998). At sublethal levels, deltamethrin was found to suppress neuronal excitability (Zhou et al. 2011), reduce queen fertility, increase larval development time (Dia et al. 2010), elevated calcium concentrations in neurons (Wang et al. 2017) in honeybees in the lab. A direct contact toxicity bioassay of deltamethrin in field relevant concentrations was associated with shortened lifespan and lower production of males in *B. impatiens* (Gradish et al. 2012a), and mortality in adult and larval *M. rotundata* (Gradish et al. 2012b). Field experiments with deltamethrin showed no significant effect on honeybee mortality or enzyme activity (Pokhrel et al. 2018).

Another field experiment showed no difference in colony health between hives near deltamethrin treated areas and control fields (Pokhrel et al. 2018).

Diazinon

There are six registered insecticides in Canada that contain diazinon. Three are used on crops and three are used on livestock (Health Canada 2016). There were >500 000 kg of diazinon sold in Canada in 2016 (Health Canada 2017). I reviewed five papers on diazinon, all of which were in the lab. Four of these were experiments on honeybees, one was on *Melipona beecheii*, one was on *Trigona nigra*, and one was on *Nannotrigona perilampoides*. Sublethal levels of diazinon were found to have lower sublethal impacts than carbaryl and resmethrin to newly emerged honeybee workers (Mackenzie & Winston 1989). When tested on three *M. beecheii*, *T. nigra*, and *N. perilampoides*, diazinon was less toxic than imidacloprid, thamethoxam, thiacloprid, and permethrin, but more toxic than methomyl (Sharma & Nath 1996). Honeybees exposed to diazinon exhibited significant changes in AChE activity in the head and thorax but not in the hemolymph (Glavan et al. 2018). Injection or topical exposure of diazinon was also associated with impaired honeybee learning (Weick & Thorn 2002). A mixture of environmentally relevant concentrations of diazinon and three other pesticides found no significant difference in honeybee survival (Nagger et al. 2015). There were differences in the toxicity of diazinon to honeybee workers depending on the season and time of day of the application (Piechowicz et al. 2016).

Dichlorvos

There are six commercial products registered in Canada containing dichlorvos. One product is used for crops, three are used for commercial food storage, and two are used for livestock (Health Canada 2016). There were <50 000 kg of dichlorvos sold in Canada in 2016

(Health Canada 2017). I reviewed six studies on dichlorvos. Four of these were performed on honeybees and three were on other bee species. In in-vitro bioassays, honeybee expression of a gene related to metabolism, growth, and reproduction transcript was induced by environmental stressors, including dichlorvos in *A. cerana* (Chi et al. 2018). A second study showed that dichlorvos exposure via rainwater could cause AChE inhibition (Hamers et al. 2000). A bioassay on *M. rotundata* found the LC50 of dichlorvos slow release vapor to be 273ug/m3 (Purdy & Kevan 2014). Honeycomb frames that were exposed to dichlorvos vapor for four months were toxic to honeybees exposed to them for up to one month after dichlorvos exposure (Clinch 1970). A field study found no effect on honeybee mortality, or behavior when exposed to dichlorvos treated clover (Palmerjones & Clinch 1968).

Dimethoate

Dimethoate is in the dithiophosphate chemical group. There are five commercial products registered in Canada that contain dimethoate four are used on crops and one is used on ornamental plants (Health Canada 2016). There were >100 000 kg of dimethoate sold in Canada in 2016 (Health Canada 2017). I reviewed nine papers on dimethoate which included ten lab experiments and three semi-field experiments; eleven of these studies were on honeybees and eight on other bee species. In lab experiments where dimethoate was used as a positive control (a treatment with a known toxic response), ingestion of dimethoate was associated with significant honeybee mortality (Dia et al. 2018; Dia et al. 2018 b). Dimethoate was conducted to have a LD50 between 0.10 and .53 ng/µg in honeybees. For honeybees, dimethoate was found to be more toxic than glyphosphate (Dia et al. 2018), tau-fluvalinate, cadmium, arsenic, propiconazole or2,4-dichlor ophenoxyacetic acid when ingested (Hesketh et al. 2016), and difubenzuran when exposed to sprayed flowers (Chon et al. 2017). It is less toxic than imidacloprid, fipronil, indoxacarb, and cypermethrin (Pashte & Patil 2018). Dimethoate does not appear to have synergistic effects with fungicides (Robinson et al. 2017b) but does have synergistic effects with *Paenibacillus larvae* (the bacterium that causes American foulbrood) (Lopez et al. 2017). In a separate study, Dimethoate sensitivity was measured for seven different bee species. The authors found the LD50 from highest to lowest was *Lasioglossum malachurum= Andrena flavipes > Colletes hederae= O. bicornis males > O. bicornis females = B. terrestris* (Uhl. et al. 2016). Dimethoate is also more toxic to *O. cornifons* than honeybees (Biddinger et al. 2013). In semi-field spray treatments, dimethoate is less toxic than fipronil, imidacloprid, and cypermethrin, but more toxic than either indoxacarb or azabirachtin (Pashte & Patil 2017).

Flupyradifurone

Flupyradifurone is in the butenolides chemical groups. There are four commercial products in Canada containing Flupyradifurone all are used on crops. There were <50 000 kg of Flupyradifurone sold in Canada in 2016 (Health Canada 2017). I reviewed three studies on Flupyradifurone, two on honeybees and one on *A. cerana*, including two lab studies and one field study. Flupyradifurone has been shown to reduce the taste and appetitive learning performance in honeybee foraging for pollen and nectar, although only the highest concentration was shown to have significant effects (Hesselbach & Scheiner 2018). In a lab study *A. cerana* larvae and adults exhibited significant impairment to memory and learning compared to controls (Tan et al. 2017). A field experiment with honeybee colonies foraging on crops treated with the maximum field rate of flupyradifurone or control crops showed no significant difference across a variety of measurements of colony health (Campbell et al. 2016).

Lambda-cyhalothrin

Lambda-cyhalothrin is in the pyrethroids, pyrethrins chemical group. There are thirteen commercial registered insecticides in Canada containing lambda-cyhalothrin six are used on crops, and seven are used on commercial buildings, food storage buildings, or livestock (Health Canada 2016). There were <50 000 kg of lambda-cyhalothrin sold in Canada in 2016 (Health Canada 2017). I reviewed ten studies on lambda-cyhalothrin including nine lab studies, two field studies, and one semi-field study. Eight studies were performed on honeybees and three on *B. terrestris.* Residue levels of lambda-cyhalothrin were not associated with any significant changes in honeybee mortality but were associated with increased invertase activity which is an important enzyme in producing honey (Zhu et al. 2017a). Lambda-cyhalothrin was discovered to have an additive effect on mortality with imidacloprid and a synergistic effect on body weight reduction with clothianidin on honeybees (Zhu et al. 2017b; Yao & Abamczyk 2018). Honeybees topically treated with lambda-cyhalothrin at night and in the spring are less susceptible than bees treated in the summer or during the day (Piechowicz et al. 2016). Honeybees exposed to lambda-cyhalothrin exhibited a reduced lifespan, homing ability, proboscis extension response, and changes in gene expression in the lab (Liao et al. 2018). Field studies on honeybees have shown that lambda-cyhalothrin does not have a repellent effect when applied to soy (Fagundez et al. 2016) but is associated with reduced locomotor activity of bees near treated orchards, although there was no significant impact on social interaction time detected (Ingram et al. 2015). *B. terrestris* exposed to lambda-cyhalothrin via pollen or sprayed filter paper produced smaller workers and prevented learning in terms of pollen foraging but showed no other sublethal effects in the field and the lab (Baron et al. 2014; Gill & Raine 2014). However, another study found effects on reproduction, survival and other sublethal effects in *B. terrestris* (Ceuppens et al. 2015).

Malathion

Malathion is in the dithiophosphates chemical group. Eleven commercially registered products in Canada contain Malathion five are used on crops four are used on livestock and two are used on stored grain (Health Canada 2016). There were >100 000 kg of malathion sold in Canada in 2016 (Health Canada 2017). I reviewed four studies on malathion. All the studies I found were on honeybees, half were performed in the lab and half were field experiments. In the field experiments malathion spray treatment was associated with honeybee mortality rates of 22–100% at distances ≤61 m (Rinkevich et al. 2017). A second field study found that honeybees only had statically higher mortality compared to control when malathion was applied using a high-density rate of spray application; no significant effects were observed with the low dosage rate (Cabrera-Marin et al. 2016). Short term exposure of a mixture of diazinon, malathion, profenofos, and chlorpyrifos to honeybees in the lab had no impact on worker bee survival but did change AChE activity (Nagger et al. 2015). A final lab study found changes in gene expression of honeybee genes related to immune function, metabolism, and AChE receptors following oral exposure to malathion with concentrations below field recommended doses (Christen & Fent 2017).

Novaluron

Novaluron is an insecticide from the acylureas chemical group. There are two commercial products registered, both are for use on crops (Table 2). It is ineffective at killing adult insects (Health Canada 2016). There were <50 000 kg of novaluron sold in Canada in 2016 (Health Canada 2017). I reviewed six studies on novaluron, including one experiment on honeybees and six on other bee species. The studies consisted of five lab and two semi-field experiments. Of these studies, four found it to be toxic to developing honeybees, *M. rotundata*, and *B. terrestris* (Fine et al. 2017; Hodgson et al. 2011; Mommaerts, et al 2006; Pitts-singer and Parbour 2017). One found it was not toxic to adult *B. impatiens*, *M. rotundata*, and *O. lignaria* (Scott-Dupree et al. 2009). One found no significant impact except for reduced drone life expectancy in *B. terrestris* (Malone et al. 2007). This indicates that novaluron is likely not toxic to adult bees but could have detrimental impacts on developing bees of a variety of species.

Permethrin

There are 51 registered insecticides in Canada containing permethrin, ten are used on crops, 37 are used for commercial, food storage, or livestock buildings, or on livestock one three are used on mosquito netting and one is registered for airplanes (Health Canada 2016). There were >50 000 kg of permethrin sold in Canada in 2016 (Health Canada 2017). I reviewed eight papers on permethrin including eight lab studies and two field studies. Seven of the studies were performed on honeybees and seven included other bee species. In a field experiment, honeybee colonies exhibited lower mortality when they were near crops treated with permethrin compared to a carbaryl+parathion treatment or amethyl+parathion treatment (Erickson et al. 1997). Another field experiment found that honeybees exhibited lower mortality than mosquitoes and low overall mortality when sprayed with permethrin at the label indicated rate (Rinkevich et al. 2017). In the lab, permethrin binds to honeybee sodium channels (Gosselin-badaroudine et al. 2015), and exposure to low levels of permethrin caused sublethal effects, including a reduction in movement and social interaction time, and difficulty standing upright (Ingram et al. 2015, Oliver et al. 2015). When applied topically to honeybees, *A. erythronii*, *M. rotundata*, and *Bombus terricola*, permethrin was consistently more toxic than mexacarbate, aminocarb, fenitrothion, carbaryl, and trichlorfon (Helson et al. 1994). A lab experiment with *M. rotundata* showed similar toxicity between permethrin and etofenprox (0.051ug/bee) and permethrin (0.057ug/bee), but less toxicity than deltamethrin (0.0016 ug/bee) (Piccolomini et al. 2018). In *M. beecheii, T. nigra*, and *N. perilampoides*, permethrin applied topically was more toxic than diazinon and methomyl but less toxic than imidacloprid, thiamethoxam, and thiacloprid (Vaidovinos-Nunez et al. 2009).

Phosmet

There are two registered insecticides in Canada that contain phosmet both are applied to crops (Health Canada 2016). There were <50 000 kg of phosmet sold in Canada in 2016 (Health Canada 2017). I reviewed five studies on phosmet. Four of these were lab studies and one was a field study. Six bee species were studied, and the field study monitored the composition of wild bee communities. In the lab studies, topical and oral application of phosmet was found to be less toxic than Chlorpyrifos to *S. bipuctata* and *Tetragonisca fiebrigi*, but chlorpyrifos was more toxic to *T. fiebrigi* than *Scaptotrigona bipunctata* (Dorneles et al. 2017). Phosmet was also less toxic than imidacloprid, l-cyhalothrin, dimethoate to honeybees and *O. cornifons* (Biddinger et al. 2013). Phosmet was less toxic than deltamethrin to *B. impatiens* when applied topically or orally but more toxic than spinetoram, spinosad and much more toxic than flubendimide (Gradish et al. 2012a). When tested topically to *M. rotundata* adults or orally to *M. rotundata* larvae, Phosmet was less toxic than spintoram, spinosad, and deltamethrin but much more toxic than flubendimide (Gradish et al. 2012b). A field experiment on *M. rotundata* found bees produced fewer offspring in nest boxes near phosmet sprayed fruit trees; the same study found no difference in wild bee abundance (Alston et al. 2007).

Spinetoram

There are three commercial products registered in Canada containing spinetoram, all are used on crops (Health Canada 2016). There were <50 000 kg of spinetoram sold in Canada in

2016 (Health Canada 2017). I reviewed six papers on spinetoram, including six lab experiments and one semi-field experiment. Three studies were performed on honeybees and three on other bee species. Lab experiments with spinetoram have found that the presence of the fungicide flusilazole increased the toxicity of spinetoram in honeybees from 11.6% mortality to 76% mortality (using spinetoram concentrations below field application rate) (Manning et al. 2017). A different study found that spinetoram applied topically or orally was much less toxic to *B. terrestris* than spinosad and that dried residue or oral exposure was less toxic than topical exposure; neither caused sublethal effects at in field-realistic concentrations (Besard et al. 2011). However, when tested on honeybees, both spinetoram and spinosad were considered topically harmful, although spinosad was less toxic than spinetoram (Abdu-Allah & Bittendrigh 2018). Spinetoram applied topically was determined to pose a moderate hazard to honeybee workers in laboratory conditions (Chen et al. 2017). It was also found to have lethal and sublethal impact on *B. impatiens* and *M. rotundata* (Gradish et al. 2012a; Gradish et al. 2012b).

Spinosad

There are eight commercial products registered in Canada containing spinosad, four are used on crops, two are used on ornamentals and turf and two are used on buildings (Health Canada 2016). There were <50 000 kg of spinosad sold in Canada in 2016 (Health Canada 2017). I reviewed thirteen papers on spinosad, including ten lab experiments and three field experiments. Five studies were performed on honeybees and thirteen were performed on other bee species. In a lab study, the field-recommended concentration of spinosad killed100%of honeybees, while lower concentrations had serious sublethal impacts (Lopes et al. 2018). Spinosad was used as a positive control in an experiment with *Friesella schrottkyil* and was found to cause mortality significantly faster than copper sulphate in topical and oral bioassays

(Rodrigues et al. 2016). In three lab studies spinosad was also shown to have lethal and sublethal impacts on three species of *M. quadrifasciata, P. helleri*, and *S. xanthotrica* and pupa after oral or contact toxicity (Barbosa et al. 2015; Tome et al. 2015a; Tome et al. 2015b). A field study found that honeybee foragers avoided contaminated honey sources when they had access to equal quality uncontaminated nectar sources in one experiment (Cabrera-Marin et al. 2015) but another study found honeybees still foraged from contaminated nectar sources while *Scaptotrigona mexicana* and *Trigona fulviventris* avoided it (Gomez-Escobar et al. 2014). Imidacloprid and spinosad were both shown to be very toxic to honeybees, with oral exposure in the lab leading to reduced flight activity (Tome et al. 2015b). In a field study, stingless bees always avoided the tainted honey, but honeybees would still go to it provided the honey was sweet enough (Gomez-Escobar et al. 2014). In a comparison study, topical application of spinosad was found to be less toxic than imidacloprid and clothianidin to *B. impatiens, M. rotundata,* and *O. ligaria*, however, it was more toxic than deltamethrin for *B. impatiens* and *M. rotundata* (Scott-Dupree et al. 2009). Topical applications of spinosad caused increased levels of mortality in *B. impatiens* at 2348 mg/L but no sublethal effects at recommended field rates (Gradish et al. 2012a). In the field, spinosad was associated with higher mortality when it was applied at high-density high application rates but not when applied at low density or low application rates (Cabrera-Marin et al. 2016). Spinosad can cause death to *M. rotundata* at field application rates (Gradish et al. 2012). Spinosad is less toxic than spinetoram to honeybees but both have high contact toxicity (Abdu-Allah & Bittendrigh 2018). Conversely, when applied topically spinosad is more toxic to *B. impatiens* than spinetoram but neither caused sublethal effects at field-realistic concentrations in the lab (Besard et al. 2011).

