

**Factors influencing bee pollinator bycatch in cutworm and armyworm moth
(Lepidoptera: Noctuidae) monitoring programs in Alberta, Canada**

by

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Abstract

Unintentional bycatch of bee pollinators in monitoring traps that target moth pests occurs in many agroecosystems. Capture of bees can have a substantial effect on the efficiency of monitoring systems and has the potential to negatively impact bee biodiversity and pollination services for both crops and wild flowering plants. As there is widespread evidence of global pollinator declines, which are largely driven by anthropogenic stressors, it is important to reduce the systematic removal of bees. In this thesis, multiple approaches were used to investigate bumble bee (Hymenoptera: Apidae) and other wild bee bycatch in green-coloured Unitraps baited with a variety of semiochemical lures. First, we examined bee attraction to traps baited with synthetic pheromones of both cutworm and armyworm moths (Lepidoptera: Noctuidae) and food bait lures in the field over three growing seasons. Electrophysiological assays tested the antennal response of bees to components of the signals tested in the field. The composition of bycatch in pheromone-baited Unitraps was dominated by bumble bees, and largely by one species, *Bombus rufocinctus* Cresson. Few other wild bees or managed honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), were captured during this study. Food bait lures that were composed of fermentation by-products were not attractive to bees in this study, however the addition of the floral volatile phenylacetaldehyde increased attraction to food baits. As food bait lures are generally unattractive to bee pollinators, they may be a valuable tool for monitoring noctuid moth populations in the Canadian Prairies. Additionally, we provide the first evidence that *Bombus* spp. can detect moth pheromone components, which provides insights into the mechanism of this attraction. Second, we examined the response of wild bees to monitoring traps baited with synthetic sex pheromone of bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae), across a large area of canola production in Alberta and evaluated the effect of environmental variables on bee bycatch. As in the first study, *B. rufocinctus* was the

most numerous species captured and few other bees were attracted to monitoring traps. We found that bertha armyworm sex pheromone-baited traps positioned in the Peace region of Northwestern Alberta had the highest bumble bee bycatch, which is likely linked to the large area of forested habitat area in the region. Local flowering plant abundance did not directly impact bee bycatch but was influential for the overall model fit. The proportion of agricultural landcover in the area surrounding monitoring traps had a negative effect on the number of bees captured, which was likely due to less natural and semi-natural habitat in those regions. Finally, we provide recommendations to reduce the bycatch of beneficial bee pollinators in monitoring networks that target lepidopteran pests, which are a necessary component of successful agriculture in the Prairie Provinces.

Preface

A version of Chapter 2 of this thesis is intended for publication as: Nicholas L. Grocock, Ronald E. Batallas, Emily McNamara, Ashton B. Sturm, Jessamyn S. Manson and Maya L. Evenden. Bumble bee (Hymenoptera: Apidae) bycatch in semiochemical-baited monitoring traps targeting cutworm and armyworm moths (Lepidoptera: Noctuidae) in central Alberta, Canada. I contributed to the experimental design, data collection, data analysis and manuscript composition. Ronald Batallas also contributed to experimental design, data collection, and manuscript editing. Emily McNamara contributed to this project as an undergraduate student; she was responsible for the morphometric measurements of bumble bee specimens. Ashton Sturm was responsible for the identification of bumble bees from early field trials (data not included here) and was instrumental in training me to identify bumble bees. Jessamyn Manson provided manuscript editing. Maya Evenden was the supervising author and was involved in the concept formation and manuscript editing.

A version of Chapter 3 of this thesis is intended for publication as: Nicholas L. Grocock and Maya L. Evenden. Local and landscape-scale features influence bumble bee (Hymenoptera: Apidae) bycatch in bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), pheromone-baited monitoring traps in Alberta, Canada. I contributed to the experimental design, data collection, data analysis and manuscript composition. Maya Evenden was the supervising author and was involved in the concept formation and manuscript editing.

This thesis is dedicated to three people, without whom I would not be the person I am today.

To my mother, Karen, and my father, Jeffry. Thank you for instilling in me the importance of education from an early age and your undying support while I follow my dreams.

To my wife, Nicole, you are beautiful inside and out and I can not imagine having completed this journey with anyone else by my side. Thank you for inspiring me daily and providing me with motivation when I needed a gentle, or not-so-gentle shove.

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Chapter 1: Introduction

Agriculture in the Canadian Prairies

Canola, *Brassica napus* L. (Brassicaceae), is a rapeseed cultivar that was first produced in Canada in the early 1970's (Shahidi 1990). Canola contains less than 2% erucic acid in the oil and is low in concentration of aliphatic glucosinolates (Shahidi 1990). The Prairie Provinces (Alberta, Saskatchewan, and Manitoba) are the largest producers of canola in Canada accounting for over 90% of the crops grown (Canola Council of Canada 2017). Wheat, *Triticum aestivum* L. (Poaceae) is another common crop in the Canadian Prairies and is often grown in rotation with canola (Blackshaw et al. 1994; Gill 2018). Crop rotations increase yield and reduce populations of pest insects and weed plant species (Blackshaw et al. 1994; McLaughlin & Mineau 1995). Canola and wheat are two of the most widely grown crops in Alberta (Statistics Canada 2018), and numerous insect pests exploit these abundant resources (Madder & Stemmerhoff 1988; Lamb 1989).

Cutworms and armyworms (Lepidoptera: Noctuidae) are generalist herbivores that can be severe insect pests in many agroecosystems (Byers & Struble 1987; Spears et al. 2016; Floate 2017). The life-history and phenology for different species of noctuid pests is highly variable (Byers & Struble 1987). Generally, cutworms cause the most damage to seedlings of annual crops in early summer (Strickland 1923). Foliage feeding is common for early instars, whereas later instars cut the seedling stems at the base, which results in the death of the plant. Armyworms cause the most damage to crops from mid to late summer during the reproductive stage of the crop (Mason et al. 1998). Groups of early instar larvae feed on plant foliage and disperse in search of new host plants when food resources are depleted. Damage from larval feeding can range from thinning of a few plants to the destruction of entire fields when larval populations are at outbreak levels (Byers & Struble 1987; Mason et al. 1998; Floate 2017). Such outbreaks are sporadic and unpredictable, but often occur when optimal conditions for larval survival coincide with local population increases (Byers & Struble 1987).

Some noctuid moths that can be pests in Alberta include redbacked cutworm (*Euxoa ochrogaster* [Guenée]), bertha armyworm (*Mamestra configurata* Walker), true armyworm (*Mythimna unipuncta* [Haworth]), pale western cutworm (*Agrotis orthogonia* [Morrison]), and

occasionally other minor species (Steck et al. 1980; Bryers & Struble 1987; Mason et al. 1998). Many of these native pests can be economically devastating when population outbreaks occur in the Prairies and can cost producers in both lost yield and additional control costs (Bryers & Struble 1980; Mason et al. 1998; Evenden et al. 2017). Management strategies for noctuid pests in the Canadian Prairies include cultural practices, natural biological control, and insecticide application (Evenden et al. 2017; Floate 2017). Detection of population outbreaks is crucial for the implementation of management strategies, which indicates the need for effective, continuous monitoring programs in the Prairies.