Spirotetramat

Spinotetramat is part of the axoles, oxazoles, thiazoles chemical group. There are three commercial products registered in Canada that have spirotetramat as an active ingredient. Two of these products are used on field crops and one is used for greenhouse crops and field ornamentals. Spirotetramat is a systematic insecticide that is most effective on immature insects (Health Canada 2016). There were <50 000 kg of spirotetramat sold in 2016 (Health Canada 2017). I reviewed two lab studies on Spirotetramat. One looked at impacts on *Osmia cornuta* and one on *B. impatiens*. The study of *O. cornuta* looked at impacts on larvae and found that larvae experienced declines of 15% (females) and 18% (males) longevity, but there was no effect on mortality and no sublethal effects were detected (Sgolastra et al. 2015). The other study was on *B. impatiens*. This study found high mortality ten days after oral exposure to spirotetramat but no difference in mortality following topical exposure (Ramanaidu & Cutler 2013).

Discussion

Knowledge gaps

The results of my literature search demonstrate that many compounds being sold have received very little published research regarding bee health. The following compounds had fewer than ten results on my initial Web of Science search: novaluron, spirotetramat, cyantraniliprole, pyridaben, phosmet, spinetoram, spiromesifen, pyrethrin, and sulfoxaflor (Table 1). The following compounds had no papers that qualified for the review criteria: pyridaben, spiromesifen, pyrethrins, sulfoxaflor, *Bacillus thuringiensis spp.*, cyclaniliprole. As a result, these compounds are not covered in the review results but do have warnings about bee health on their PMRA labels. There is a need for more research on the effects of these compounds on bee health.

This paper does not cover compounds that were not registered as commercial insecticides on the PMRA label database as of November 2018 with “Harvanta 50sl insecticide” (registration number 32889) being the last registered insecticide considered for my review. It also does not cover compounds that did not have warnings about bee health on their label. This means that compounds that PMRA has not identified as toxic to bees were not included in this review despite the possibility of discovering hazards to use around bees in the future. It should also be noted that I did not review more than 20 papers for any compound even when there was more research available. This was due to time constraints, and because I wanted to get an overall view of registered pesticides in Canada instead of focusing on a few specific compounds.

Relative toxicities

All compounds in this review are known to be toxic to bees at some concentration, and this is stated on the product label. Reducing the amount of these bee-toxic insecticides could be beneficial to bee health. However, the degree and impact varied widely in the studies reviewed. Many studies in my review directly measured the relative toxicities of different insecticides on bees. While no individual study directly compared all insecticides considered in this review, comparative studies do provide some useful information about the relative toxicity of some active ingredients. I will now discuss how each insecticide relates in terms of toxicity to the other reviewed pesticides and the overall findings on each compound and classify them in order of compounds with the least number for studies reviewed to the most reviewed. Many of these compounds were tested alongside other active ingredients not formally included in this review. For clarity, I will focus on their relationship with the other reviewed insecticides as well as the three neonicotinoids imidacloprid, thiamethoxam, and clothianidin.

I have listed the compounds from least to most studies reviewed (Table 1). The following compounds had no relevant studies that fit my search criteria: *Bacillus thuringiensis spp.*, Cyclaniliprole, sulfoxaflor, pyrethrins, spiromesifen, pyridaben. The following compounds had 1-5 studies: cyantraniliprole (1), spirotetramat (2), acephate (3), Flupyradifurone (3), malathion (4), diazinon (5), phosmet (5). The following compounds had 6-10 relevant studies that fit my search criteria: abamectin (6), novaluron (6), spinetoram, dichlorvos, chlorantraniliprole (7), permethrin (8), acetamiprid (8), chlorpyrifos (8), carbaryl (8), bifenthrin (8), dimethoate (9), cypermethrin (10), lambda-cyhalothrin (10). The following compounds had 11-20 studies: spinosad (13), deltamethrin (15).

Active Ingredients

Cyantraniliprole

Cyantraniliprole appears to pose a low risk to *B. terrestris* based on a greenhouse, field, and lab experiments (Dinter et al. 2015).

Spirotetramat

Spirotetramat can reduce the longevity of *O. cornuta* and *B. impatiens* but was not associated with changes in direct mortality (Ramanaidu & Cutler 2013; Sgolastra et al. 2015). Overall, spirotetramat poses risk to bees under some circumstances.

Acephate

Acephate can cause sublethal effects in the lab (Yao et al. 2018: Zhu et al. 2017b) and has synergistic effects with several other insecticides on honeybee body weight (Yao et al. 2018). Acephate also caused large reductions in reproduction, food storage, and honeycomb construction (Fiedler 1987). Acephate is very toxic to bees, especially when used with synergistic compounds.

Flupyradifurone

Flupyradifurone showed some sublethal effects on honeybees and *A. cerana* in the lab but no effects on colony health measures (including the number of bees, weight of honey, etc) in a field study (Campbell et al. 2016; Hesselbach & Scheiner 2018; Tan et al. 2017).

Malathion

Malathion can cause bee death when applied at a high-density rate spray application or from less than 61m (Gabrera-marin et al. 2016; Rinkevich et al. 2017). Malathion is also associated with sublethal effects on honeybees in the lab with the ingestion of environmentally relevant concentrations but was not associated with decreased survival (Christen & Fent 2017; Nagger et al. 2015). As all the studies I found were on honeybees, nothing is known about the effects on other species of bees.

Diazinon

Diazinon can cause sublethal effects to bees in the lab (Glavan et al. 2018; Weick & Thorn 2002) but did not cause differences in survival when applied orally at environmentally relevant levels in combination with three other pesticides (Nagger et al. 2015).

Phosmet

Phosmet was less toxic to *S. bipunctata* and *T. fiebrigi* than imidacloprid, dimethoate, deltamethrin, cyhalothrin, spinetoram, and spinosad (Dorneles et al. 2017; Gradish et al. 2012). A field experiment on *M. rotundata* found a reduction in bee reproduction in nesting boxes near trees treated with phosmet (Grandish et al. 2012). Phosmet could pose risks to *S. bipunctata* and *T. fiebrigi* (Dorneles et al. 2017).

Abamectin

Despite being less toxic than four other pesticides it was tested against in a lab ingestion study, abamectin was still found to reduce honeybee lifespan (Aljedani 2017). It is also more toxic to *B. terrestris* and *M. quadrifasciata* than to honeybees (Besard et al. 2010; Del Sarto et al. 2014). Abamectin was found to be more toxic than dimethoate and novaluron with oral and topical applications in the lab (Aljedani 2017; Zhang et al. 2016). Overall, despite being less toxic than several other insecticides, abamectin is still highly toxic to bees (Costa et al. 2014).

Novaluron

Novaluron was less toxic than imidacloprid, clothianidin, deltamethrin, and spinosad (Scott-Dupree et al. 2009). All studies reviewed on novaluron showed that it was toxic to honeybee, *M. rotundata*, *B. terrestris* larvae but not adult bees (Fine et al. 2017; Hodgson et al. 2011; Malone et al. 2007; Mommaerts, et al 2006; Pitts-singer and Parbour 2017; Scott-Dupree et al. 2009).

Spinetoram

Spinetoram was found to be less toxic than spinosad, with direct and dried residue exposure (to *B. terrestris*) and dimethoate, but more toxic than spinosad topical and oral exposure (to honeybees) and phosmet (Abdu-Allah& Bittendrigh 2018; Gradish et al. 2012a). It also reacts synergistically with the fungicide fusilazole (Manning et al. 2017).

Dichlorvos

Honeycomb frames that were treated with slow release dichlorvos were toxic to honeybees for up to one month after treatment (Clinch 1970). Dichlorvos treated clover was also found to be toxic to foraging honeybees (Palmerjones & Clinch 1968). Overall, dichlorvos appears to pose a risk to honeybees under some circumstances. As all the studies I found were on honeybees, nothing is known about the effects on other species of bees.

Chlorantraniliprole

Chlorantraniliprole was less toxic than spinosad, imidacloprid (to *P. helleri and S. xanthotrica*), clothianidin (to *P. helleri, S. xanthotrica*, and *B. impatiens*) clothianidin+bifenthrin, or abamectin (to *B. impatiens*) (Gradish et al. 2010; Larson et al. 2014; Tome et al. 2015). Five of the seven studies I reviewed on chlorantraniliprole showed no negative impact on honeybees, *Bombus spp.*, *P. helleri, S. xanthotrica* exposed in lab, field or semi-field experiments (Dinter et al. 2015; Gradish et al. 2010; Larson et al. 2014; Larson et al. 2013; Tome et al. 2015). The other two studies on chlorantraniliprole found changes in gene expression (Christen & Fent 2017) or toxicity with oral but not topical exposure (Smagghe et al. 2013).

Permethrin

Permethrin was more toxic (lower LD50) than carbaryl, and diazinon but less toxic than deltamethrin, imidacloprid, and thiamethoxam to *M. beecheii*, *T. nigra*, and *N. perilampoides* in the lab (Valdovinos-Nunez et al. 2009). Permethrin is associated with sublethal impacts on honeybees in the lab (Ingram et al. 2015, Oliver et al. 2015). Permethrin overall was associated with lower mortality than some compounds but can be toxic under certain circumstances.

Acetamiprid

Acetamiprid is a newer type of neonicotinoid that is not being considered in the pollinator re-evaluation or the Ontario regulation with imidacloprid, thiamethoxam, and clothianidin (Ontario Ministry of Agriculture, Food, and Rural affairs 2015; Health Canada 2019). The papers I reviewed indicate that it is less toxic to honeybees, *A. cerana* and *O. cornifons* than many other compounds, including chlorpyriphos, dichlorvos, malathion, spinosad, lambda-cyhalothrin, dimethoate, or phosmet (Biddinger et al. 2013; Iwasa et al. 2004; Stanley et al. 2015). It is also less toxic to honeybees, and *O. cornifons* than the three main neonicotinoids currently in use in

Canada and has a lower transference rate from plants to honeybees than imidacloprid or thiamethoxam (Biddinger et al. 2013; Iwasa et al. 2004; Silvina et al. 2017). It does have synergistic effects with piperonyl butoxide and some fungicides (Biddinger et al. 2013; Iwasa et al. 2004; Manning et al. 2017). It is unlikely to significantly impact honeybee colonies at field recommended doses but could impact *M. rotundata* (Baines et al. 2017; Iwasa et al. 2004; Yue et al. 2018). Overall it appears to pose a smaller risk compared to many other insecticides including commonly used neonicotinoids.

Chlorpyrifos

Chlorpyrifos was the only insecticide in this review that was more toxic than imidacloprid and carbaryl (Dia et al. 2017; Mackenzie & Winston 1989). It has been shown to reduce bumblebee colony survival in a semi-field experiment (Gels et al. 2002). It has also been shown to be toxic to *S. bipunctata*, *T. fiebrigi*, and *B. impatiens* (Dorneles et al. 2017; Gels et al. 2002), even when applied at levels that may not be toxic to honeybees (Nagger et al. 2015). Ingestion by honeybees is associated with changes in gene expression in a lab study (Christen & Fent 2017). Overall, chlorpyrifos has been shown to pose serious risks to many bee species.

Carbaryl

Carbaryl was less toxic than permethrin, diazinon, and chlorpyrifos to *B. impatiens*, but it has been shown to reduce bumblebee colony growth and survival (Gels et al. 2002). Carbaryl caused only low levels of mortality in a honeybee field experiment (Erickson et al. 1997), carbaryl bran bait was not associated with increased alfalfa leafcutter bee mortality but carbaryl spray treatments were (Gregory et al. 1992).

Bifenthrin

Bifenthrin was shown to be more toxic to honeybees than thiamethoxam in a semi-field study (Thomazoni et al. 2009). Bifenthrin was shown to be highly toxic to honeybees and bumblebees, causing acute effects at low levels and a variety of sublethal effects at very low levels (Dia et al 2010; Liao et al. 2017; Mao et al. 2011; Thomazoni et al. 2009; Zhou et al. 2011). Bifenthrin also had synergistic effects on the LD50 value of honeybees with the miticide fluvalinate but not with clothianidin on *B. impatiens* colony strength in a semi-field study (Ellis et al. 1997; Larson et al. 2014).

Dimethoate

Dimethoate was less toxic than imidacloprid and cypermethrin but was found to be more toxic than eight other insecticides not considered in this review in two lab studies and one semi-field study (Chon et al. 2017; Dia et al. 2018; Hesketh et al. 2016). Dimethoate is also frequently used as a positive control in some experiments and is toxic at low levels (Dia et al. 2018; Dia et al. 2018b).

Cypermethrin

Cypermethrin was found to be more toxic (lower LD50 value) than dimethoate and less toxic than imidacloprid to honeybees (Pashte & Patil 2018). Cypermethrin is associated with some sublethal impacts on honeybees and can have synergistic effects with some fungicides (Alquisira-Ramirez et al. 2017; Christen & Fent 2017; Kadala et al. 2014; Raimets et al 2018).

Lambda-cyhalothrin

Lambda-cyhalothrin can cause a range of sublethal effects on bees in lab and field experiments (Baron et al. 2014; Ceuppens et al. 2015; Gill & Raine 2014; Ingram et al. 2015; Liao et al. 2018; Zhu et al. 2017a). Lambda-cyhalothrin has also been shown to have synergistic effects with clothianidin in honeybee reducing body weight with oral exposure (Yao &

Abamczyk 2018). Overall, lambda-cyhalothrin appears to pose a risk to honeybees and *B. terrestris* under some circumstances, especially when used in combination with synergistic compounds.

Spinosad

Spinosad was found to be less toxic than imidacloprid, clothianidin, dimethoate and spinetoram (to honeybees, and *B. impatiens*) but more toxic than deltamethrin, and spinetoram (*B. terrestris*) (Abdu-Allah& Bittendrigh 2018; Gradish et al 2012; Scott-Dupree et al. 2009). Spinosad is very toxic to bees and has been shown to cause a range of acute and sublethal effects even at low doses (Barbosa et al. 2015; Grandish et al. 2012; Lopes et al. 2018; Tome et al. 2015; Tome et al. 2015b).