Moth pest monitoring systems

Sex pheromones are species-specific chemical signals used by many insects to locate potential mates (Witzgall et al. 2010). The chemical constituents of hundreds of pheromones have been identified, and many of these are signals used by insects in the order Lepidoptera (Witzgall et al. 2010). Most lepidopteran sex pheromones are a blend of straight-chain compounds ranging from 10-18 carbon atoms in length and with acetate, aldehyde, or alcohol functional groups (Bjostad et al. 1987). Female moths release a sex pheromone plume, which males detect and use to orient and locate the calling female (Cardé & Willis 2008). There is selection for rapid recognition of the pheromone signal and navigation to the female, as males compete for females via scramble competition (Cardé & Willis 2008).

As moths are reliant on sex pheromones for detecting and locating mates, these semiochemical cues can be exploited for the purposes of integrated pest management (IPM) monitoring programs (Witzgall et al. 2010). Sex pheromone-baited traps can be used to detect the presence and abundance of target insects, which can inform management decisions (Witzgall et al. 2010). Unfortunately, sex pheromone-baited traps may not reflect moth breeding populations (Gerber & Walkof 1992), as only males are attracted (Witzgall et al. 2010). Noctuid moths are strong fliers that can orient to pheromone traps from long distances (Schneider 1999), and trap capture typically does not reflect larval densities or economic damage in the following growing season (Ayre & Lamb 1990; Gerber & Walkof 1992). Feeding attractant lures are a possible alternative to species-specific pheromones for monitoring noctuid moth populations and may be useful to assess breeding populations as they attract both male and female moths

(Landolt et al. 2007; Witzgall et al. 2010; Batallas 2018) over shorter distances than pheromone signals.

Insect bycatch

Although monitoring programs are designed to be species-specific, features of traps and lures can be highly attractive to non-target insects, or bycatch (Spears & Ramirez 2015). Visual elements of monitoring traps (e.g. colour, trap design) can attract non-target species as many insects use visual cues to find resources, such as flowering plants (Briscoe & Chittka 2001). For example, in apple orchards in British Columbia, Canada, yellow-coloured traps capture large numbers of beneficial insect pollinators including many Hymenoptera and Diptera (Aurelian et al. 2015). Olfactory cues released from semiochemical (e.g. pheromone or other volatile chemical cue) lures can also attract large numbers of insects as unintentional bycatch. In some cases, synthetic pheromone lures attract species that are closely related to the target species (Weber & Ferro 1991; Spears et al. 2016). Additionally, the signal emitted from pheromone lures may be used as a kairomone, a semiochemical cue that is exploited by another species such as natural enemies of the target pest (Zuk & Kolluru 1998). Feeding attractant lures are much less species-specific than pheromone signals and attract many groups of insects (e.g. Aurelian et al. 2015). For example, floral volatiles and fermentation by-products attract some species of noctuid moths as well as non-target Hymenoptera (Meagher & Mitchell 1999; Landolt et al. 2007).

Bycatch of non-target species in monitoring programs can increase trap processing time as well as decrease the overall trap effectiveness for the target species (Cha et al. 2015; Landolt & Zhang 2016). Large numbers of non-target bycatch can degrade target specimens, which increases processing time and reduces the reliability of identification, particularly for lepidopteran pests (Weber & Ferro 1991). If the bycatch contains high numbers of beneficial insect species, monitoring traps have the potential to negatively impact these populations and the important ecosystem services they provide. Efforts to reduce bycatch of beneficial insects (Clare et al. 2000; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016) can cause some traps or lures to become unviable as a monitoring tool (e.g. Aurelian et al. 2015).

Much of the insect bycatch in semiochemical-baited traps in a variety of farming systems consists of beneficial bee pollinators (Hendrix III & Showers 1990; Gross & Carpenter 1991;

Meagher & Mitchell 1999; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016). This capture of bees in semiochemical-baited traps is often facilitated by both visual (e.g. trap style, colour) and olfactory cues (Gauthier et al. 1991; Stephen & Rao 2005; Spears et al. 2016). Studies that examine bee response to colour are plentiful; however, colour preference among bee taxa is inconsistent (Stephen & Rao 2005). Foraging bees often use visual cues while searching for nectar and pollen (Chittka & Spaethe 2007; Kulahci et al. 2008; Junker & Parachnowitsch 2015). As such, bees orient more commonly to yellow, blue or white traps than to green traps positioned in a variety of agroecosystems (Hamilton et al. 1971; Hendrix III & Showers 1990; Clare et al. 2000; Mori & Evenden 2013; Spears et al. 2016). Interestingly, traps baited with moth pheromones consisting of unsaturated carbon-10-18 acetates, aldehydes, and alcohols commonly capture wild bee pollinators and especially bumble bees (Hymenoptera: Apidae) as bycatch (Gross & Carpenter 1991; Meagher & Mitchell 1999; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016). For example, in alfalfa and corn growing regions of Utah, USA, bumble bees and other wild bees are commonly captured in traps that target *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) moths (Spears et al. 2016). Although this is a widespread phenomenon, the mechanism of attraction remains unknown.

As pheromone-baited traps capture beneficial insects as bycatch and may not provide accurate information about noctuid pest breeding populations, other semiochemical lures should be investigated for monitoring. Synthetic feeding attractant lures attract some noctuid moth pests and in some cases beneficial hymenopterans (Landolt et al. 2006; Landolt et al. 2007). For example, in agricultural production in Alaska, USA, floral volatiles (e.g. phenylacetaldehyde, benzyl acetate, β -myrcene) attract *Bombus* spp., whereas fermentation by-products (e.g. acetic acid, 3-methyl-1-butanol) attract many vespids (Hymenoptera: Vespidae) (Landolt et al. 2007). The removal of hymenopteran species in monitoring traps may have ramifications for these beneficial communities and consequentially ecosystem services.

Bee importance and ecology

Insects are important pollinators that provide ecosystem services for wild plants and crops in agricultural landscapes (Goulson 2003; Klein et al. 2007; Potts et al. 2010; Goulson et al. 2015). Pollination by insects, especially bees, is crucial for 75% of global food crop production (Klein et al. 2007; Potts et al. 2010). Estimates of the value of pollination services at

the global level are over 170 billion USD per year (Gallai et al. 2009). Additionally, with an ever-increasing human population comes an increased reliance on pollinators for human food production (Aizen & Harder 2009; Senapathi et al. 2017). Wild bees are highly efficient pollinators of diverse crops across multiple continents and can even outperform honey bees, *Apis mellifera* L., (Hymenoptera: Apidae), which are used globally to supplement pollination services in managed ecosystems (Garibaldi et al. 2013; Connelly et al. 2015). Unfortunately, global declines of bee populations are increasing and appear to be driven by anthropogenic factors (Winfree et al. 2009; Potts et al. 2010; Goulson et al. 2015).

Habitat loss and fragmentation, decreased resource availability, and the increased use of insecticides are major threats to bee communities (Winfree et al. 2009; Potts et al. 2010; Goulson et al. 2015). Additionally, climate change is anticipated to intensify these effects on bees in the future (Goulson et al. 2015). Global bee declines have been linked to the destruction of natural and semi-natural habitat as a result of increased human land use, especially for agricultural intensification (Potts et al. 2010; Goulson et al. 2015; Senapathi et al. 2017). The control of pestiferous insect and weed plant species in agriculturally dominated ecosystems often relies on the use of agrochemicals, which can have both direct and indirect effects on bee communities (Goulson et al. 2015). Many classes of insecticides (e.g. organophosphates, carbamates, pyrethroids, and neonicotinoids) can cause bee toxicity (Grixti et al. 2009; Potts et al. 2010; Goulson et al. 2015). Neonicotinoids are one of the classes of pesticides most strongly implicated in the decline of bee pollinators (Goulson et al. 2015), as they persist in nectar and pollen of treated plants and wildflowers (Krupke et al. 2012; Stanley et al. 2016). Bees exposed to field-relevant levels of neonicotinoids also show sublethal effects including impaired learning, foraging, and homing ability (Goulson et al. 2015; Stanley et al. 2016). The use of herbicides to control weed plants has an indirect impact on bee communities through the reduction of wild flowering plant availability, which can make agricultural monocultures even more uninhabitable (Morandin & Winston 2005; Goulson et al. 2015). Wild bee declines are clearly driven by anthropogenic factors, and multiple, interacting, and sometimes synergistic stressors can be involved (Brown & Paxton 2009, Potts et al. 2010; Goulson et al. 2015).

The Canadian Prairies support six families (Apidae, Andrenidae, Halictidae, Colletidae, Megachilidae, and Melittidae) and over 380 species of bees, many of which are unique to the

grasslands of the Prairie Ecozone (Sheffield et al. 2014). Included in this diversity are twenty-eight of the forty bumble bee species found in Canada (Sheffield et al. 2014). Intensive agriculture, mostly for food production, also occurs in this region (Shorthouse 2010; Vankosky et al. 2017). Over 75% of native grasslands have been converted to agriculture to support livestock grazing or crop production (Shorthouse 2010; Vankosky et al. 2017). Additionally, more natural landscapes will be converted to agricultural land as the human population continues to rise (Senapathi et al. 2017). Agriculturally dominated landscapes are notoriously linked with low bee diversity and abundance (Potts et al. 2010; Goulson et al. 2015; Senapathi et al. 2017), however mass-flowering crops such as canola can provide resource pulses for pollinators (Westphal et al. 2003; Westphal et al. 2009). Conversely, both forest and grassland habitats are important for supporting diverse wild bee communities as they provide nesting resources and plentiful native flowering plants (Bailey et al. 2014; Bennett & Isaacs 2014; Hopfenmüller et al. 2014; Mallinger et al. 2016; Kammerer et al. 2016). There have been global efforts to mitigate the effects of intensive agriculture on bees, such as floral provisioning as part of agri-environment schemes (Pywell et al. 2006; Scheper et al. 2015; Wood et al. 2015). Local flowering plant abundance can increase bee diversity and abundance but may not translate to population increases (Pywell et al. 2006; Potts et al. 2009; Scheper et al. 2015; Wood et al. 2015). Although the influence of local and landscape-scale habitat features on wild bee community composition has been extensively explored (e.g. Senapathi et al. 2017), the impact of these factors on bee pollinator bycatch in monitoring traps has not been investigated to date.

Research objectives

Conservation of wild bees may be imperative for the sustainability of agricultural productivity and ecosystem function in the Canadian Prairies. Currently, bees face many stressors and although the ecological impact of removal in monitoring systems is unknown, changes to monitoring protocols to reduce pollinator bycatch would be beneficial. The attraction of bee pollinators to pheromone cues of a distantly related assemblage of moth pests is a widespread, yet poorly understood phenomenon that warrants further exploration.

In this thesis, we evaluate the abundance and diversity of hymenopteran pollinator bycatch in semiochemical-baited monitoring traps targeting noctuid moth pests in agroecosystems in Alberta, Canada. Chapter 2 focuses on bee attraction to a variety of noctuid

pheromone and feeding attractant lures in traps positioned in canola and wheat fields in central Alberta. Additionally, we investigate the electrophysiological response of two *Bombus* spp. to pheromone lure components. In Chapter 3, we evaluate the attraction of bumble bees and other wild bees to bertha armyworm pheromone-baited traps across the canola growing regions of Alberta. We also explore the impact of local and landscape-level habitat features on bee bycatch in monitoring traps.

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Chapter 2: Bumble bee (Hymenoptera: Apidae) bycatch in semiochemical-baited monitoring traps targeting cutworm and armyworm moths (Lepidoptera: Noctuidae) in central Alberta, Canada

Abstract

Cutworms and armyworms (Lepidoptera: Noctuidae) are native pests in North America that affect many crops grown on the Canadian Prairies. Semiochemical-baited traps are used to monitor noctuid moth populations and inform management decisions. The unintentional capture of bee pollinators in baited traps targeting moth pests occurs in many agroecosystems. Capture of bees reduces monitoring system efficiency and may negatively impact biodiversity and pollination services for crops and wild flowering plants. The first objective of this study was to assess the abundance and diversity of wild bees captured in semiochemical-baited monitoring traps in central Alberta, Canada. We performed a series of field experiments in paired canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) fields. Green-coloured Unitraps were baited with either a noctuid pheromone or a food bait lure and bee bycatch was compared to capture in unbaited traps. Bumble bees (Hymenoptera: Apidae), and especially *Bombus rufocinctus* Cresson, were captured in traps baited with all tested pheromone lures. Traps baited with food bait lures that consisted of fermentation by-products captured a similar number of bees as unbaited control traps. Incorporation of the floral volatile phenylacetaldehyde with food bait lures increased bee capture compared to the food bait alone. In general, more *Bombus* spp. were captured in traps positioned at canola crops as compared to wheat, but the community composition captured in both crops was similar. Few other wild bees or honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae), were captured during this study. A second experiment assessed the antennal response of two *Bombus* spp. to field-tested moth pheromone components using electroantennogram (EAG) assays. This study provides the first evidence that *B. rufocinctus* Cresson and *B. impatiens* Cresson perceive chemical signals used for sexual communication in a distant insect order. Pheromone-baited traps are common and necessary components of many integrated pest management programs that target moth pests. Modification of traps to reduce attraction of pollinators could reduce the impact on beneficial insect populations.

Introduction

Cutworms and armyworms (Lepidoptera: Noctuidae) are generalist herbivores that are pestiferous in many agroecosystems including the Prairies of western Canada (Byers & Struble 1987; Floate 2017). In Alberta, some of the native noctuid moths are field crop pests that can cause economic loss when larval populations reach outbreak levels (Byers & Struble 1987; Mason et al. 1998; Floate 2017). Noctuid larvae are generalist herbivores that feed on many field crops in the Prairie Provinces. Feeding damage ranges from plant removal causing minor patchiness to destruction of entire fields when larvae are at outbreak levels (Floate 2017). Strategies to manage cutworms and armyworms in the Prairie Provinces include cultural practices, natural biological control, and insecticide application (Mason et al. 1998; Evenden et al. 2017; Floate 2017). Reliable monitoring tools for this guild of noctuid pests are less well developed.

Monitoring pest populations is the foundation of integrated pest management (IPM) programs (Witzgall et al. 2010; Flint & Van den Bosch 2012). The temporal and spatial distribution of lepidopteran pests in agroecosystems can be assessed through moth capture in traps that are attractive to a target species (Mason et al. 1998; Mori & Evenden 2013; Spears et al. 2016). Traps baited with synthetic copies of species-specific pheromones of noctuid moths detect the presence and provide a measure of density of target populations (Steck et al. 1980; Byers & Struble 1987; Mason et al. 1998). As pheromone signals travel far down wind and noctuid moths are strong fliers (Schneider 1999), the capture of moths in pheromone-baited traps may not reflect the breeding population nor successfully predict future larval densities and subsequent economic damage (Gerber & Walkof 1992). Capture of moths in monitoring traps baited with food bait lures (e.g. fermentation products, plant volatiles, etc.) that attract both male and female moth pests over short distances may better reflect breeding populations (Landolt et al. 2007). Unfortunately, trap capture of non-target insects, or bycatch, occurs in both pheromone-baited (Spears & Ramirez 2015) and food bait traps (Aurelian et al. 2015).