Deltamethrin

Deltamethrin was more toxic than novaluron (in *B. impatiens, M. rotundata,* and *O. lignaria*), and deltamethrin (in honeybees), and less toxic than abamectin (in honeybees), clothianidin, and imidacloprid (in *B. impatiens, M. rotundata,* and *O. lignaria*) with oral exposure and spray treatments (Aljedani 2017; Scott-Dupree et al. 2009; Zhang et al. 2016). Deltamethrin was associated with sublethal impacts to honeybees in the lab (Dia et al. 2010; Grandish et al. 2012; Shou et al. 2011; Wang et al. 2017;). However, in field experiments it had a negative impact (Pokhrel et al. 2018). Overall it is unclear what the effects of deltamethrin are to bees in the field.

Comparison to neonicotinoids

All ten compounds in this review that were tested against imidacloprid were less toxic except for chlorpyrifos at the same concentrations. All of the five compounds tested against the neonicotinoid clothianidin were less toxic than clothianidin (Baines et al. 2017; Biddinger et al.

2013; Chen et al. 2017; Dia et al. 2017; Gels et al. 2002; Gradish et al. 2010; Pashte & Patil 2017; Pashte & Patil 2018; Scott-Dupree et al. 2009; Stanley et al. 2015; Tome et al. 2015a; Tome et al. 2015b; Yue et al. 2018). Of the three compounds that were compared against thiamethoxam, two were less toxic and bifenthrin was more toxic. I also reviewed the neonicotinoid acetamiprid and found that it was less toxic than all of the compounds it was compared to and seems to pose a much lower risk to honeybees and *O. cornifrons* than the other three neonicotinoids that are being considered for higher regulation (Baines et al. 2017; Biddinger et al. 2013; Costa et al. 2014; Silvia et al. 2017; Stanley et al. 2015; Yue et al. 2018;). However, it is important to note that the volume of neonicotinoid pesticides used in seed treatment (their most widespread use) is typically much lower than the volumes of pesticides required to treat the same crop area through other applications. Older classes of pesticides are mainly applied as a foliar spray, so direct toxicity comparisons should be taken according to the context and application rate. It is also important to consider that not all insecticides can be applied as seed coats like neonicotinoids and therefore might not make suitable alternatives. Proposed alternatives to neonicotinoids in Canada include sulfoxaflor, cyantraniliprole, flonicamid, spirotetramat, Flupyradifurone, spinosad, permethrin all of which had warnings about bee toxicity and were included in this review except for flonicamid.

Top-selling compounds of 2016

According to the PMRA, in 2016, sales carbaryl totaled between 50 000 and 100 000 kg, while dimethoate, malathion, and chlorpyrifos each sold between 100 000 and 500 000 kg, and diazinon sold over 100 000 kg of the active ingredient in 2016 (Health Canada 2017). All other insecticides covered in this review sold less than 50 000 kg of active ingredients (Health Canada 2017). It should also be noted that chlorpyrifos was the only insecticide that was found to be more toxic than imidacloprid in a direct comparison toxicity study on honeybees (Dia et al. 2018; Mackenzie & Winston 1989). Carbaryl has a wide array of approved use, including on trees, livestock, crops, lawns, ornamentals, and crops all of which are potential exposure routes for bees. Dimethoate is approved for use on crops, trees, and livestock quarters (Table 2). Malathion is approved for use on crops, grain in storage, and livestock (Table 2). Chlorpyrifos is used mainly on crops but also used on trees, in greenhouses, on industrial and non-residential buildings, and on ornamentals (Table 2). It should be noted that these sales data in addition to not giving exact numbers measure mass sold which doesn't take into account the differing toxicity of each compound.

Compounds with synergistic effects

The papers I reviewed found synergistic effects in the following active ingredients: Acephate, Bifenthrin, Cypermethrin, Deltamethrin, Lambda-cyhalothrin, Acetamiprid, Dimethoate, and Spinetoram. These synergistic effects lead to death or sublethal health effects with insecticide at lower doses than expected by an additive effect. Acephate had synergistic effects with clothianidin, lambda-cyhalothrin, oxamyl, tetraconazole, and chlorpyrisos on honeybee body weight (Yao et al. 2018). Bifenthrin had synergistic effects on mortality with the miticide fluvalinate (Ellis et al. 1997) but had no synergistic effects with clothianidin (Larson et al. 2014). Cypermethrin had synergistic effects on mortality with the fungicide imazalil (Raimets et al. 2018). Deltamethrin had synergistic effects with prochloraz (Meled et al. 1998). Lambda-cyhalothrin was found to have synergistic effects with clothianidin (Yeo&Abamczyk 2018). Acetamiprid has synergistic effects with several fungicides (Biddinger et al. 2013). Dimethoate has synergistic effects with *P. larvae* (causes American foulbrood in honeybees) (Lopez et al. 2017). Spinetoram reacts synergistically on honeybee mortality with flusilazole (Manning et al.

2017). When these compounds are used in combination with other compounds that potentially act as synergists, the application rates may not be adequately protective. Other insecticides in this paper may also have synergistic effects that have not been discovered as many of the insecticides I reviewed had little or no scientific research on their synergistic effects on bees.

Other bee species and development stages

The toxicity of some insecticides was dependent on the bee species, sex, and development stage tested. The following insecticides showed differential toxicity between bee species and development stages: Spinetoram, Spinosad, Phosmet, Acetamiprid, and Diazinon. Spinetoram was more toxic than Spinosad to honeybees but less toxic than spinosad to *B. terrestris* (Abdu-Allah & Bittendrigh 2018; Besard et al. 2011). Acetamiprid was less toxic than phosmet to honeybees but more toxic than phosmet when tested on *O. cornifrons* (Biddinger et al. 2013; Iwasa et al. 2004). Diazinon was more toxic to adult honeybees than carbaryl but less toxic to honeybee larvae than carbaryl (Mackenzie &Winston 1989). This indicates that in many cases honeybees are not an appropriate proxy for determining if an insecticide is toxic to other bee species; moreover, the development stage of honeybees may also impact study findings.

Some bee species showed major differences in their sensitivity to different insecticides reviewed. When contact toxicity of abamectin was tested on five different bee species it was found that *L. malachurum* and *A. flavipes* had the highest sensitivity, followed by *C. hederae* and *O. bicornis* males, with *O. bicornis* females and *B. terrestris* having the lowest sensitivity (Uhl. et al. 2016). Dimethoate applied topically is more toxic to *O. cornifons* than honeybees (Biddinger et al. 2013). Phosmet applied directly or indirectly on filter paper was less toxic than Chlorpyrifos to *S. bipunctata* and *T. fiebrigi*, but chlorpyrifos was more toxic to *T. fiebrigi* in a lab study (Dorneles et al. 2017). *M. quadrifasciata* is more susceptible to abamectin with ingestion or contact application than honeybees (Del Sarto et al. 2014). *M. quadrifasciata* and *P. helleri* are more susceptible to deltamethrin than honeybees (Del Sarto et al. 2014 ; Tome et al. 2017)**.** Phosmet applied topically was less toxic than acetamiprid to *O. corniforons* but not to honeybees (Biddinger et al. 2013; Iwasa et al. 2004). The toxicity of spinosad and spinetoram varied a lot depending on species. When phosmet was tested topically in a lab bioassay on *M. rotundata* it was found to be less toxic than spinetoram or spinosad, but spinosad was more toxic than phosmet when tested on *B. impatiens* (Gradish et al. 2012a; Gradish et al. 2012b). Direct contact with spinosad is more toxic than deltamethrin to *B. impatiens* and *M. rotundata* but not *O. ligaria* (Scott-Dupree et al. 2009). Spinetoram wet or dry residue was 52 and 8 times less toxic to *B. terrestris* than spinosad respectively (Besard et al. 2011). However, when applied topically or orally tested on honeybees, spinosad was found to be less toxic than spinetoram (Abdu-Allah & Bittendrigh 2018).

Different bee species exhibited different behavioral responses to different insecticides. Insecticides also differed in terms of their toxicity, depending on the timing of the application, type of exposure (oral or topical), duration of exposure, and development stage. *T. fulviventris, S. mexicana,* and *A. mellifera* always avoided foraging on honey that was contaminated with spinosad, but honeybees would still forage on contaminated honey when the honey was sweet enough (Gomez-Escobar et al. 2014). Honeybees sprayed at night and in the spring had lower mortality in response to deltamethrin treatment than those sprayed in the summer or during the day (Piechowicz et al. 2016). Honeybees sprayed in the summer were also more susceptible to synergistic effects between deltamethrin and prochloraz compared to winter bees (Meled et al. 1998). Diazinon was found to be less toxic than carbaryl to newly emerged honeybee workers but more toxic than carbaryl when tested on workers over 14 days old (Mackenzie & Winston

1989). Novaluron was only toxic to bee larvae but not adult bees with oral, spray, and direct contact applications (Fine et al. 2017; Hodgson et al. 2011; Mommaerts, et al 2006; Pitts-singer and Parbour 2017; Scott-Dupree et al. 2009). Some insecticides in this review were only tested on honeybees so it is not known if other bee species have differential responses. Acephate, and malathion were only tested on honeybees, and most of the other compounds reviewed had much more research on honeybees than all other bee species. This highlights the importance of testing compound on other bee species as well as considering the timing and type of an insecticide application. It should also be noted that almost all studies reviewed were on commercially reared bee species including all of the bumblebee species, the leafcutter bees and mason bees, and some of the neotropical stingless bee species. There was only one study that looked at wild bee diversity and abundance (Alston et al. 2007). To minimize the risk of insecticides to important wild pollinators, honeybees, and other commercially reared bee species it is important to study native bee species.

Application method, duration, and concentration

It was difficult to compare toxicity reports among studies as they varied greatly in their methodology for application type and duration of exposure. Forty-four studies performed only oral exposures, 20 studies only direct contact exposures, and six indirect contact. Twenty-four studies used field applications of pesticides and 18 performed multiple experiments with a combination of different exposure types. Oral application of insecticides is usually more toxic then direct contact toxicity (Abdu-Allah & Bittendrigh 2018; Del Sarto et al. 2014.; Ramanaidu & Cutler. 2013). Field and greenhouse applied pesticides were typically applied at field recommended rate sometimes with additional treatments at higher or lower rates (Hodgson et al. 2011; Lopes et al. 2018). Many of the oral or direct contact treatments were used multiple concentrations of insecticides to preform a bioassay and determine the LD50 of the pesticide (Abdu-Allah & Bittendrigh 2018; Besard et al. 2010; Chen et al. 2017; Del Sarto et al. 2014.; Gradish et al. 2012b; Iwasa et al. 2004; Purdy & Kevan 2014).

Studies also varied in the time frame that effects were measured after exposure, with most doing acute toxicity bioassays (which typically test for survival after 24 hours of exposure); other studies looked at the effects of chronic exposure which is based on longer term observations of several weeks (Baines et al. 2017; Ceuppens et al. 2015; Fine et al. 2017; Gill & Raine 2014; Glavan et al. 2018; Pitts-singer and Parbour 2017; Ramanaidu & Cutler 2013; Tan et al. 2017). All these factors are known to impact the reported toxicity of insecticides in bees.

Other pesticides

This review did not explicitly include herbicides, fungicides, antimicrobials or other pesticides not specifically classified as insecticides by the PMRA. However, some of these compounds have direct or indirect impacts on bees. For example, some fungicides have synergistic effects with many of the compounds studied here, which can increase their toxicity and may lead to an underestimation of the risk that many bee species face in field-realistic conditions. In Canada, insecticides only make up a small percent (5.7%) of overall pesticides used in Canada for agricultural purposes, with herbicides making up the vast majority of active ingredient sold (72.9%) and fungicides being the second biggest class of pesticides used (10.1%) in 2016 (Health Canada 2017).

Conclusion

After reviewing 29 commonly used registered insecticides that have warnings regarding bee toxicity on their labels, I have found that seven of them had no relevant research on bee health. Ten of these compounds were directly tested against clothianidin, imidacloprid, or thiamethoxam and the only compound found to be more toxic than imidacloprid was chlorpyrifos, which is also one of the top-selling insecticides by weight in Canada (Health Canada 2017). Many compounds also had synergistic effects with other insecticides or fungicides. Insecticide sensitivity can change with bee species and development stages, so it is important to consider these factors when determining the impact of a specific insecticide. Many of these compounds had very little discoverable research, and it was especially difficult to determine the risks of these insecticides to bees, especially species other than honeybees. More research is needed on current and newly emerging insecticides to minimize the risk to bees. Focusing exclusively on one class of insecticide can cause other important risks to be overlooked.

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Table A1.1. Summary of number of papers reviewed for each active ingredient, number of registered products and sales data. The number of search results for each active ingredient on web of science combined with the search term and ((Bee OR Bees OR Apis melliferea OR Honeybees OR Bumblebee OR Bumblebees OR Stenotritidae OR Colletidae OR Andrenidae OR Halictidae OR Melittidae OR Megachilidae OR Apidae OR Apis OR Bombus)), the number of studies that were actually reviewed after eliminating irrelevant studies, the number of registered products used on field crops that use that active ingredient on the PMRA database and the amount of active ingredient (in kg) sold in Canada in 2016.

Chemical groups	Active ingredients	Number of search results Web of Science	Number of reviewed studies	Number of registered products used on field crops	Amount sold in Canada 2016 (kg)
Acylureas	Novaluron	9	6	2	<50 000
Azoles, Oxazoles, Thiazoles	Spirotetramat	3	2	2	<50 000
	Chlorantraniliprole	14	7	4	<50 000
Benzamides	Cyantraniliprole	6	1	11	<50 000
Carbamates	Carbaryl	45	8	4	>50 000
Diazines	Pyridaben	3	0	3	<50 000
	Dimethoate	63	9	4	>100 000
	Malathion	65	4	5	>100 000
Dithiophosphates	Phosmet	9	5	2	<50 000
	Clothianidin	217	N/A	13	<50 000
	Imidacloprid	447	N/A	25	<50 000
Guanidines	Thiamethoxam	221	N/A	19	>100 000
	Abamectin	13	6	3	<50 000
	Spinetoram	7	6	3	<50 000
	Spinosad	47	13	4	<50 000
Organic Acids	Spiromesifen	2	0	2	<50 000
Phosphates	Dichlorvos	19	6	1	<50 000
Phosphoramidothioates	Acephate	15	3	2	<50 000
	Bifenthrin	25	8	1	<50 000
	Cypermethrin	51	10	4	<50 000
Pyrethroids, Pyrethrins	Deltamethrin	105	18	8	<50 000

	Lambda-cyhalothrin	32	10	6	<50 000
	Permethrin	51	8	9	<50 000
	Pyrethrins	9	0	3	<50 000
Pyridines	Acetamiprid	86	8	2	<50 000
	Sulfoxaflor	8	0	3	<50 000
	Flupyradifurone	6	3	2	<50 000
	Chlorpyrifos	82	8	14	>100 000
Thiophosphates	Diazinon	24	5	3	>500 000
Microbials	Bacillus thuringiensis ssp. Aizawai	0	0	1	N/A
2017 registration	Cyclaniliprole	0	0	N/A	N/A

Table A1.2. Summary table of registered commercial insecticides in Canada that are labelled as toxic to bees (2018). Including product registration number, product name, active ingredients, uses and hazards listed on the online product labels from the online label database (Health Canada 2017).