Bycatch of non-target insects in monitoring traps increases trap processing time and decreases trap effectiveness for the target species (Cha et al. 2015; Landolt & Zhang 2016). If the bycatch consists of beneficial arthropod species that provide important ecosystem services, monitoring traps might negatively impact these populations and the services they provide.

Beneficial hymenopteran pollinators make up much of the insect bycatch in pheromone-baited traps positioned in a variety of agroecosystems (Hendrix III & Showers 1990; Gross & Carpenter 1991; Meagher & Mitchell 1999; Aurelian et al. 2015; Spears et al. 2016). Although the impact of bee removal from agroecosystems in monitoring traps that target moth pests is unknown (Meagher & Mitchell 1999), it may negatively influence local bee abundance and species diversity, which could alter pollination services for both wild plants and managed crops and could potentially reduce crop yields (Goulson 2003; Potts et al. 2010). As there is already widespread recognition of global declines of wild bee populations (Winfree et al. 2009; Potts et al. 2010; Goulson et al. 2015), practices that systematically remove bees from agricultural landscapes should be avoided.

Attraction of bees to semiochemical-baited traps that target moth pests might be mediated by both visual (e.g. trap style, colour) and olfactory cues (Gauthier et al. 1991; Stephen & Rao 2005; Spears et al. 2016). Bees use visual cues to locate floral resources when foraging for nectar and pollen (Chittka & Spaethe 2007; Kulahci et al. 2008; Junker & Parachnowitsch 2015). It is therefore unsurprising that more bees orient to and are captured in yellow, blue and white traps than in green traps positioned in several cropping systems (Hamilton et al. 1971; Hendrix & Showers 1990; Gross & Carpenter 1991; Clare et al. 2000; Mori & Evenden 2013; Spears et al. 2016). Wild bee pollinators and especially bumble bees (Hymenoptera: Apidae) are frequently captured as bycatch in traps baited with moth pheromones, which consist of unsaturated carbon-10-18 acetates, aldehydes, and alcohols (Gross & Carpenter 1991; Meagher & Mitchell 1999; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016). The attraction of wild bees to pheromone signals used as mating cues in a distantly related insect assemblage raises questions about the mechanism of this unique attraction. This attraction may be explained by similarities in the acetate-based pheromone components produced by both moths and bumble bees. To our knowledge, this is the first study to investigate the mechanisms driving wild bee bycatch in lepidopteran pheromone-baited monitoring traps.

Here, we assess the abundance and diversity of *Bombus* spp. and other wild bees captured in green non-saturating Unitraps baited with synthetic noctuid pheromone or food bait lures when positioned in canola, *Brassica napus* L. (Brassicaceae), and wheat, *Triticum aestivum* L. (Poaceae), fields in Alberta. We further test the electrophysiological response of two bumble bee

species to moth pheromone components in an attempt to determine if bees can detect these compounds. This work could lead to recommendations for moth monitoring systems that will minimize capture of non-target pollinator species.

Materials and methods

Study area

We monitored bycatch between 2014 and 2016 in the prairies of the Aspen Parkland Ecoregion in Alberta, Canada. Like most prairies, this region has been extensively modified over the past century to support agriculture (Shorthouse 2010). This region is located in the Canadian Prairie Ecozone, which contains twenty-eight of Canada's forty bumble bee species (Sheffield et al. 2014). The landscape is characterised by extensive agricultural plains with discontinuous clusters of trembling aspen (*Populus tremuloides* Michx.) (Salicaceae), balsam poplar (*Populus balsamifera* L.) (Salicaceae), and mixed stands (Shorthouse 2010).

Seven sites were selected across central Alberta, over an area of approximately 7350 km² throughout five counties (Table 2-1). All experimental sites were separated by at least 20 km. Each site was comprised of a paired canola and wheat field, separated by at least 500 m. All experiments were conducted at the same sites each year. Due to crop rotation practices, a field farmed to canola in the first year was rotated to wheat in the second year and back to canola in the third year of the study.

Bycatch sampling methods

Non-saturating green Unitraps (Contech Enterprise Inc, Delta, BC) were deployed to monitor for cutworm and armyworm moths throughout the growing season. Traps were positioned 1.5 m above ground and spaced 25 m apart in a linear transect along the field edge. At each site, one Unitrap replicate was baited with each tested synthetic sex pheromone or feeding attractant lure designed to target moth pests. An unbaited control trap was included for comparison at each field for all experiments. Moth sex pheromone lures were prepared by Contech Enterprise Inc. (Delta, BC) and loaded onto pre-extracted red rubber septa (Table 2-2). Sex pheromone lures were placed inside the roof of the Unitrap in a basket and were replaced

every four weeks, as recommended by the manufacturer. Feeding attractant lures were prepared in the laboratory following the methods of Landolt et al. (2007) (Table 2-2). Feeding attractant lures were dispensed in 15 mL Nalgene HDPE vials (Thermo Scientific, Rochester, NY). Vials were packed with cotton balls, loaded with 10 mL of the chemical mixture (Table 2-2), and secured inside the bucket of the Unitrap. A 3.0 mm diameter hole drilled in the centre of the vial cap allowed the release of the volatile components. Feeding attractant lures were replaced every two weeks (Landolt et al. 2007). An insecticidal strip of Hercon Vaportape II (10% dichlorvos; Hercon Environmental, Emigsville, PA) was placed inside the bucket of each trap to kill captured insects. Insecticidal strips were replaced every four weeks. Traps were intentionally deployed in the field to coincide with target moth flight periods and to limit the capture of bumble bee queens.

Trap-catch was collected every week and frozen at -20 °C. Bee bycatch was separated from the total trap contents and individual bees were pinned and dried for identification. *Bombus* spp. were identified to species according to Williams et al. (2014). Males were excluded from identification and subsequent analyses, as few were captured. Honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) were identified and all other bees were identified to family using a combination of identification aids (Packer & Ratti n.d.; Packer et al. 2007). Identifications were verified using comparisons with our own reference collections and specimens housed in the E. H. Strickland Entomological Museum, University of Alberta, Edmonton, Canada.

Field experiments

In the first experiment, pollinator bycatch was assessed from traps baited with lures based on by-products of fermented sugar baits (hereafter ‘FB’) and positioned in canola and wheat fields in 2014. The FB lure consisted of glacial acetic acid (Fisher, Fair Lawn, NJ; 99.7% purity) and 3-methyl-1-butanol (Sigma Aldrich, St. Louis, MO; 98.5% purity) mixed in equal parts by weight (Landolt et al. 2007) (Table 2-2). Bycatch in traps baited with the FB lure was compared to that in traps baited with one of three commercially prepared synthetic noctuid pheromone lures: redbacked cutworm (‘RBC’; *Euxoa ochrogaster* [Guenée]), bertha armyworm (‘BAW’; *Mamestra configurata* Walker) and true armyworm (‘TAW’; *Mythimna unipuncta* [Haworth]) and an unbaited control (Table 2-2). All lures were present concurrently at each site from 23 June to 2 September 2014. After bee identification, morphometric measurements were taken for

each female *B. rufocinctus* to distinguish between worker and queen females in the bycatch. IT length is a robust estimate of body size (Cane 1987; Greenleaf et al. 2007) and was measured as the distance between the wing bases using a stereomicroscope equipped with an ocular micrometer at 12x magnification (Wild Heerbrugg, Switzerland). Wing measurements were completed by processing scanned images with ImageJ (Rasband 2017) to measure forewing length, forewing area, and the radial cell length on the forewing. All wing measurements were performed on the right forewing; the left forewing was used if the right was missing or damaged.

A second experiment tested the effect of additional semiochemicals combined with the original FB lure on bee bycatch in baited traps positioned in wheat and canola fields in 2015. Tested semiochemicals included an additional sugar fermentation by-product, the short chain alcohol 2-methyl-1-propanol (Acros Organic, Fair Lawn, NJ; > 99% purity), and a floral volatile phenylacetaldehyde (Acros Organic, Fair Lawn, NJ; > 98% purity), in combination with the original FB. The feeding attractant lures tested included: 1) FB; 2) acetic acid + 3-methyl-1-butanol + 2-methyl-1-propanol ('FBMP'); 3) acetic acid + 3-methyl-1-butanol + phenylacetaldehyde ('FBPAA'); and 4) acetic acid + 3-methyl-1-butanol + 2-methyl-1-propanol + phenylacetaldehyde ('FB4') (Table 2-2). All feeding attractant lures were prepared in equal proportions by weight. The capture of bycatch in traps baited with the various feeding attractant lures was compared to four moth pest pheromone lures: RBC, BAW, TAW, and pale western cutworm ('PWC'; *Agrotis orthogonia* [Morrison]) and an unbaited control (Table 2-2). All lures were present from 29 June to 4 August 2015.

A final field experiment conducted 7 June to 28 July 2016 assessed bycatch in traps baited with the original FB as compared to the floral volatile phenylacetaldehyde (Acros Organic, Fair Lawn, NJ; > 98% purity) alone ('PAA') (Table 2-2). This lure was assessed to determine the pollinator attraction to PAA in the absence of fermentation by-products. Bycatch in the FB and PAA traps was compared to that in traps baited with one of two moth pest pheromone lures (RBC and BAW), an unbaited control trap, and a trap baited with a known quantity of dead moths (Table 2-2). Traps baited with dead moths were included as a second control to rule out the possibility that pollinators sensed, and were attracted to, the volatiles produced from dead target moths commonly captured in pheromone-baited traps. The 'dead moth' treatment consisted of a mesh bag filled with 10 g of dead moths obtained from previous

capture in pheromone-baited Unitraps and stored at -20°C until use. The bag was positioned in the bucket of Unitraps to mimic the odour release from moth trap capture and was replaced every two weeks.

Electrophysiological experiment

To test the hypothesis that bumble bees can detect the various moth pheromones used to bait Unitraps in the field, electroantennogram (EAG) assays using bumble bee antennae were conducted in response to a subset of pheromone components tested in the field. We tested two different species of bumble bees to compare antennal response of a local species, *B. rufocinctus*, that responded strongly to components in field tests and *B. impatiens* Cresson, which is commonly available from commercial breeders and is often used in field and laboratory-based studies in North America (Shipp et al. 1994; Stubbs & Drummond 2001; Cnaani et al. 2002). A standard colony of *B. impatiens* (Biobest Canada, Leamington, ON) was maintained in a growth chamber (Percival Intellus Environmental Controller Model I30VL; Percival Scientific, Perry, IA) at 23±2 °C on a 12:12 hour L:D cycle and provisioned with BIOGLUC sugar solution (Biobest Canada, Leamington, ON). Similar sized workers were randomly selected and removed from the colony one month later, in May 2018, for use in EAG assays. In early July 2018, we collected *B. rufocinctus* workers from field margins of a canola field near Sunnybrook, Alberta (53°09'00.8"N 114°07'25.0"W). Bees were captured using insect nets (0.3 m diameter) and were housed in refrigerated containers for transport to the University of Alberta, where they were placed in individual containers and provided with 10% sugar solution. Bees were placed into a growth chamber, under the environmental conditions detailed above, for ~18 hours prior to EAG assays.

The EAG system consisted of an IDAC-02 data acquisition controller system, a Syntech EAG probe (Type PRG-2, internal gain 10X), and EAG 2000 software (Syntech, Hilversum, The Netherlands). Bumble bees were chilled at 4 °C for 10-15 minutes before the right antenna was excised using micro-dissecting scissors; the left antenna was used if the right was missing or damaged. The antenna was cut at the base of the flagellum and at the tip of the terminal segment. The cut antenna was attached to a stainless-steel antenna holder using a small quantity of conductive gel (Spectra 360; Parker Laboratories, Orange, NJ). The stimuli tested were synthetic moth pest pheromone components Z11-16Ac, Z5-12Ac, and Z7-12Ac (Pherobank, Wijk bij

Duurstede, Netherlands), which were selected because they are the major components of the moth pests targeted in field experiments (Table 2-2). Each test compound was serially diluted in hexane (Sigma Aldrich, St. Louis, MO) to obtain 1, 10, and 100 $\mu\text{g}/\mu\text{L}$ hexane solutions. 25 μL of each solution was pipetted onto 0.2 cm x 7.0 cm strips of filter paper (Whatman No. 1), which was inserted into individual Pasteur pipettes 30 minutes prior to the assay so that the solvent could evaporate. Hexane-treated filter paper inserts were created in the same manner and positioned in Pasteur pipettes as a negative control. Linalool (Sigma Aldrich, St. Louis, MO; 97% purity) was diluted to 10 $\mu\text{g}/\mu\text{L}$ with hexane and was used as a positive control (Anfora et al. 2011) to ensure bumble bee antennae remained alive for the duration of the trial. Carbon-filtered and humidified air, from a Syntech CS-55 stimulus controller, flowed at 50 mL/minute over the mounted antenna. Stimulus puffs were triggered by hand via the stimulus controller and had a pulse duration of 0.2 seconds and flow rate of 10 mL/second. Test compounds were presented in ascending order of dosage with 30 second inter-stimuli intervals (i.e. hexane, linalool, 1 $\mu\text{g}/\mu\text{L}$ Z11-16Ac, linalool, 10 $\mu\text{g}/\mu\text{L}$ Z11-16Ac, linalool, 100 $\mu\text{g}/\mu\text{L}$ Z11-16Ac, hexane) and stimuli cartridges were replaced after every five antennae tested. Antennae from 10 individuals of both *B. impatiens* and *B. rufocinctus* were tested and EAG responses were recorded as the maximum amplitude of depolarization (mV) induced by the test compound.