Registration Number	Product Name	Active Ingredients	Uses	Hazards
4590	Fyfanon 50% Emulsifiable Concentrate Insecticide	Malathion	Crop and grain storage	toxic to bees and people and birds and aquatic systems
5821	Malathion 500 Emulsifiable Concentrate Insecticide	Malathion	Crops	toxic to bees and people and birds and aquatic systems
7442	Dibrom Insecticide	Naled	Crops	toxic to aquatic organisms
8277	Cygon 480 Systemic Insecticide	Dimethoate	Crops and trees (including soy)	toxic to bees and people and birds and aquatic systems
8372	Malathion 85e	Malathion	Crops	toxic to bees and people and birds and aquatic systems
9337	Fyfanon Ulv Ultra Low Volume Concentrate Insecticide	Malathion	Crops	toxic to bees
9382	Lagon 480 E Insecticide	Dimethoate	Crops and trees (including soy)	toxic to bees and people and birds and aquatic systems
9542	Bartlett Superior 70 Oil Emulsifiable Insecticide	Mineral Oil	Crops mostly fruit	
9807	Cygon 480 Ec Systemic Insecticide	Dimethoate	Crops and trees (including soy)	toxic to bees and people and birds and aquatic systems
11252	Dipel Wp	Bacillus Thuringiensis	Crops	Don't apply in water
11302	Thuricide-Hpc High Potency Aqueous Concentrate	Bacillus Thuringiensis		Don't apply in water
11889	Diazinon 500 E	Dichlorvos Plus Related Active Compounds	Crops	toxic to bees and people and birds and aquatic systems

13816	Dimilin 25% Wp Insecticide - Insect Growth Regulator	Diflubenzuron	for mosquito control, gypsy moth control, and forest insect control	toxic to aquatic organisms
14225	Orthene 75% Soluble Powder Systemic Insecticide	Acephate	Crops including corn, vegetables, trees, berries	toxic to bees and people and birds and aquatic systems
14669	Safer's Insecticide Soap Concentrate	Potassium Salts of Fatty Acids	Crops including vegetables, fruits, shrubs trees, or greenhouse	toxic to aquatic organisms
14879	Lorsban 4e Insecticide	Chlorpyrifos	Crops including corn, vegetable, trees, berries	toxic to bees and people and birds and aquatic systems
14882	Ambush 500ec Insecticide	Permethrin	Crops including vegetables, fruits, trees, and, ornamentals	toxic to aquatic ecosystems and beneficial insects
14976	Ambush 50ec Emulsifiable Concentrate Insecticide	Permethrin	Greenhouse ornamentals, tomatoes, and cucumbers	toxic to aquatic ecosystems
14981	Superior 70 Oil	Mineral Oil	Crops (fruits, rutabagas, and ornamentals)	toxic to aquatic ecosystems
15738	Ripcord 400ec Agricultural Insecticide	Cypermethrin	Crops including corn	toxic to bees, beneficial insects and aquatic ecosystems
16458	Lorsban* 15g Insecticide	Chlorpyrifos	Crops	toxic to birds, mammals, bees, beneficial insects, and aquatic systems
16465	Lime Sulphur Insecticide Miticide Fungicide	Lime Sulphur or Calcium Polysulphide	Crops (fruits and berries)	toxic to aquatic organisms
16653	Sevin T&O Insecticide	Carbaryl	trees	toxic to bees, birds, mammals, and aquatic organisms
16688	Pounce 384 Ec Insecticide	Permethrin	Crops, fruits, vegetables, trees, farm buildings	toxic to bees and aquatic organisms

17534	Sevin 5-D Insecticide Dust	Carbaryl	Livestock and crops	toxic to bees
17734	Decis 5 Ec Insecticide (Praries And Peace River Region of BC)	Deltamethrin	crops (western provinces)	Toxic to mammals, bees, aquatic organisms, beneficial insects
19455	Vectobac 600l Biological Larvicide	Bacillus Thuringiensis, Serotype H-14	Greenhouse vegetables	
19466	Vectobac 200g Biological Larvicide Granules	Bacillus Thuringiensis, Serotype H-14	Mosquito control	
20015	Hornet & Wasp Killer Ii	Propoxur And Piperonyl Butoxide And Pyrethrins and N-Octyl Bicycloheptene Dicarboximide	Kills wasps hornets and bees	toxic to bees
20078	Decis Flowable Insecticide (Prairies and Peace River Region of BC)	Deltamethrin	crops (western provinces)	toxic to bees and aquatic organisms and beneficial insects
20384	Flying & Crawling Insect Killer I	Pyrethrins and Piperonyl Butoxide	A concentrated insecticide for use in Hospitals, Food Processing Plants, Restaurants, Hotels and other Commercial and Industrial Buildings, to kill flying and crawling insects. For use in Milk Parlours and Milk houses. Also for use on Beef and Dairy Cattle to kill flying insects	toxic to aquatic organisms

20385	Flying & Crawling Insect Killer Ii	Pyrethrins And Piperonyl Butoxide	A concentrated insecticide for use in Hospitals, Food Processing Plants, Restaurants, Hotels and other Commercial and Industrial Buildings, to kill flying and crawling insects. For use in Milk Parlours and Milk houses. Also for use on Beef and Dairy Cattle to kill flying insects.	
20575	Dursban T Insecticide	Chlorpyrifos	Turf, greenhouses, industrial sites	Toxic to wildlife, birds, aquatic organisms, beneficial insects, bees
20836	Treflan Granular Herbicide	Trifluralin	Grasses, shrubs, and trees	Avoid runoff into water sources
20880	Pro Magic Mist Ds Insecticide	Pyrethrins and N-Octyl Bicycloheptene Dicarboximide and	Insecticide is designed for use in hospitals, restaurants, food processing plants, food service establishments, stables, barns and poultry houses while they are in operation. Also for use in meat processing and meat packaging plants, when not in operation. Each can contains enough product to protect an area of at least 170 m3 of free flow air space for up to 30 days.	Toxic to fish and birds

20944	Lorsban 50w Insecticide	Chlorpyrifos	Crops	Toxic to mammals, bees, aquatic organisms, beneficial insects, and birds
21568	Acecap 97 Systemic Insecticide Implants	Acephate	Trees	Toxic to bees, birds, aquatic organisms
21655	Sunspray Ultra-Fine Spray Oil	Mineral Oil	Crops (fruit rutabaga and trees)	Toxic to aquatic organisms
21997	Dursban Water Soluble Insecticide	Chlorpyrifos	Turf, around non residential buildings, ornamentals	toxic to birds, mammals, bees, beneficial insects, and aquatic systems
22122	Buzz-Off Wasp & Hornet Blaster	N-Octyl Bicycloheptene Dicarboximide And Pyrethrins And Piperonyl Butoxide And Propoxur	Kills wasps hornets and bees	toxic to bees
22339	Chipco Sevin Rp2 Carbaryl Insecticide Liquid Suspension	Pyrethrins And Piperonyl Butoxide and N-Octyl Bicycloheptene Dicarboximide	For control of insect pests on field, vegetable, and fruit crops, ornamentals, lawns, and poultry.	toxic to bees and some non target plants
22478	Decis 5 Ec Insecticide (Eastern Canada & British Columbia)	Deltamethrin	Crops	Toxic to mammals, aquatic organisms, bees, beneficial insects
22661	Terand Wasp & Hornet Killer	N-Octyl Bicycloheptene Dicarboximide And Pyrethrins And Piperonyl Butoxide And Propoxur	Kills wasps hornets and bees	Toxic to bees

23006	Imidan 50-Wp Instapak	Phosmet	Crops	Toxic to birds, mammals, aquatic organisms, bees, beneficial insects
23216	Spray-Pak Flying Insect Killer Metered Pressurized Spray	Pyrethrins And Piperonyl Butoxide	To control: flies, mosquitoes, small flying moths and gnats use areas: food plants, restaurants, stores, hotels, motels, public buildings, industrial settings, dairies, stables, cow barns, poultry houses and other areas with at least 170 m3 of free flow air space	Toxic to fish and birds
23433	Bugwacker Tall Insecticide	Pyrethrins And Piperonyl Butoxide	A concentrated insecticide for use in Hospitals, Food Processing Plants, Restaurants, Hotels and other Commercial and Industrial Buildings, to kill flying insects and crawling insects. For use in Milk Parlours and Milk Houses. Also for use on Beef and Dairy Cattle to kill flying insects.	
23704	Pyrate 480 Ec Insecticide	Chlorpyrifos	Adult and Larval Mosquitoes, Mountain Pine Beetle in Forestry Situations	Toxic to birds, mammals, aquatic organisms, bees, beneficial insects
23705	Pyrinex 480ec For Food Crops	Chlorpyrifos	Crops	Toxic to birds, mammals, aquatic organisms, bees, beneficial insects

23917	Force 3.0g Insecticide	Tefluthrin	Crops	Toxic to aquatic organisms birds and wild animals
24071	Bio-Environmental Permethrin Water Based Insecticide for Food and Orna	Permethrin	Crops	Toxic to fish and bees
24094	Admire 240 Flowable Systemic Insecticide	Imidacloprid	Crops	Toxic to aquatic ecosystems bees and birds
24175	Dragnet Ft Emulsifiable Concentrate Insecticide	Permethrin		Toxic to bees, predatory mites, fish, aquatic invertebrates, birds, wildlife
24212	Country Mist Insecticide Metered Pressurized Spray	Piperonyl Butoxide And Pyrethrins N-Octyl Bicycloheptene Dicarboximide	Food handling and food preparation areas of restaurants and food processing plants and in stores, farms, dairies, barns, poultry houses, hotels, motels, supermarkets, food warehouses, hospitals and nursing homes. Also for use in meat processing and packaging plants when not in operation	Toxic to fish and birds
24363	Safer's Trounce Insecticide Concentrate	Pyrethrins And Potassium Salts of Fatty Acids	Crops (field, greenhouse and trees)	Toxic to aquatic organisms

24400	K-G Insecticide I	Pyrethrins and N-Octyl Bicycloheptene Dicarboximide And Piperonyl Butoxide	Homes, Food Handling Establishments, Commercial or Institutional Kitchens, Dining Areas and Pantries of Restaurants, Supermarkets, Food Plants, Food Warehouses, Bottling Plants, Hospitals, Hotels, Motels, Stores, Dairies, Stables, Nursing Homes, Daycare Centers, Egg and Milk Handling Areas of Dairies and Chicken Houses	
24464	Governor 75wp Insecticide	Cyromazine	Insect Growth Regulator for the control of larval stages of Colorado potato beetle on potatoes in Ontario, Quebec and Atlantic Canada ONLY.	
24465	Citation 75wp Insecticide	Cyromazine	greenhouse ornamentals and outdoor ornamentals	
24485	Avid 1.9% Ec Miticide Insecticide	Abamectin	greenhouse ornamentals and greenhouse vegetables	Toxic to predatory mites, bees, fish, wildlife
24503	Confirm 240f Agricultural Insecticide	Tebufenozide	crops	Toxic to aquatic ecosystems
24551	Agri-Mek 1.9% Ec Insecticide/Miticide	Abamectin	Crops	Toxic to bees, aquatic organisms, and wildlife
24648	Pyrifos 15g Insecticide	Chlorpyrifos	Crops	Toxic to bees, beneficial organisms, birds, mammals, fish and aquatic organisms

24711	K-G Insecticide Ii	Pyrethrins and N-Octyl Bicycloheptene Dicarboximide And Piperonyl Butoxide	Food handling Establishments, Meat Packing, Food Processing Plants, Food Storage Areas, Food Transportation Vehicles, Commercial or Institutional Kitchens, Dining Areas and Pantries of Restaurants, Supermarkets, Bottling Plants, Hospitals, Hotels, Motels, Homes, Stores, Nursing Homes, Day-care Centers, Campgrounds, Buses, Trains and Ships	
24712	K-G Insecticide Vi Pressurized	Permethrin	Homes, Hotels, Motels, Non-food Areas of Hospitals, Transportation Equipment [Buses, Boats, Ships, Trains, Trucks (empty), Planes], Meat Packing and Food Processing Plants, Storage Areas, Restaurants, and other Food Handling Areas, Utilities, Warehouses, Animal Quarters, Milk Rooms & to Treat Livestock.	
24927	Evergreen Emulsifiable 60-6	Pyrethrins And Piperonyl Butoxide	ornamentals, stored products and livestock areas	toxic to fish

24978	Foray 48ba Biological Insecticide Aqueous Suspension	Bacillus Thuringiensis Subspecies Kurstaki (All Strains)	Crops, tree, and ornamentals	
24984	Matador 120ec Insecticide	Lambda-Cyhalothrin	Crops	Toxic to bees and aquatic organisms
25134	Sanmite Miticide/Insecticide	Pyridaben	greenhouse crops and outdoor ornamentals (insecticide/miticide)	Toxic to aquatic ecosystems
25135	Nexter Wp Miticide/Insecticide	Pyridaben	Crops	Toxic to bees
25229	Dyno-Mite Miticide/Insecticide Wettable Powder Formulation	Pyridaben	greenhouse crops	Toxic to aquatic ecosystems and terrestrial plants
25556	Gaucho 75 St	Imidacloprid	systemic insecticide seed treatment for early season protection of canola, mustard (condiment-type only) and rapeseed seed and seedlings from flea beetles	toxic to birds and aquatic invertebrates
25573	Decis Flowable Insecticide (Eastern Canada And B.C.)	Imidacloprid	greenhouse ornamentals and vegetables	Toxic to aquatic invertebrates
25636	Merit 60 Wp Greenhouse and Nursery Insecticide	Imidacloprid	greenhouse ornimentals and vegtables	Toxic to aquatic invertibrates
25638	Malathion 95 Ulv Insecticide	Malathion	Crops	Toxic to bees
25650	Cygon 480-Orn Systemic Insecticide	Dimethoate	Crops and animal quarters	Toxic to birds, bees, fish and other wildlife

25651	Cygon 480-Ag Systemic Insecticide	Dimethoate	Crops and animal quarters	Toxic to birds, bees, fish and other wildlife
25831	Nufos 4e Insecticide	Chlorpyrifos	Crops	Toxic to birds, bees, fish and other wildlife and other beneficial insects
25932	Merit Solupack Insecticide	Imidacloprid	Turf grass	Toxic to bees and aquatic invertebrates
25933	Merit Granular	Imidacloprid	Turf grass	Toxic to aquatic organisms
26124	Gaucho 480 Fl Insecticide	Imidacloprid	Crops	Toxic to birds, aquatic organisms and bees
26508	Dipel 2x Df Biological Insecticide Dry Flowable	Bacillus Thuringiensis Subspecies Kurstaki (All Strains)	Crops	
26514	Disvap Fog	Piperonyl Butoxide and D-Trans-Allethrin		
26533	Virosoft Cp4	Cydia Pomonella Granulosis Virus (Strain Cmgv4)	Crops (Apple trees)	toxic to aquatic systems
26637	Helix Liquid Seed Treatment	Difenoconazole And Fludioxonil And Thiamethoxam And Metalaxyl-M And S-Isomer	Crops	Toxic to bees, aquatic organisms, wild mammals and birds
26834	Conserve 480sc Naturalyte Insect Control Product	Spinosad	Ornamentals and turf grass	Toxic to bees and aquatic organisms
26835	Success Insecticide	Spinosad	Crops	Toxic to bees and aquatic organisms