Statistical analysis

All statistical analyses were conducted using the statistical software R in ‘RStudio v1.1.447’ (R v3.5.0; R Core Team 2018). Results from each field experiment were modelled independently using the ‘glmer.nb’ function (package ‘lme4’; Bates et al. 2015) to construct generalized linear mixed models with a negative binomial distribution. Models initially included all possible interactions, which were removed if they were not statistically significant ($\alpha = 0.05$). Although traps were deployed at different times to target various moth pest species, the total number of bumble bees trapped was determined for the time period when all lure types were present at each site (2014: 23 June to 2 September; 2015: 29 June to 4 August; 2016: 7 June to 28 July). Date was not included as a predictor variable due to low capture rates, which prevented models from running when bycatch was not pooled across the season. For each experiment, a separate model was created specifying the summed number of *B. rufocinctus* trapped throughout the season as the dependent variable, different lure and crop types as independent variables, and

site as a random blocking term (Table A1). In the experiment conducted in 2014, the total of all other bumble bee species ('other *Bombus*') was summed and specified as the dependent variable in an additional model. In the experiment conducted in 2015, the number of *B. rufocinctus* captured was analyzed as in the first experiment, but the low capture of other *Bombus* required pooling bycatch according to lure type (unbaited, pheromone, and fermentation- or floral-based feeding attractant), which was used, along with crop, as an explanatory variable. The 2015 model for the other *Bombus* included an 'offset', which accounted for the different number of traps representing the different lure types (e.g. 4 pheromone lure traps vs. 1 unbaited control trap). In the 2016 experiment, *B. rufocinctus* bycatch was analyzed as before, but the capture rate of other *Bombus* was low and prevented further analysis. Other species of wild bees (non-*Bombus*) were captured in low numbers for all experiments and were not included in statistical models. We performed Wald chi-square analyses using the 'Anova' function (package 'car'; Fox 2012) to test for differences in the number of bumble bees captured in traps baited with different lures and positioned in different crop types for all models. This was followed with post-hoc means separation using the Tukey method ($\alpha = 0.05$; package 'lsmeans'; Lenth & Hervé 2015).

We used non-metric multidimensional scaling (NMDS) ordination and ANOSIM analysis to assess differences in the species richness of bumble bee bycatch in traps baited with the different lures and positioned in the different crop types for each year of the study. Analyses were performed excluding *B. rufocinctus* to assess differences for less frequently captured species represented by >1 individual. NMDS ordinations, based on Bray-Curtis pairwise distance matrices ('bcdist' function, package 'ecodist'; Goslee & Urban 2007), were generated using the package 'vegan' (Oksanen et al. 2013). ANOSIM analyses were completed using the 'anosim' function (package 'vegan'). ANOSIM analysis uses a ranked dissimilarity matrix to compare the similarity of the community within and between treatment groups. ANOSIM generates the R test statistic, which indicates treatment differences if significantly different from zero (Clarke 1993).

In the electrophysiological experiment, EAG response data were transformed using the $[\ln(x + 1)]$ function to satisfy assumptions of normality. We analyzed the effects of each test compound separately using the 'lmer' function (package 'lme4') to construct generalized linear mixed models for both bumble bee species. EAG responses were analysed with a random intercept and slope to account for the repeated measures on the same bee antenna. For each

model, we specified the transformed EAG response as the dependent variable and compound concentration as a fixed factor. Compound concentration of the stimulus was specified as the random intercept and the antenna identification number was considered as the random slope (~ Concentration | Antenna ID). We used the 'anova' function to test for differences in EAG response at different concentrations for each test compound, compared to the hexane control. This was followed with post-hoc means separation using the Tukey method ($\alpha = 0.05$).

Results

Field experiments

Bees captured in 2014 included a total of 603 female bumble bees from 13 species as well as 55 males that were not identified to species (Table 2-3). The capture rate was ~1.9 bumble bees per trap/day. *Bombus rufocinctus* was the most abundant species captured and represented ~73.6% of the bumble bee bycatch, excluding males. All morphometric measurements indicate that the majority of *B. rufocinctus* females captured were similar in size and were likely workers. We also captured a total of 76 other bees from 3 families: Andrenidae, Apidae, and Megachilidae (Table 2-4). There were significant differences in the number of *B. rufocinctus* captured in traps baited with the different lure types (Wald $\chi^2=60.949$, $df=4$, $p<0.0001$). There were significantly more *B. rufocinctus* per trap captured in the noctuid moth pheromone-baited Unitraps than in unbaited control traps (Figure 2-1). There was no difference in *B. rufocinctus* capture in the FB lure and the unbaited control trap. These trends did not exist for the capture rates of other *Bombus*, which were not influenced by lure type (Wald $\chi^2=4.381$, $df=4$, $p=0.357$). On average, more *B. rufocinctus* were captured in Unitraps positioned along canola fields compared to wheat, but this difference was not statistically significant (Wald $\chi^2=2.971$, $df=1$, $p=0.085$). Significantly more other *Bombus* spp. were captured in traps positioned in canola compared to wheat fields (Wald $\chi^2=4.159$, $df=1$, $p=0.041$).

A total of 693 female and 4 male bumble bees were captured in 2015. *Bombus* females were from 16 species, while males were not identified to species (Table 2-3). The capture rate was ~2.14 bumble bees per trap/day. *Bombus rufocinctus* was the most abundant species captured and represented ~79.1% of the bumble bee bycatch, excluding males. We also captured a total of 83 non-*Bombus* bees from 4 families: Andrenidae, Apidae, Halictidae, and

Megachilidae (Table 2-4). Lure type significantly affected the number of *B. rufocinctus* captured in traps baited with the different lure types during the 2015 field season (Wald $\chi^2=72.682$, $df=8$, $p<0.0001$). More *B. rufocinctus* were captured in pheromone-baited Unitraps compared to the unbaited control traps, but only traps baited with the PWC pheromone lure captured significantly more bees than the control trap (Figure 2-2). The FB and FBMP feeding attractant lures, which consisted of sugar bait fermentation products, did not significantly influence capture of *B. rufocinctus*. Alternatively, traps baited with the FB4 and FBPA lures, which contained fermentation and floral components, caught more *B. rufocinctus*, but only traps baited with the FBPA lure caught significantly more *B. rufocinctus* than the unbaited control trap (Figure 2-2). After correction for the number of traps, lure classification (e.g. pheromone, fermentation by-product, floral volatile) significantly impacted capture of other *Bombus* spp. (Wald $\chi^2=16.876$, $df=3$, $p=0.0007$). The fermentation-based feeding attractant lures elicited the lowest capture of other *Bombus*, which was significantly lower than trap catch in both the pheromone-baited and feeding attractant-baited traps that contained floral components (Figure 2-3). Unbaited control traps captured an intermediate number of other *Bombus* (Figure 2-3). More *B. rufocinctus* were captured in Unitraps positioned along canola fields compared to wheat (Wald $\chi^2=7.522$, $df=1$, $p=0.006$), whereas there was no effect of crop type on the number of other *Bombus* spp. captured (Wald $\chi^2=2.481$, $df=1$, $p=0.115$).

A total of 450 female bumble bees representing 9 species were captured in 2016 (Table 2-3). The capture rate was ~1.8 bumble bees per trap/day. *Bombus rufocinctus* was the most abundant species captured and represented the vast majority of bumble bee bycatch at ~92.9%. We also captured a total of 70 non-*Bombus* bees, from 4 families: Andrenidae, Apidae, Halictidae, and Megachilidae (Table 2-4). Lure type impacted the number of *B. rufocinctus* captured (Wald $\chi^2=91.77$, $df=5$, $p<0.0001$). The most *B. rufocinctus* were captured in traps baited with the PAA lure (Figure 2-4). There were significantly more *B. rufocinctus* captured in both the RBC and BAW pheromone-baited Unitraps than in unbaited control traps. As in previous years, traps baited with the FB lure captured similar numbers of *B. rufocinctus* as the unbaited control trap. Additionally, there was no significant difference in the number of *B. rufocinctus* captured in the unbaited control and the ‘dead moth’ treatment. More *B. rufocinctus* were captured in Unitraps positioned along canola fields compared to wheat, but this difference was not significant (Wald $\chi^2=2.79$, $df=1$, $p=0.095$).

For all years of the study, there was an overlapping pattern in the NMDS plots of bumble bee species compositions for both lure (only 2014 data presented; Figure 2-5) and crop type (only 2014 data presented; Figure 2-6). There were no significant differences in captured bumble bee species composition among all tested lure types (2014: ANOSIM $R=-0.026$, $p=0.734$; 2015: ANOSIM $R=0.047$, $p=0.119$; 2016: ANOSIM $R=-0.040$, $p=0.641$). Similarly, there were no differences in species composition between *Bombus* spp. captured in traps positioned along canola or wheat fields (2014: ANOSIM $R=-0.007$, $p=0.551$; 2015: ANOSIM $R=0.001$, $p=0.362$; 2016: ANOSIM $R=0.004$, $p=0.412$).

Electrophysiological experiment

In general, antennae of both species tested showed a dose dependent EAG response to increasing doses of test compounds compared to the hexane control, however species responses to test components varied (Figure 2-7). The EAG response to Z11-16Ac was only significantly higher than hexane at 100 $\mu\text{g}/\mu\text{L}$ for *B. impatiens* ($F_{3, 27}=4.51$, $p=0.011$) and not for *B. rufocinctus* at any concentration. The opposite was observed for Z5-12Ac, in which *B. rufocinctus* had higher responses at 100 $\mu\text{g}/\mu\text{L}$ compared to hexane ($F_{3, 27}=10.84$, $p<0.0001$) but the *B. impatiens* response was not significantly different than the control at any of the tested concentrations. Both bumble bee species showed significantly greater EAG responses for Z7-12Ac at 10 and 100 $\mu\text{g}/\mu\text{L}$ as compared to hexane ($F_{3, 27}$, $p<0.0001$). The EAG response to the linalool (10 $\mu\text{g}/\mu\text{L}$) standard was numerically higher than the responses to pheromone components (Figure 2-8) and was similar to previous studies using *B. terrestris* L. (Anfora et al. 2011).

Discussion

Trapping experiments in canola and wheat fields in Alberta, Canada show that bumble bee bycatch is prevalent in traps baited with pheromones of noctuid moth pests native to the Prairie Provinces, whereas relatively few other wild bees were captured. In general, more bumble bees were captured in traps positioned along canola fields than in those along wheat fields. Although more bees were captured in traps positioned in canola, the community composition of bee bycatch did not vary with crop type. Feeding attractant lures based on sugar bait

fermentation products were unattractive to bumble bees but attraction was enhanced with the addition of floral volatiles to food bait lures. Traps baited with the floral volatile PAA alone resulted in high bumble bee bycatch. *Bombus rufocinctus* was the most commonly captured species across all three years of our study, and there were relatively low numbers of other *Bombus* spp. captured. *Bombus rufocinctus* perceives the moth pheromone components tested here and is attracted to pheromone lures targeting four common noctuid moth pests (RBC, BAW, TAW, and PWC) in the field. This attraction is driven by olfactory signals rather than visual cues of the moth traps, as more *B. rufocinctus* are captured in baited traps than in unbaited control traps, or those experimentally baited with dead moths.

Although the number of bees varied with lure treatment, the number of *Bombus* spp. captured in this study was similar regardless of the semiochemical treatment used to bait the monitoring traps. The number of *Bombus* spp. bycatch captured in the variously baited Unitraps in this study was similar to that collected from pan traps and active netting in Alberta (Kohler 2017; Sturm 2017). *Bombus rufocinctus*, *B. ternarius* Say, and *B. borealis* Kirby are the most frequently captured bumble bee species in previous work (Kohler 2017; Sturm 2017) and in this study in Alberta. A higher proportion of *B. rufocinctus* was captured in this study, compared to studies in which bees were captured with relatively unbiased sampling techniques (Westphal et al. 2008; Kohler 2017; Sturm 2017). This is another indication that *B. rufocinctus* is more attracted to pheromone lures, as other bees are captured in numbers that reflect abundance in the sampling area. The number of *B. rufocinctus* captured was the same in the various pheromone-baited traps tested. This differs from other studies in which bumble bee bycatch varies with the type of pheromone lure used to attract different noctuid moth species. For example, in alfalfa and corn growing regions of Utah, traps baited with pheromone lures that target *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) moths have high bee bycatch, whereas traps baited with *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) and *S. littoralis* Boisduval (Lepidoptera: Noctuidae) pheromone lures do not capture more bees than unbaited control traps (Spears et al. 2016). Traps in corn fields baited with *S. frugiperda* J.E. Smith (Lepidoptera: Noctuidae) pheromone lures, however, attract more *Bombus* spp. than the floral volatile PAA (Meager and Mitchell 1999), which was highly attractive in our study. Differential bumble bee attraction to pheromone lures that target closely related moth pests could be due to differences in agroecosystems but is most likely driven by differences in the molecular structure of the

pheromone components used to bait traps that target different moth species. It appears pheromone components comprised of straight chain hydrocarbons (10 - 18 carbons long) with one double bond and a terminal aldehyde, alcohol, or acetate functional group are attractive to *Bombus* spp. (Meager & Mitchell 1999; Spears et al. 2016). Release of these types of compounds from the pheromone-baited traps in the current study especially attract *B. rufocinctus* to baited traps positioned in both canola and wheat fields in central Alberta. Monitoring traps that target *S. litura* and *S. littoralis* are baited with pheromone components that have two double bonds (e.g. Z9E11-14Ac, Z9E12-14Ac) and are less attractive to *Bombus* spp. (Spears et al. 2016).

Long chain monounsaturated hydrocarbons appear to be widely attractive to *Bombus* spp., but the mechanism of this attraction remains unknown. Bumble bees could be preadapted to sense these molecules because of the structural similarity of the moth pheromones to signals used by the bees. Male bumble bees produce species-specific pheromone blends in the cephalic portion of the labial gland ('CLG'; De Meulemeester et al. 2011). These pheromones can include fatty acid derivatives, straight chain saturated and monounsaturated hydrocarbons, and acyclic terpenes with alcohol, aldehyde, or acetate functional groups (Appelgren et al. 1991; Bergström et al. 1996). Some North American *Bombus* spp., including *B. nevadensis* Cresson, *B. griseocollis* De Geer, and *B. rufocinctus* perch on prominent landscape features and mark the perches with pheromones to attract females (O'Neill et al. 1991). The CLG secretions of *B. rufocinctus* and other perching males are predominately acetate based (Bertsch et al. 2008). Interestingly, *B. rufocinctus* in the current study was the predominant species captured in traps baited with acetate-based pheromone lures.