26837	Warrior Insecticide	Lambda-Cyhalothrin	Crops	Toxic to bees and aquatic organisms
26854	Bioprotec Caf	Bacillus Thuringiensis Subspecies Kurstaki (All Strains)	Trees	
26862	Aquabac (200g) Biological Larvicide Granules (5/8) (10/14)	Bacillus Thuringiensis, Serotype H-14	Mosquito control	
26873	Chipco Sevin T&O Carbaryl Insecticide	Carbaryl	Lawns and ornamentals	Toxic to bees
26911	Doktor Doom Residual Surface Insecticide Spray	Permethrin	Homes, Hotels, Motels, Non-food Areas of Hospitals, Transportation Equipment (Buses, Boats, Ships, Trains, Trucks (empty), Planes) Meat Packing and Food Processing Plants, Storage Areas, Restaurants, and other Food Handling Areas, Utilities, Warehouses, Animal Quarters, Milk Rooms and to Treat Livestock	
26981	Isomate-M100 Oriental Fruit Moth Pheromone	E-8-Dodecen-1-Yl Acetate And Z-8-Dodecen-1-Ol and Z-8-Dodecen-1-Yl Acetate	Crops (Fruit trees)	
27045	Cruiser 5fs Seed Treatment	Thiamethoxam	Crop	toxic to bees, aquatic organisms, birds, mammals

27127	Tristar 70 Wsp Insecticide	Acetamiprid	Ornamentals	Toxic to bees and aquatic organisms
27128	Assail 70 Wp Insecticide	Acetamiprid	Crops	Toxic to bees and aquatic organisms
27141	Isomate-P Pheromone	E,Z)-3,13-Octadecadien-1-Yl Acetate And (Z,Z)-3,13-Octadecadien-1-Yl Acetate	Crops (Fruit trees)	
27147	Check Mite + Bee Hive Pest Control Strip	Coumaphos	Control of mites and beetles in honeybee hives	toxic to birds and aquatic organisms
27170	Gaucho 600 Fl Insecticide	Imidacloprid	Crops	toxic to wildlife, bees, and aquatic systems
27174	Gaucho Cs Fl (Insecticide/Fungicide Seed Treatment)	Thiram And Carbathiin And Imidacloprid	Crops	toxic to birds and aquatic organisms
27220	Prescription Treatment Brand P.I. Contact Insecticide	Pyrethrins And Piperonyl Butoxide	Apartments, Campgrounds, Food Storage Areas, Homes, Hospitals, Hotels, Motels, Nursing Homes, Resorts, Restaurants and other Food Handling Establishments, Schools, Supermarkets, Transportation Equipment (Buses, Boats, Ships, Trains, Trucks), Utilities, Warehouses, and other Commercial and Industrial Buildings.	

27265	De-Cide(Tm)	Silicon Dioxide (Present As 100% Diatomaceous Earth) - Fresh Water Fossils	Livestock, commercial buildings, gardens, food processing plant	Don’t use in aquatic habitats
27273	Endeavor 50wg Insecticide	Pymetrozine	Ornamentals (outdoor and greenhouse), and greenhouse vegetables	toxic to aquatic organisms
27274	Fulfill 50wg Insecticide	Pymetrozine	Crops (leafy vegetables)	toxic to aquatic organisms
27278	Conserve 120 Sc Naturalyte Insect Control Product	Spinosad	Ornamentals and turf	toxic to bees and other beneficial insects and aquatic invertebrates
27339	Isomate-M Rosso Oriental Fruit Moth Pheromone	E-8-Dodecen-1-Yl Acetate And Z-8-Dodecen-1-Yl Acetate And Z-8-Dodecen-1-Ol	Crops (fruit trees)	
27349	Genesis 240 Flowable Systemic Insecticide	Imidacloprid	Crops (potatoes)	toxic to wildlife
27357	Intercept 60 Wp Greenhouse Insecticide	Imidacloprid	Greenhouse crops and ornamentals	toxic to bees, aquatic invertebrates and other beneficial insects
27376	Aquabac Ii Xt Biological Larvicide	Bacillus Thuringiensis, Serotype H-14	Mosquito control	
27449	Titan Insecticide	Chlorpyrifos	Crop (potatoes)	toxic to aquatic organisms, birds, small mammals, and bees
27453	Poncho 600 Fs Seed Treatment Insecticide	Chlorpyrifos	Crops	toxic to aquatic organisms, birds, small mammals, and bees

27479	Citadel 480ec Insecticide	Chlorpyrifos	Crops	toxic to birds, wildlife, aquatic organisms, bees, beneficial insects
27525	Isomate-Gbm Plus Grape Berry Moth Pheromone	(Z)-9-Dodecenyl Acetate	Crop (grapes)	
27538	Diazinon 50 Ec Insecticide	Diazinon	Crops (fruits and vegetables)	toxic to aquatic organisms, birds, small mammals, and beneficial insects, and bees
27564	Prosper Fl Flowable Insecticide And Fungicide Seed Treatment	Metalaxyl And Clothianidin And Thiram And Carbathiin	Crops	toxic to aquatic organisms, birds, small mammals, bees
27666	Purespray Green Spray Oil 13e	Mineral Oil	Crops	toxic to aquatic organisms
27682	Konk 404 Residual Crack, Crevice and Surface Insecticide with Permethr	Permethrin	Homes, Hotels, Motels, Non-food Areas of Hospitals, Meat Packing and Food Processing Plants, Storage Areas, Restaurants, and other Food Handling Areas, Utilities, Warehouses , Transportation Equipment (Buses, Boats, Ships, Trains, Trucks (empty), Planes), Animal Quarters, Milk Rooms & to Treat Livestock and Horses	
27702	Admire 240 Spt Flowable Systemic Insecticide	Imidacloprid	Crops (potatoes)	toxic to wildlife and aquatic environments
27750	Bioprotec 3p Dry Flowable Biological Insecticide	Bacillus Thuringiensis Subspecies	Crops	

		Kurstaki (All Strains)		
27786	Intrepid Insecticide	Methoxyfenozide	Crops	toxic to aquatic organisms
27825	Entrust 80 Insecticide	Spinosad	Crops	toxic to bees and aquatic organisms
27863	Prescription Treatment Brand Avert Plus Canadian Carpenter Ant Bait	Abamectin	Commercial and Other Structures, Food Storage Areas, Homes, Hotels, Inedible Product Areas of Meat Packing Plants, Non-Food / Feed Areas of Commercial Buildings, Motels, Residential Areas, Schools, Supermarkets, Non-Occupied Patient Areas of Hospitals and Nursing Homes, and Warehouses	toxic to birds, mammals and aquatic organisms
27864	Prescription Treatment Brand Avert Granular Carpenter Ant Bait	Abamectin	Commercial and Other Structures, Food Storage Areas, Homes, Hotels, Inedible Product Areas of Meat Packing Plants, Non-Food / Feed Areas of Commercial Buildings, Motels, Residential Areas, Schools, Supermarkets, Non-Occupied Patient Areas of Hospitals and Nursing Homes, and Warehouses	toxic to birds, mammals and aquatic organisms

27876	Sevin Xlr Carbaryl Insecticide Liquid Suspension	Carbaryl	Crops	toxic to bees, aquatic organisms, birds, mammals
27886	Neudosan Commercial	Potassium Salts of Fatty Acids	For use indoors, outdoors and in greenhouses; fruit trees, vegetables, houseplants, ornamental and bedding plants, ornamental and shade trees.	toxic to aquatic organisms
27932	Bugcon Schmack Formula 1409 Metered Spray for Food Establishments	Pyrethrins And Piperonyl Butoxide and N-Octyl Bicycloheptene Dicarboximide	Mosquito control	
27954	Saber Er Premise Insecticide	Thiamethoxam	Crops	toxic to bees and aquatic organisms
27986	Cruiser 350fs Seed Treatment Insecticide	Thiamethoxam	Crops	toxic to bees, aquatic organisms, birds, mammals
28119	Vault 50 Fs Insecticide Seed Treatment	Acetamiprid	Crops	toxic to aquatic organisms bees and birds
28124	Landscape Oil Spray Emulsifiable Insecticide	Mineral Oil	greenhouse ornamental, nursery and landscape pests	toxic to aquatic organisms
28146	Opal Insecticidal Soap	Potassium Salts of Fatty Acids	outdoors and in greenhouses; fruit trees, vegetables, houseplants, ornamental and bedding plants, ornamental and shade trees	toxic to aquatic organisms
28219	Ipco Pivot 418 Ec	Propiconazole	Crops	toxic to aquatic organisms and non target terrestrial plants and beneficial organisms

28336	Gf-120 Fruit Fly Bait	Spinosad	Crops (Fruit trees)	toxic to bees, and aquatic organisms, and beneficial insects
28351	Syllit 400 Fl	Dodine	Crops (Fruit trees)	toxic to aquatic organisms and non target terrestrial plants
28373	Knock Down X-Max Flying Insect Killer (1.8 Pyrethrin From Chrysanthemu	Pyrethrins And Piperonyl Butoxide	Mosquito control	
28402	Doktor Doom 6% Pyrethrin Knockdown Insect Killer	Pyrethrins And Piperonyl Butoxide	ornamentals, stored products and livestock areas	toxic to fish
28407	Actara 240sc Insecticide	Thiamethoxam	crops	toxic to aquatic organisms bees and beneficial insects
28408	Actara 25wg Insecticide	Thiamethoxam	crops	toxic to aquatic organisms bees and beneficial insects
28414	Distance	Pyriproxyfen	greenhouse ornamentals and greenhouse vegetables	toxic to aquatic organisms and beneficial insects
28429	Calypso 480 Sc Insecticide	Thiacloprid	Crops (Fruit trees)	toxic to aquatic organisms and beneficial insects
28475	Alias 240 Sc Systemic Insecticide	Imidacloprid	crops	toxic to bees and aquatic systems
28515	Rimon 10 Ec	Novaluron	Crops	toxic to aquatic organisms, non target terrestrial plants, bees and beneficial insects
28584	Knock Down Max Flying Insect Killer (1.0 Pyrethrin From Chrysanthemum	Pyrethrins And Piperonyl Butoxide and N-Octyl Bicycloheptene Dicarboximide	Mosquito control	
28590	Forbid 240 Sc Insecticide/Miticide	Spiromesifen	Greenhouse ornamentals and vegetables	toxic to aquatic organisms, non target terrestrial plants, bees and beneficial insects

28691	Flying Insect Killer Metered I (1.8% Pyr-Commercial)	Pyrethrins And Piperonyl Butoxide	Mosquito control	toxic to fish and birds
28726	Grapple Insecticide	Imidacloprid	Crops	toxic to aquatic ecosystems, bees
28777	Radiant Sc Insecticide	Spinetoram	Crops	toxic to bees, wild mammals, beneficial insects, non target terrestrial plants
28778	Delegate Insecticide	Spinetoram	Crops	toxic to bees, wild mammals, beneficial insects, non target terrestrial plants
28791	Deltagard Sc Insecticide	Deltamethrin	Crops	toxic to aquatic organisms, bees, beneficial insects
28792	K-G Flying Insect Killer Metered Ii (0.975% Pyr-Commercial)	Piperonyl Butoxide And Pyrethrins N-Octyl Bicycloheptene Dicarboximide	Mosquito control	toxic to fish and birds
28795	Up-Cyde 2.5 Ec	Cypermethrin	Crops	toxic to aquatic organisms bees
28814	Isomate-Cm/Lr Tt	(Z)-11-Tetradecen-1-Ol and (Z)-11-Tetradecenal and 1-Tetradecanol and (Z)-9-Tetradecen-1-Yl Acetate And (Z)-11-Tetradecenyl Acetate And 1-Dodecanol And Codlelure	Crop (fruit and nut trees)	

28821	Cruiser Maxx Beans Seed Treatment	Metalaxyl-M And S-Isomer and Fludioxonil Casn = 131341-86-1 (Guar = 1.12 % Nominal)	Crops	toxic to aquatic organisms, and bees, birds and wild mammals
28877	Perm-Up Emulsifiable Concentrate Insecticide	Permethrin	Crops	toxic to aquatic organisms and bees
28881	Rimon 10 Ec Insecticide	Novaluron	Crops	toxic to aquatic organisms, bees, beneficial insects
28905	Oberon Flowable Insecticide-Miticide	Spiromesifen	Crops	toxic to aquatic organisms, non target terrestrial plants, beneficial insects and bees
28946	Lambda-Cyhalothrin Cs Insecticide	Lambda-Cyhalothrin	turf, ornamentals, animal housing, and buildings	toxic to aquatic organisms and bees
28953	Movento 240 Sc Insecticide	Spirotetramat	crops	toxic to bees, aquatic organisms, beneficial insects, and non target plants
28954	Movento 150 Od Insecticide	Chlorpyrifos	Crops	toxic to bees and aquatic organisms
28975	Nipsit Inside 600 Insecticide	Chlorpyrifos	Crops	toxic to bees and aquatic organisms
28980	Acelepryn (Tm) Insecticide	Chlorantraniliprole	turf, outdoor and greenhouse ornamentals	toxic to aquatic organisms, beneficial insects
28981	Altacor Insecticide	Chlorantraniliprole	Crops (fruit and berries)	toxic to aquatic organisms, beneficial insects
28982	Coragen Insecticide	Chlorantraniliprole	Crops	toxic to aquatic organisms, beneficial insects
29048	Grapple-2 Insecticide	Imidacloprid	Crops	toxic to aquatic organisms, birds, bees, and beneficial insects

29052	Silencer 120 Ec Emulsifiable Concentrate Insecticide	Lambda-Cyhalothrin	Crops	toxic to aquatic organisms, bees
29064	Imidan Wp Insecticide	Phosmet	Crops	Toxic to birds, mammals, aquatic organisms, bees
29127	Cruiser Maxx Cereals Commercial Seed Treatment	Thiamethoxam And Difenoconazole And Metalaxyl-M And S-Isomer	Crops	toxic to aquatic organisms, birds, mammals, and bees
29130	Quali-Pro Imidacloprid 75 Wsp Insecticide	Imidacloprid	turf	Toxic to bees and aquatic invertebrates
29158	Prosper T 200 Flowable Insecticide and Fungicide Seed Treatment	Metalaxyl And Carbathiin And Clothianidin And Trifloxystrobin	Crops (seed treatment)	toxic to aquatic organisms, small mammals, bees
29159	Prosper Fx Flowable Insecticide and Fungicide Seed Treatment	Carbathiin And Metalaxyl And Clothianidin And Trifloxystrobin	Crops (seed treatment)	toxic to aquatic organisms, small mammals, bees
29185	Quali-Pro Imidacloprid 0.5 Granular Insecticide	Imidacloprid	turf	Toxic to aquatic invertebrates
29192	Cruiser Maxx Cereals Seed Treatment	Thiamethoxam And Difenoconazole And Metalaxyl-M And S-Isomer	Crops	toxic to aquatic organisms bees birds and small mammals
29197	Nolo Bait Biological Insecticide	Nosema Locustae Canning, (Spore Of)	Rangeland and crops control of grasshoppers	not for use in aquatic systems

29320	Botanigard Es	Beauveria Bassiana Strain Gha	Greenhouse crops and ornamentals	
29321	Botanigard 22 Wp	Beauveria Bassiana Strain Gha	Greenhouse crops and ornamentals	Toxic to bees and beneficial insects
29352	Isomate-Cm/Ofm Tt	1-Tetradecanol and Z-8-Dodecen-1-Yl Acetate And E-8-Dodecen-1-Yl Acetate And Z-8-Dodecen-1-Ol And Codlelure and 1-Dodecanol	Crop (fruit trees)	
29382	Clutch 50 Wdg Insecticide	Chlorpyrifos	Crops	Toxic to aquatic organisms, birds, small wild mammals and non-target terrestrial plants, bees and beneficial insects
29383	Arena 50 Wdg Insecticide	Chlorpyrifos	turf	Toxic to aquatic organisms, birds, small wild mammals and non-target terrestrial plants, bees and beneficial insects
29384	Clothianidin Insecticide	Chlorpyrifos	turf and crops	Toxic to aquatic organisms, birds, small wild mammals and non-target terrestrial plants, bees and beneficial insects
29499	Orthene 97% Pellet	Acephate	crop	toxic to bees birds mammals and aquatic organisms
29567	Kontos Insecticide	Spirotetramat	greenhouse and outdoor crops	toxic to aquatic organisms, bees, beneficial insects
29609	Stress Shield for Cereals	Imidacloprid	Crops	toxic to birds and aquatic invertebrates
29610	Stress Shield for Cereals and Soybeans	Imidacloprid	Crops	toxic to birds aquatic organisms and bees

29611	Concept Liquid Insecticide	Deltamethrin And Imidacloprid	Crops	toxic to aquatic organisms and bees
29650	Lorsban Nt Insecticide	Chlorpyrifos	Crops	toxic to birds, wildlife, aquatic organisms, bees, beneficial insects
29661	Enstar® Ew	S-Kinoprene	Greenhouse ornamentals	
29703	Confidor 200 Sl	Imidacloprid	trees	Toxic to aquatic invertebrates and bees
29796	Beleaf 50sg Insecticide	Flonicamid	Crops	toxic to beneficial insects non target terrestrial plants,
29824	Weedaway Pivot 418 Ec	Propiconazole	Crops	toxic to aquatic organisms, non target terrestrial plants
29849	Chlorpyrifos 480 Ec Insecticide	Chlorpyrifos		toxic to birds, wildlife, aquatic organisms, mammals, bees, beneficial insects
29886	Tengard Emulsifiable Concentrate Insecticide	Permethrin	Crops (Fruit trees ornamental trees and farm buildings)	toxic to bees predatory mites fish birds and other wildlife
29984	Warhawk 480 Ec Insecticide	Chlorpyrifos	Crops	toxic to fish, birds, mammals, bees, beneficial insects
30026	Foray® Wg Biological Insecticide Water Dispersible Granules	Bacillus Thuringiensis Subspecies Kurstaki (All Strains)	forestry woodland and residential use	
30042	Isomate-Ptb Dual	(E,Z)-3,13-Octadecadien-1-Yl Acetate And (Z,Z)-3,13-Octadecadien-1-Yl Acetate	Crop (stone fruits and almonds)	
30120	Cyd-X	Cydia Pomonella Granulovirus (Strain M)	Crop (apples)	

30164	Pyganic Crop Protection Ec 1.4 Ii	Pyrethrins	Crops	toxic to bees, aquatic organisms,
30184	Bas 516 F St	Pyraclostrobin And Boscalid	Crop	toxic to birds and aquatic organisms
30312	Hercon Disrupt Bio-Flake Sbw	(E,Z)-11-Tetradecenal	Forestry and woodlands	
30316	Mako Insecticide	Cypermethrin	Crop	toxic to aquatic organisms
30325	Voliam Xpress Insecticide	Lambda-Cyhalothrin And Chlorantraniliprole	Crops	toxic to aquatic organisms beneficial insects bees
30362	Emesto Quantum	Penflufen And Clothianidin	Crops (potatoes) insecticide and fungicide	toxic to birds, mammals, bees, aquatic organisms
30363	Prosper Evergol	Clothianidin And Trifloxystrobin And Metalaxyl And Penflufen	Crops fungicide/insecticide	toxic to birds, mammals, bees, aquatic organisms
30375	Green Way Ant & Roach Bait Gel	Disodium Octaborate Tetrahydrate		
30382	Entrust Insecticide	Spinosad	Crops	toxic to bees and beneficial arthropods aquatic organisms
30388	A18046a Seed Treatment	Azoxystrobin And Metalaxyl-M And S-Isomer and Thiamethoxam And Fludioxonil	Crops (soy)	toxic to aquatic organisms, bees, birds, and mammals
30404	Endigo Insecticide	Thiamethoxam And Lambda-Cyhalothrin	Crops	toxic to non target plants, aquatic organisms, bees, beneficial insects
30436	Cruiser Maxx Vibrance Cereals Seed Treatment	Metalaxyl-M And S-Isomer and Difenoconazole	Crops	toxic to aquatic organisms, birds, mammals, and bees

		And Sedaxane And Thiamethoxam		
30437	Vibrance Xl Seed Treatment	Metalaxyl-M And S-Isomer and Sedaxane And Difenoconazole	Crops	toxic to aquatic organisms, birds, and mammals
30505	Sombrero 600 Fs	Imidacloprid	Crops	toxic to wildlife, aquatic organisms, birds, bees
30546	Balence Biological Beetle Bait	Beauveria Bassiana Strain Hf23		toxic to bees
30559	Treeazin Systemic Insecticide	Azadirachtin	trees	toxic to aquatic organisms and bees
30589	Isomate Dwb		Crops (fruit trees, blueberries, ornamentals)	
30605	Zone Guard, Pro Flying Insect Killer 1.80%	Piperonyl Butoxide And Pyrethrins	Mosquito and fly control	toxic to fish and birds
30607	Zone Guard, Flying Insect Killer 1	Piperonyl Butoxide And Pyrethrins	Mosquito and fly control	toxic to fish and birds
30615	Purge I Insecticide	Piperonyl Butoxide And Pyrethrins	Mosquito and fly control	toxic to fish and birds
30616	Purge Iii Insecticide	Piperonyl Butoxide And Pyrethrins N-Octyl Bicycloheptene Dicarboximide	Mosquito and fly control	toxic to fish and birds
30666	Pylon Miticide Insecticide	Chlorfenapyr	Greenhouse crops and ornamentals (insecticide/ miteacide)	toxic to bees and beneficial insects
30668	Stress Shield 600	Imidacloprid	Crops	toxic to birds aquatic organisms bees

30723	Flagship Insecticide	Thiamethoxam	outdoor ornamentals, greenhouse peppers and Viburnum in outdoor nurseries and landscapes	toxic to aquatic organisms, bees, beneficial insects
30825	Transform Wg Insecticide	Sulfoxaflor	Crops	toxic to beneficial insects and bees
30826	Closer Insecticide	Sulfoxaflor	Crops	toxic to beneficial insects and bees
30892	Verimark Insecticide	Cyantraniliprole	Crops	toxic to aquatic organisms, bees
30893	Benevia Insecticide	Cyantraniliprole	Crops	toxic to non target plants, aquatic organisms, bees
30894	Dupont Lumiderm Insecticide Seed Treatment	Cyantraniliprole	Crops	toxic to aquatic organisms and bees
30895	Exirel Insecticide	Cyantraniliprole	Crops	toxic to non target plants, aquatic organisms, beneficial insects, bees
30898	Fortenza Red	Cyantraniliprole	Crops	toxic to aquatic organisms, bees
30899	Fortenza	Cyantraniliprole	Crops	toxic to aquatic organisms and bees
30900	Minecto Duo 40wg	Thiamethoxam And Cyantraniliprole	Crops	toxic to bees and aquatic organisms
30901	Mainspring X Insecticide	Thiamethoxam And Cyantraniliprole	greenhouse and outdoor ornamentals	toxic to non target plants, bees, beneficial insects, aquatic organisms
30972	Sepresto 75 Ws	Imidacloprid And Clothianidin	Crops	toxic to bees, birds, mammals, aquatic invertebrates
30985	Mpower Krypton	Chlorpyrifos	Crops	toxic to aquatic organisms, birds, mammals, bees, beneficial insects

31024	Cruiser Maxx Potato Extreme	Thiamethoxam And Fludioxonil And Difenoconazole	Crop (potatoes)	toxic to bees, birds, beneficial insects, aquatic organisms, small animals
31068	Acceleron® Ix-409 Insecticide Seed Treatment	Imidacloprid	Crops (beans)	Toxic to birds, aquatic organisms and bees
31300	Masterline Lambdacy Insecticide	Lambda-Cyhalothrin		Commercial and industrial buildings and livestock buildings
31355	Nipsit Suite Canola Seed Protectant	Metalaxyl And Metconazole and Clothianidin	Crop (insecticide/ fungicide)	toxic to aquatic organisms, birds, small wild animals, bees
31357	Nipsit Suite Cereals of Seed Protectant	Metalaxyl And Clothianidin And Metconazole	Crops (insecticides/fungicide)	toxic to aquatic organisms, birds, small wild animals, bees
31375	Ima-Jet	Imidacloprid	trees	Toxic to aquatic invertebrates and bees
31396	Capture 240 Ec	Bifenthrin	Crop (potatoes and blueberries)	Toxic to aquatic organisms and bees and beneficial insects
31408	Vibrance Quattro	Difenoconazole And Fludioxonil And Metalaxyl-M And S-Isomer and Sedaxane	Crops	Toxic to birds, mammals, and aquatic organisms
31419	Isomate Ofm Tt	E-8-Dodecen-1-Yl Acetate And Z-8-Dodecen-1-Yl Acetate And Z-8-Dodecen-1-Ol	Crops (fruit trees)	
31433	Kopa Insecticidal Soap	Potassium Salts of Fatty Acids	Crops and ornamental trees	Toxic to aquatic organisms
31442	Twinguard Insecticide	Spinetoram And Sulfoxaflor	Crops (fruit and potatoes)	toxic to beneficial insects, bees, wild mammals, non target plants

31451	Byi 02960 480 Fs	Flupyradifurone	Crops (soy)	toxic to aquatic organisms, birds, mammals
31452	Sivanto Prime Insecticide	Flupyradifurone	Crops	toxic to aquatic organisms, (maybe toxic to bees?), beneficial insects
31453	Cruiser Vibrance Quattro	Thiamethoxam And Metalaxyl-M And S-Isomer and Difenoconazole And Fludioxonil And Sedaxane	Crops	toxic to aquatic organisms, birds, bees
31454	Helix Vibrance	Metalaxyl-M And S-Isomer and Fludioxonil And Sedaxane And Thiamethoxam And Difenoconazole	Crops	toxic to aquatic organisms and bees birds and wild mammals
31479	Ima-Jet 10	Imidacloprid	trees	toxic to aquatic organisms, and bees
31557	Xentari Wg Biological Insecticide	Bacillus Thuringiensis Ssp. Aizawai	Crops	toxic to bees, and beneficial insects
31589	Isomate Cm Flex	1-Tetradecanol	Crops	
31607	Agri-Mek Sc	Abamectin	Crops (insecticide/miticide)	toxic to aquatic organisms, wildlife, bees
31773	Terminator Wasp & Hornet Killer	N-Octyl Bicycloheptene Dicarboximide And Pyrethrins And Piperonyl Butoxide And Propoxur	Outdoor crack and crevice wasp and hornet killer	toxic to bees
31808	Bugwacker 240	Permethrin	toxic to aquatic organisms	toxic to aquatic organisms

31943	Prozap Annihilator-Xp	Pyrethrins And Piperonyl Butoxide	ornamentals, stored products and livestock	toxic to fish
32047	Semios Cm Plus	Codlelure	Crops (fruit trees)	
32082	Force 10cs Insecticide	Tefluthrin	Crop (corn)	toxic to aquatic organisms, birds, and mammals
32083	Force 15cs Insecticide	Tefluthrin	Crop (corn)	toxic to aquatic organisms, birds, and mammals
32084	Force 25cs Insecticide	Tefluthrin	Crop (corn)	toxic to aquatic organisms, birds, and mammals
32152	Aquasurf Non-Ionic Spray Adjuvant	Chlorantraniliprole	Crops (corn)	toxic to aquatic organisms and birds
32154	Dupont Lumivia Seed Treatment	Chlorantraniliprole	Crops (corn)	toxic to aquatic organisms and birds
32245	Fenpyroximate 5sc Miticide/Insecticide	Fenpyroximate	Greenhouse crops and ornamentals (insecticide/ miteacide)	toxic to aquatic organisms, and beneficial insects
32250	Rascendo	Sulfoxaflor	Crops	toxic to birds, bees, mammals
32270	Hgw86 200 Sc Potato Seed Treatment Insecticide	Cyantraniliprole	Crops (potatoes)	toxic to aquatic organisms, bees
32302	Fujimite Miticide/Insecticide	Fenpyroximate	indoor and greenhouse and ornamentals and greenhouse vegetables	toxic to aquatic organisms and beneficial insects
32341	Applaud Insect Growth Regulator	Buprofezin	Crops	
32368	Okina Insect Control	Cyantraniliprole	greenhouse crops	toxic to bees, beneficial insects, aquatic organisms
32383	Talus Insect Growth Regulator	Buprofezin		
32408	Vegol Crop Oil	Canola Oil	Crops (insecticide/miticide/fungicide)	toxic to aquatic organisms, beneficial insects

32425	Bioprotec Plus	Bacillus Thuringiensis Subspecies	Crops	
32427	Silencer 120 Ec Low Voc	Lambda-Cyhalothrin	Crops	toxic to aquatic organisms and bees
32446	Poleci 2.5 Ec Eastern Insecticide	Deltamethrin	Crops	toxic to aquatic organisms, bees, beneficial insects
32447	Poleci 2.5 Ec Western Insecticide	Deltamethrin	Crops	toxic to aquatic organisms, bees, beneficial insects
32563	Ship 250 Ec Insecticide	Cypermethrin	Crops	toxic to fish, beneficial arthropods, bees
32656	Deltagard 20ew	Deltamethrin	Outdoor mosquito control	toxic to bees, beneficial insects, aquatic organisms
32730	Confound Sbw	(E,Z)-11-Tetradecenal	forestry	
32768	Sharphos Insecticide	Chlorpyrifos	Crops	toxic to fish and aquatic organisms, toxic to birds and wild mammals, bees, beneficial insects
32819	Doktor Doom Formula 420 Professional Use 3-In-1 Crop & Plant Rescue Co	Canola Oil	Crops (Insecticide/Miticide/Fungicide)	toxic to aquatic organisms, beneficial insects
32837	Force Evo Insecticide	Tefluthrin	Crops	toxic to aquatic organisms, birds, mammals
32862	Cyclaniliprole 50sl Insecticide	Cyclaniliprole And Carboxanilide	Crops	toxic to aquatic organisms, beneficial insects, and bees
32889	Harvanta 50sl Insecticide	Cyclaniliprole	Crops	toxic to aquatic organisms, beneficial insects, bees
32993	Velifer	Beauveria Bassiana Strain Ppri 5339	Greenhouse	toxic to beneficial organisms and bees
33023	Minecto Pro	Cyantraniliprole And Abamectin	Crops(insecticide/miticide)	toxic to bees and aquatic organisms

33099	Suffoil-X	Mineral Oil	Crops(insecticide/miticide/ fungicide)	toxic to aquatic organisms
33113	Pyrinex 450 Lv Ec	Chlorpyrifos	Crops	toxic to aquatic organisms, birds, mammals, bees, beneficial insects
33191	Revokbtk	Bacillus Thuringiensis Subspecies	Crops	

Appendix 2 table A2.1 2018 pilot study data. Summary including site, sampling date, avg wind speed, number of bees collected, number of floral units, crop type, adjacent crop type, avg soil hardness measurement in the crop and hedgerow.

Site ID	Date	Temp ˚ C	Wind speed avg Km/hr	Number of bees collected	Floral units	Crop	Adjacent crop	crop soil hardness	hedgerow soil hardness
2	August 28 2018	29.4	1.6	15	3735.2	Soy	Soy	2.56	2.5
2	July 30 2018	24.9	1.6	14	114401.2	Soy	Soy		
3	August 24 2018	26.6	4.4	5	7178.4	Corn	N/A	1.22875	1.1
3	July 26 2018	28.2	2.5	6	229.6	Corn	N/A		
4	August 27 2018	26.2	1.6	4	1795.8	Corn	Corn	1.2225	1.1
4	July 20 2018	33.3	0	3	167232	Corn	Corn		
6	August 20 2018	33.1	0	1	697	Corn	Corn	2.40625	1.4
6	July 27 2018	29.3	0	3	86578	Corn	Corn		
8	August 22 2018	22.3	4.4	5	32777	Corn	Soy	0.06625	0.1
8	July 19 2018	32	1	4	2604073	Corn	Soy		
12	August 29 2018	28.7	3.8	2	4005.6	Corn	Hay	2.25125	1.4
12	July 17 2018	28.9	2	2	112212.8	Corn	Hay		
17	August 22 2018	20	6.5	1	683.2	Soy	Corn	0.7375	0.3
17	July 19 2018	27.3	2.7	6	409	Soy	Corn		
18	Aug 27 2018	26.8	1.2	5	1841.8	Soy	Soy	1.0925	0.3
18	July 17 2018	30.1	4.6	4	56.4	Soy	Soy		
19	August 22 2018	21.8	2.5	9	17667.6	Soy	Soy	0.94375	0.9
19	July 23 2018	28.6	9	6	10243.4	Soy	Soy		
20	August 20 2018	31.7	0.5	2	0	Corn	Alfalfa	0.30625	2.1
20	July 27 2018	28.8	4.2	2	3120	Corn	Alfalfa		
21	August 16 2018	28.4	1.1	3	3235.6	Soy	Corn	1.71875	2.1
21	July 23 2018	30.2	6.5	2	483.4	Soy	Corn		
22	August 28 2018	30	7.5	9	3376	Corn	Hay	1.78375	2.0
22	July 30 2018	27.1	5.8	1	1503.4	Corn	Hay		
23	Aug 24 2018	25.4	0	13	63293	Soy	Hay	0.6175	4.0

23	July 26 2018	28.5	6.2	0	243	Soy	Hay		
24	August 27 2018	26.1	2	4	3048.2	Soy	Hay	0.415	1.9
24	July 20 2018	33.7	1.8	7	92060.4	Soy	Hay		
25	August 16 2018	28.4	0.7	2	127.2	Corn	Hay	1.64375	1.0
25	July 10 2018	32	2.6	2	26870.8	Corn	Hay		
26	August 16 2018	24.5	0	12	33375.2	Corn	Corn	0.63125	0.
26	July 10 2018	31.3	7.05	2	16264.4	Corn	Corn		

Appendix 3 table A3.1 Bee genera summary separated by neonicotinoid category and site visit

Neonicotinoid category	Genera	Sampling period 1	Sampling period 2	Sampling period 3	Sampling period 4	Nesting habitat*	Nesting Classification
No detection	*Agapostemon*	0	1	2	0	Ground	Ground
	Andrena	13	8	0	0	Ground	Ground
	Anthidium	0	0	0	1	Cavity	Non-Ground
	Anthophora	0	1	1	0	Cavity/Ground	Non-Ground
	Apis	3	1	8	11	Cavity	Non-Ground
	Augochlorella	0	1	0	2	Ground	Ground
	Augochloropsis	0	1	0	1	Ground	Ground
	Bombus	2	4	2	4	Cavity	Non-Ground
	Ceratina	5	5	0	4	Pith	Non-Ground
	Colletes	0	0	0	1	Ground	Ground
	Halictus	6	4	4	2	Ground	Ground
	Hoplitis	0	2	0	0	Cavity/Pith	Non-Ground
	Hylaeus	0	2	1	7	Cavity	Non-Ground
	Lasioglossum	13	45	42	20	Mostly Ground	Ground
	Megachile	0	0	0	2	Cavity/Wood/Ground	Non-Ground
	Melissodes	0	0	8	20	Ground	Ground
	Nomada	1	0	0	0	Parasitic of Ground nesters	Non-Ground
	Osmia	0	1	0	0	Cavity	Non-Ground
Low concentration	*Agapostemon*	4	1	1	2	Ground	Ground
	Andrena	49	0	0	4	Ground	Ground
	Apis	0	2	1	13	Cavity	Non-Ground
	Augochlorella	0	0	2	0	Ground	Ground
	Bombus	18	1	9	27	Cavity	Non-Ground
	Ceratina	10	9	2	3	Pith	Non-Ground
	Chelostoma	0	0	1	0	Cavity	Non-Ground

	Colletes	1	0	0	0	Ground	Ground
	Halictus	10	2	6	2	Ground	Ground
	Heriades	0	0	1	1	Cavity	Ground
	Hoplitis	0	4	0	0	Cavity/Pith	Non-Ground
	Hylaeus	0	3	9	12	Cavity	Non-Ground
	Lasioglossum	54	27	60	4	Mostly Ground	Ground
	Megachile	0	1	3	5	Cavity/Wood/Ground	Non-Ground
	Melissodes	0	0	17	9	Ground	Ground
	Nomada	5	0	0	1	Parasitic of Ground nesters	Non-Ground
	Osmia	1	0	0	0	Cavity	Non-Ground
	Peponapis	0	0	0	2	Ground	Ground
	Perdita	0	0	0	1	Ground	Ground
	Sphecodes	0	0	0	4	Parasitic of Ground nesters	Non-Ground
	Stelis	0	0	1	0	Parasitic	Non-Ground
High concentration	*Agapostemon*	0	0	2	0	Ground	Ground
	Andrena	12	16	1	3	Ground	Ground
	Anthophora	0	0	1	0	Cavity/Ground	Non-Ground
	Apis	24	2	7	4	Cavity	Non-Ground
	Augochlora	0	2	1	0	Wood	Non-Ground
	Augochlorella	0	0	1	2	Ground	Ground
	Bombus	3	3	2	6	Cavity	Non-Ground
	Ceratina	4	8	0	2	Pith	Non-Ground
	Chelostoma	0	0	1	0	Cavity	Non-Ground
	Halictus	4	1	9	3	Ground	Ground
	Hoplitis	0	0	1	0	Cavity/Pith	Non-Ground
	Hylaeus	3	16	15	25	Cavity	Non-Ground
	Lasioglossum	32	34	49	20	Mostly Ground	Ground

Megachile	0	0	1	0	Cavity/Wood/Ground	Non-Ground
Melissodes	0	0	13	75	Ground	Ground
Nomada	2	1	0	1	Parasitic of Ground nesters	Non-Ground
Osmia	0	0	1	0	Cavity	Non-Ground
Pseudopanurgus	0	0	0	1	Ground	Ground

* Nesting habitats determined by using literature sources on the next page.

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Appendix 4 Table A4.1 Flower morphospecies summary separated by neonicotinoid category and sampling period.

			Total number of floral units counted			
Neonicotinoid category	Morphospecies	Floral unit	Sampling period 1	Sampling period 2	Sampling period 3	Sampling period 4
No detection	*Achillea millefolium*	Flower	0	0	0	94
No detection	*Anemone canadensis*	Flower	0	6	0	0
No detection	*Arctium minus*	Capitulum	0	0	0	133
No detection	*Asclepias syriaca*	Flower	0	0	137	0
No detection	*Brassica sp.*	Flower	0	83	95	29
No detection	*Capsella bursa-pastoris*	Flower	0	3	0	0
No detection	*Cirsium arvense*	Capitulum	0	0	12	0
No detection	*Cornus sericea*	Flower	0	481	0	0
No detection	*Daucus carota*	Flower	0	0	0	2520
No detection	*Echinocystis lobata*	Flower	0	0	0	1764
No detection	*Erigeron pulchellus*	Capitulum	0	5	0	0
No detection	*Erigeron sp.*	Capitulum	0	0	4	3
No detection	*Erigeron strigosus*	Capitulum	0	0	0	4
No detection	*Erysimum cheiranthoides*	Flower	0	0	90	0
No detection	*Fragaria ananassa*	Flower	45	0	0	0
No detection	*Fragaria sp.*	Flower	23	0	0	0
No detection	*Galium sp*	Flower	0	14	0	0
No detection	*Crataegus sp.*	Flower	818.5	0	0	0
No detection	*Hieracium sp.*	Capitulum	0	0	7	0
No detection	*Leucanthemum vulgare*	Capitulum	0	0	1	0
No detection	*Linaria vulgaris*	Flower	0	0	0	13
No detection	*Lithospermum officinale*	Flower	0	281	0	0

No detection	*Malus sp.*	Flower	2956	0	0	0
No detection	*Medicago lupulina*	Inflorescence	0	0	0	1
No detection	*Medicago sativa*	Flower	0	0	321	261.6
No detection	*Nepeta cataria*	Flower	0	0	59	8
No detection	*Oenothera perennis*	Flower	0	0	1	0
No detection	*Pastinaca sativa*	Flower	0	0	2120	660
No detection	*Prunus sp.*	Flower	6	0	0	0
No detection	*Rubus sp.*	Flower	0	34	2	0
No detection	*Solanum sp.*	Flower	0	0	0	3
No detection	*Solidago sp.*	Flower	0	0	0	16538
No detection	*Sonchus arvensis*	Capitulum	0	0	0	127
No detection	*Taraxacum agg*	Capitulum	72	0	0	0
No detection	*Taraxacum officinale*	Flower	0	1	0	0
No detection	*Viburnum nudum*	Flower	0	6750	0	0
No detection	*Viburnum sp.*	Flower	0	3180	0	0
No detection	*Vicia cracca*	Flower	0	46	516	252
Low concentration	*Achillea millefolium*	Flower	0	1410	1786	0
Low concentration	*Alliaria petiolata*	Flower	23	0	0	0
Low concentration	*Amelanchier bartramiana*	Flower	30	0	0	0
Low concentration	*Arctium minus*	Capitulum	0	0	10	24
Low concentration	*Brassica sp.*	Flower	34	0	0	0
Low concentration	*Cerastium sp.*	Flower	0	17	0	0
Low concentration	*Cirsium arvense*	Capitulum	0	0	19	1
Low concentration	*Cirsium vulgare*	Capitulum	0	0	0	10
Low concentration	*Clematis virginiana*	Flower	0	0	0	101
Low concentration	*Daucus carota*	Flower	0	0	10500	15120
Low concentration	*Erigeron canadensis*	Capitulum	0	0	0	378
Low concentration	*Erigeron pulchellus*	Capitulum	0	0	299	13

Low concentration	*Fragaria sp.*	Flower	0	2	0	0
Low concentration	*Galium sp*	Flower	0	127	250	0
Low concentration	*Hieracium sp.*	Capitulum	0	0	8	0
Low concentration	*Lactuca biennis*	Capitulum	0	0	0	8
Low concentration	*Lonicera morrowii*	Flower	661	0	0	0
Low concentration	*Lysimachia punctata*	Flower	0	0	0	1
Low concentration	*Medicago lupulina*	Inflorescence	0	0	6	8
Low concentration	*Oenothera biennis*	Flower	0	0	0	1
Low concentration	*Oenothera villosa*	Flower	0	0	3	2
Low concentration	*Pastinaca sativa*	Flower	0	0	1800	1589
Low concentration	*Potentilla argentea*	Flower	0	0	0	3
Low concentration	*Potentilla norvegica*	Flower	0	0	35	0
Low concentration	*Ranunculus acris*	Flower	0	0	1	0
Low concentration	*Ranunculus sp.*	Flower	0	0	1	0
Low concentration	*Ribes americanum*	Flower	376	0	0	0
Low concentration	*Rudbeckia hirta*	Capitulum	0	0	26	15
Low concentration	*Solidago spp.*	Flower	0	0	0	20667
Low concentration	*Sonchus arvensis*	Capitulum	0	0	0	29
Low concentration	*Spiraea alba*	Flower	0	0	470	0
Low concentration	*Stellaria graminea*	Flower	6	0	0	0
Low concentration	*Taraxacum agg*	Capitulum	19	0	0	0
Low concentration	*Trifolium hydbridum*	Inflorescence	0	0	0	2
Low concentration	*Trifolium pratense*	Inflorescence	0	0	14	6
Low concentration	*Trifolium repens*	Inflorescence	0	0	23	0
Low concentration	*Verbascum sp.*	Flower	0	0	0	27
Low concentration	*Verbascum thapsus*	Flower	0	0	26	0
Low concentration	*Verbena hastata*	Flower	0	0	65	0
Low concentration	*Vicia cracca*	Flower	0	461.4	164	17
High concentration	*Anemone canadensis*	Flower	6	97	0	0

High concentration	*Arctium minus*	Capitulum	0	0	30	0
High concentration	*Brassica sp.*	Flower	60	0	0	0
High concentration	*Cichorium intybus*	Flower	0	0	0	6
High concentration	*Cinquefoil sp.*	Flower	0	0	6	0
High concentration	*Cirsium arvense*	Capitulum	0	0	2	0
High concentration	*Cornus sericea*	Flower	72	0	0	0
High concentration	*Daucus carota*	Flower	0	0	75600	39480
High concentration	*Erigeron pulchellus*	Capitulum	0	15	15	0
High concentration	*Erigeron sp.*	Capitulum	0	0	0	70
High concentration	*Erysimum cheiranthoides*	Flower	0	5	0	0
High concentration	*Fragaria ananassa*	Flower	8	0	0	0
High concentration	*Fragaria sp.*	Flower	4	0	0	0
High concentration	*Galium sp*	Flower	0	135	0	0
High concentration	*Geum canadense*	Flower	0	8	0	0
High concentration	*Glechoma hederacea*	Flower	26	0	0	0
High concentration	*Lotus corniculatus*	Flower	0	73	195	35
High concentration	*Lythrum salicaria*	Flower	0	0	8	11
High concentration	*Medicago lupulina*	Inflorescence	0	0	0	8
High concentration	*Medicago sativa*	Flower	0	3045.6	10163.4	215
High concentration	*Pastinaca sativa*	Flower	0	1702	39160	1644
High concentration	*Rubus sp.*	Flower	0	16	0	0
High concentration	*Solidago spp.*	Flower	0	0	0	19725
High concentration	*Sonchus arvensis*	Capitulum	0	0	0	25
High concentration	*Tanacetum vulgare*	Capitulum	0	0	0	431
High concentration	*Taraxacum agg*	Capitulum	125	0	0	0
High concentration	*Trifolium repens*	Inflorescence	0	29	9	0
High concentration	*Vicia cracca*	Flower	0	1284.6	450	14
High concentration	*Viola sororia*	Flower	3	0	0	0

High concentration	*Grand Total*	304	6410.2	125638.4	61664

Appendix 5 Table A5.1 University of Guelph Agriculture and Food Laboratory Service (AFL) pesticide results. Clothianidin MDL=7ppb, clothianidin MQL=20ppb.

Site ID	Detection level crop	Detection level hedgerow
1	0.025 ppm	ND
2	Clothianidin <MDL, Pyraclostrobin <MDL, Picoxystrobin <MDL	Clothianidin <MDL
3	Clothianidin <MQL	Clothianidin <MDL
4	Clothianidin <MDL	ND
5	Clothianidin <MDL Picoxystrobin <MDL	Picoxystrobin <MDL, Clothianidin <MDL
6	Chlorantraniliprole <MDL, Clothianidin <MDL	Clothianidin <MDL
7	Clothianidin <MQL	ND
8	Sulfentrazone <MDL, Clothianidin <MDL	ND
9	Clothianidin <MDL, Dimethenamid 0.028 ppm	Clothianidin <MDL , Dimethenamid <MDL
10	Clothianidin <MDL, Dimethenamid 0.023 ppm, Pyraclostrobin <MDL	ND
11	ND	ND
12	ND	ND
13	ND	ND
14	ND	ND
15	ND	ND
16	Clothanidin <MDL	ND

Appendix 6. Ground nesting bee rearing pilot study

Introduction

Recent public concern for pollinator health has led to an increase in research of the impacts of pesticides on bees, mainly focused on the European honeybee and a handful of other managed bee species (Lundin et a., 2015). However, the ecological traits of these bee species do not reflect those of most wild bee species. Most bee species are ground nesting, solitary or semi social, are only active for a short period each year, and are frequently much smaller than honeybees or bumblebees. This means they can have different exposure routes to pesticide, smaller flight ranges, and require flowers to be present near their nests during their activity period (Zurbuchen et al., 2010). Soil can be a major pesticide exposure route for ground nesting bees during burrow construction (Chan et al., 2019).

The lack of studies on ground nesting bees is partially due to the difficulty studying them, and lack of standardized methodology in rearing them in captivity. Various methods have been attempted to rear ground nesting bees including buckets of soil, glass observation nests, and semi- field studies (Leonard & Harmon-Threatt, 2019). A recent review by Leonard et al. (2019) summarized all these methods and determined the strengths and weaknesses of these designs and how to improve and standardize their methods. They report that only a small number of ground nesting bee species have been studied in an experimental setting, and little research had been done in semi-field experiments. This portion of my study aimed to determine which ground nesting bees found in southern Ontario are suitable for semi-field experiments. I tested the survival and reproduction of a variety of ground nesting bee species throughout the summer in a semi-field experiment to determine their suitability as test organisms for future experiments.

Methods

Study site and enclosure set up

At the Canada Food Inspection Agency (CFIA) experimental farm in Ottawa, ON, I tested which native ground nesting bee species were well-suited for use in semi field experiments. To this end, I set up two 3 m^3 modified gazebos (https://www.canadiantire.ca/en/pdp/gazebo-10-x-10-ft-2994560p.html) and replaced the top fabric with netting to prevent bees from escaping while allowing for exposure to the environment. The replacement top of the gazebos was initially attached with spray glue and Gorilla tape ™ but this did not withstand the rain and was replaced with one solid piece of mesh which was sewn on (Figure 1). A 20 cm long piece of plastic skirting was attached to the bottom of the gazebos, which was buried in the sandy soil below the gazebo. The gazebos were also tethered down using rope and wooden stakes. I chose a sandy soil because it had better drainage and is easier for bees to construct burrows compared to harder soil sites (Cane., 1991).

Bee collection

I collected ground nesting bees from four morphospecies in Gatineau Park (NCC, Gatineau, QC) and the Fletcher Wildlife Garden in central Ottawa, ON, using sweep nets. The bees collected in Gatineau Park were found in sandy soil similar to that at the experimental farm. I tried to collect already mated females (indicated by the presence of pollen on their scopa). I also collected male bees in the hopes that unmated females would mate in the gazebo. The captured bees were stored in a cooler in small jars with mesh tops as they were transported to the experimental farm. Bees were fed sugar water and marked with enamel paint on their thorax prior to release into the gazebo.

Floral resources

Within the gazebos, bees were provided flowers from untreated organic seeds from natural seed bank including: red clover (*Trifolium*), wild thyme (*Thymus*), candytuft (*Iberis*), California poppy (*Eschscholzia*), dandelions (*Taraxacum*) , chicory (*Cichorium*), spearmint (*Mentha*), wild pansy (*viola*), and hyssop (*Hyssopus*) (https://www.seed-bank.ca/). I chose these species in order to provide food for the bees throughout the summer as they have different expected flowering times. I started growing the seedlings in a greenhouse several weeks before snow melt. Once the snow melted, I also planted seeds directly in the soil inside the gazebo. The greenhouse plants were not flowering in May and June, so I also used flowering tree branches of maple, apple, willow, cherry, magnolia, and lilac (genera: *Acer, Malus, Salix, Prunus, Magnolia Syringa*) to provide bee foraging resources. I also transplanted already flowering wildflowers from the field margin of the experimental farm into the gazebo to provide additional forage; these included daisies (*Bellis*), fleabane (*Erigeron*), and mustards (*Brassica*) species. I collected soil samples from both gazebos to determine soil characteristics (see chapter 2 methods), and measured soil hardness using a penetrometer.

Data collection

Once the bees were released into the gazebo, I visited once every 1-4 days when bees were active to record the number of burrows they constructed using visual surveys, the survival of the adult bees, as well as the wind speed, weather and temperature. I also ensured that adequate flowers were available at each visit. I marked visible burrows with small wooden sticks with flagging tape. In the spring of 2020, I planned to place emergence traps over the burrows to determine how many offspring emerge, however, these plans were thwarted by the effects of the covid-19 crisis on university research activities.

Results

Bee species

Over the course of the summer I tested the suitability of four groups of bees for semi field experiments, two of which were identified to genus (*Andrena* and *Halictus*) and two of which were identified to species (*C. inaequalis* and *Melissodes agilis*) (Table 1). *C. inaequalis* were assessed form May 15-June 5, multiple species of *Andrena* were assessed from May 15-July 10, one *Halictus* species was assessed from June 5- June 6, and *Melissodes agilis* were assessed from July 15-July 19 (Table 1). *C. inaequalis* was the only species confirmed to burrow (Figure 2 A) in the enclosure. However, at one-point *Andrena* were in the enclosure with *C. inaequalis,* so it is possible that they constructed some of the burrows. *Melissodes agilis* died within hours after transport with or without paint markings, *Andrena* were released only a couple at a time throughout the season and could not be confirmed to burrow, and only a few *Halictus* were released and were not seen again after release.

Discussion

Colletes inaequalis

I was able to collect many *C. inaequalis* from a sandy area in Gatineau Park during two separate visits. These were the only bees tested that readily burrowed. However, due to rainstorms which damaged the gazebos in the spring during the period of highest *C. inaequalis* activity, these bees either escaped or died. One *C. inaequalis* female immediately started burrowing after being fed sugar water. *C. inaequalis* were also observed feeding on apple, cherry, and dandelion flowers (Figure 2 B). The first release of *C. inaequalis* included males because we were unsure if the females were mated. The male *C. inaequalis* were observed in small burrows that they constructed on two occasions. In total, *C. inaequalis* constructed 22 burrows (2 may have been *Andrena sp*). *Colletes* spp. were not common in the agricultural areas near Ottawa where I conducted my field experiment, with only two *Colletes* specimins collected in total over the whole summer (Chapter 2). However, they may be useful for assessing other ecological questions about ground nesting bees. If released in a sturdier structure with more early morning light, *Colletes inaequalis* could be a promising species for studying ground nesting bees in a semi field setting.

Andrena spp.

A total of 14 individual *Andrena* from several species were released throughout the season in groups of one, two, or three at a time. *Andrena* were collected from the Fletcher wildlife garden, or from the CFIA experimental farm. They were not observed again after release; many probably escaped when the gazebo was damaged. It was also not possible to identify them to species in the field. The difficulty in finding enough *Andrena* of one species in one place, and identifying them to species, makes them a poor candidate for use in semi-field experiments.

Halictid sp.

Like *Andrena, Halictus* bees were not found in large enough numbers in one place at one time to be a good candidate for a semi field experiment in this area. Their relatively small body size also made it difficult to find them again when released in an enclosure of this size. Halictids do have the advantage of being a lot easier to identify to species than *Andrena* and are therefore better suited to experiments in smaller enclosures where they can be monitored more effectively and are less likely to escape.

Melissodes agilis

Melissodes agilis initially appeared to be suitable for this type of experiment due to their relatively large body size, and aggregated nesting habits, making it easy to collect many specimens simultaneously from one location at one time. *M. agilis* were also common near agricultural fields in my field study, making them a good surrogate bee for ground nesters near agriculture. However, for unknown reasons, *M. agilis* is much more sensitive to transport and being enclosed than *Colletes inaequalis.* I attempted to release them twice. The first time I marked their thorax with enamel paint, and they died within a couple hours of release (Figure 2 C). I then collected more and released them without marking them with paint and they all died within a day. Both times most bees were observed flying to the top corners of the gazebo where they would not come down and it is likely that they exhausted themselves trying to escape. I attempted to get them to feed on flowers after release by placing them on flowers. They drank from the flowers then would fly up to the top of the gazebo again. This species' sensitivity and tendency to fly to the top of the enclosure made them unsuitable subjects in this experiment.

Site issues

Both gazebos were set up on the edge of a forest on the CFIA experimental farm. The forest provided shade in the morning, thereby reducing the temperature in the enclosure, and inhibiting bee activity, especially during what turned out to be an unusually cold, wet spring. Furthermore, the gazebos were not sturdy enough to withstand serval wind and rainstorms that occurred during the spring. Early in the season four triangular sections of the top of the gazebos were replaced with mesh using gorilla tape and spray glue (Figure 1A). Soon after there were several wind and rainstorms that detached these top mesh pieces. On June 11 the top of the gazebos were removed and replaced with one continuous piece of mesh (Figure 1 B). This replacement top did not break during the rest of the season and allowed more light to pass through. However, there weren't any large rainstorms following this replacement. Throughout the season other insects were found in the gazebo some may have been in the soil when the gazebo was assembled such as ants and June beetles, other insects managed to get into the gazebo through holes. This indicated that the bees may have been able to escape, and these invading insects could have killed the bees as observed when ants were found in one of the *Colletes inaequalis* burrows.

Future directions

Future efforts to use ground nesting bees should focus on using sturdy enclosure structures, preventing invasion from predatory insects, and minimizing stress to the bees during transport. My study has shown that despite these challenges some bee species are more suited to nesting in semi-field enclosures. *M. agilis* seemed promising initially but was extremely sensitive to transport and being enclosed. *Colletes inaequalis* constructed many burrows in a short amount of time despite adverse weather conditions. Focusing on bees that can be caught in relatively large numbers from a single source and can be identified in the field will also help with future efforts. Matching the soil type of the bee species is found in with the soil type of the experimental enclosure will also help in encouraging burrow construction. I also observed male *Colletes inaequalis* constructing small burrows which to my knowledge is a previously undocumented behaviour. While many methods have been attempted for studying ground nesting bees with various levels of success there are no standardized protocols for these experiments (Leonard et al. 2019). Future research should focus on standardizing their research methods.

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Table A6.1. Summary of bee species released in a gazebo experiment over the summer of 2019. Several *Andrena* species were used during the summer but I was not able to identify them in the field. Two of the burrows could have been constructed by either *Colletes inaequalis* or *Andrena sp.* The time period is from the first release date to the last day of observed activity (direct observation of bee or new burrow constructed).

Species	Total Number Bees Released	Total Number Burrows Constructed	Time Period	Flowers
Colletes inaequalis	19	22±2	May 15- Jun 5	Maple, Dandelion, Magnolia, apple, willow, cherry
Andrena (Multiple species)	14	0±2	May 15-July 10	Maple, Dandelion, Magnolia, apple, willow, cherry, brassica, lilac, daisies, fleabane
Halictid sp.	3	0	Jun 5-Jun 6	Apple, Lilac
Melissodes agilis	15	0	Jul 15-Jul 19	California poppy, candy tuft, daisy

A) **B)**

Figure A6.1 Pictures of gazebo enclosure used for semi-field experiment. **A)** Initial gazebo design with partial replacement of top with mesh triangles. **B)** Gazebo design after top was replaced with one solid piece of mesh.

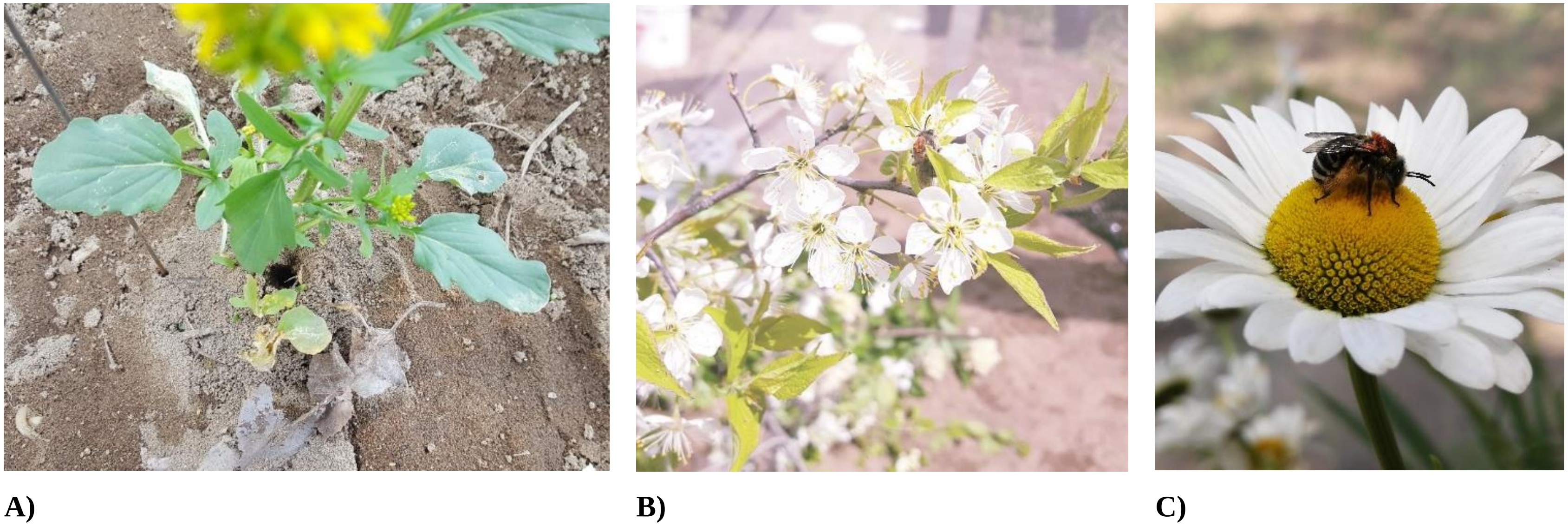

Figure A6.2 Pictures of bee activity during semi-field experiment. **A)** *Colletes inaequalis* burrow constructed under a mustard plant in the gazebo (May 31 2019). **B)** *Colletes inaequalis* marked with paint foraging on apple flowers (May 29 2019). **C)** *Melissodes agilis* foraging on daisies after being marked with paint (July 15 2019).