Although the primary function of male-produced pheromones in *Bombus* spp. is to attract unmated queens for reproduction (Bergström et al. 1985; Appelgren et al. 1991; Šobotník et al. 2008), it is likely that conspecific workers and males may also be attracted to this signal. Bumble bee workers, males, and young queens have similar EAG responses to a variety of floral volatiles and bumble bee pheromone components (Fonta & Masson 1984). In our electrophysiological experiment, both *B. impatiens* and *B. rufocinctus* had significant and similar EAG responses to the synthetic noctuid moth pheromone component Z7-12Ac, a component of the sex pheromone of RBC and PWC. Antennae from the two *Bombus* species responded differently to the other pheromone components tested. *Bombus rufocinctus* responded to Z5-12Ac but not to Z11-16Ac

and the opposite was observed for *B. impatiens*. The pheromone blends of RBC and PWC moths contain Z5-12Ac and these lures captured high numbers of *B. rufocinctus* in field tests. Numerically but not statistically more *B. rufocinctus* were captured in pheromone traps targeting RBC and PWC than those targeting BAW and TAW. The main pheromone component of BAW and TAW is Z11-16Ac, to which *B. rufocinctus* did not respond during the EAG bioassay. These EAG results demonstrate that workers of both *B. impatiens* and *B. rufocinctus* can perceive individual lepidopteran pheromone components and are differentially responsive to different components, however we are unable to confirm that this difference in response is not a result of differences in volatility associated with the size of the test compounds. Many studies use *B. impatiens* or *B. terrestris* as a focal bumble bee species (Shipp et al. 1994; Cnaani et al. 2002), but these results indicate the importance of considering the response of multiple species, especially for studies of olfactory response.

Many species of bumble bees likely perceive the chemicals released from baited Unitraps but only some species will respond behaviourally. It is important to note that pheromone lures tested in field experiments released blends of various pheromone components, which may induce different physiological and behavioural responses than individual components. Antennal electrophysiological response, as measured by EAG, informs perception capability but not the resulting behavioural response, which is often highly context dependent. For example, volatiles released by the obligate nest parasite *B. vestalis* Geoffroy repel *B. terrestris* workers (Lhomme et al. 2015), whereas in our study similar semiochemicals attract *B. rufocinctus*. The orchid *Cypripedium calceolus* L. (Orchidaceae) can also attract *B. terrestris* workers through mimicry of the acetate-based bumble bee pheromone components (Coppée et al. 2011; Przybyłowicz et al. 2012). Male *Bombus* spp. may try to usurp the scent-marked territories of other perching males (O'Neill et al. 1991) and use these chemicals to locate mating sites (Bertsch et al. 2008). Approximately 79% of the male bumble bee bycatch in our study was in traps baited with noctuid moth pheromone lures. The orientation exhibited by *Bombus* species to pheromone-baited traps must be due to the recognition and response to noctuid pheromone components as fewer bees were captured in unbaited control traps and bees were not attracted to volatiles released from large numbers of dead moths.

The lures based on fermentation products of sugar baits were tested as potential lures for monitoring multiple species of noctuid moths (Landolt et al. 2007; Batallas 2018). In general, fewer bees were captured in traps baited with feeding attractant lures than in those baited with moth pheromones. The addition of floral volatiles to baits releasing the fermentation products enhanced bee capture in traps baited with food bait lures. Bee bycatch in traps baited with the PAA floral volatile alone was far greater than in food bait traps and even exceeded bycatch in pheromone-baited traps. Similarly, Landolt et al. (2007) captured more *Bombus* spp. in traps baited with a floral lure (PAA, β -myrcene, methyl-salicylate, and methyl-2-methoxy benzoate) than in traps baited with the same type of food bait lures tested in the current study. As such, although floral volatiles can be attractive to many noctuid pests, they are not commonly recommended for monitoring because of high pollinator bycatch (Meagher & Mitchell 1999; Landolt et al. 2007; Batallas 2018). Fermentation based food bait lures could be used for the effective monitoring of many species of noctuid moth pests (Batallas 2018), while reducing the impact of monitoring on beneficial pollinator populations.

Generally, we found that traps positioned in the field margins of canola crops had higher bumble bee bycatch than traps adjacent to wheat fields, but the differences were often only marginally significant. The availability of mass-flowering crops such as canola provide a highly rewarding food resource for many pollinators and can increase bumble bee colony growth and abundance during a growing season (Westphal et al. 2003; Westphal et al. 2009; Senapathi et al. 2017). The community composition of *Bombus* spp. bycatch captured in traps was not influenced by crop type. In Alberta, canola and wheat crops are often grown in close proximity, which may explain the relatively minor differences in bycatch community composition. Bumble bees are capable of long-distance flights during foraging trips, especially in agriculturally dominated landscapes (Rao & Strange 2012). This allows them to access high quality floral resources far from nesting areas and creates opportunities for interacting with monitoring traps.

Four other families of hymenopteran pollinators (Andrenidae, Apidae, Halictidae, and Megachilidae) were captured in relatively low numbers in this study. At the family level, wild bee richness captured in this study was similar to that collected from pan traps and active netting in Alberta (Kohler 2017; Sturm 2017). The proportion of non-*Bombus* bees captured using these sampling techniques was much higher than found in the current study. Although other non-

Bombus bees were likely present in the agroecosystems where we conducted our study, they were not captured in the monitoring traps in this study. Our results differ from previous studies that report capture of non-*Bombus* bees in monitoring traps baited with pheromone lures (Meagher & Mitchell 1999; Spears et al. 2016). For example, Spears et al. (2016) captured significantly greater numbers of both *Lasioglossum* (Hymenoptera: Halictidae) and *Agapostemon* (Hymenoptera: Halictidae) in multicoloured traps baited with *H. armigrera* pheromone than in unbaited control traps. Overall, our results corroborate previous studies that show visual cues from green-coloured monitoring traps do not attract non-*Bombus* pollinators (Clare et al. 2005; Stephen & Rao 2005; Mori & Evenden 2013; Spears et al. 2016).

This study demonstrates that acetate-based noctuid pheromone lures attract bumble bees, and especially *B. rufocinctus*, in Alberta agroecosystems. This attraction may be due to the similarities between the components of male-produced bumble bee pheromones and synthetic noctuid pheromone lures. This study also provides the first electrophysiological evidence that bumble bee workers can perceive the components of pheromone lures. Future work assessing the response of *Bombus* spp. to acetate, aldehyde, and alcohol-based pheromone lures as well as to male-produced bumble bee pheromones could provide additional understanding of the mechanisms driving this response. Although the level of bee bycatch in this study may not pose a significant threat to pollinator populations, any reduction in bycatch would be beneficial as monitoring is essential for the success of agricultural production in the Canadian Prairies. To limit the bycatch of wild bee pollinators in monitoring traps that target noctuid moth pests in the Prairie Provinces, managers should continue to use green monitoring traps that are deployed when queen and male bumble bees are not active. Additionally, lures based on fermentation by-products are attractive to multiple noctuid moth species (Landolt et al. 2007; Batallas 2018) and do not attract *Bombus* species. Further research on the efficacy of using food bait lures for widescale monitoring and the impact of these lures on other beneficial insects such as vespid wasps (Hymenoptera: Vespidae) (Landolt et al. 2007), lady beetles (Coleoptera: Coccinellidae) (Spears et al. 2016), and parasitic wasps and flies is needed before they can be widely adopted for monitoring. Future research in this area should also investigate the environmental factors that influence wild bee bycatch in pheromone baited traps so monitoring protocol changes can be implemented to reduce bycatch and lower the impact of pest monitoring on beneficial wild pollinators.

Table 2-1. Site locations for seven canola and wheat sites in central Alberta, Canada.

County	Site	Field	Coordinates	2014	2015	2016
Leduc	1	A	53.23790 N 113.34226 W	Canola	Wheat	Canola
		B	53.24722 N 113.34219 W	Wheat	Canola	Wheat
	2	A	53.28640 N 113.87867 W	Canola	Wheat	Canola
		B	53.27595 N 113.85422 W	Wheat	Canola	Wheat
Parkland	3	A	53.44492 N 113.71344 W	Canola	Wheat	Canola
		B	53.43946 N 113.71339 W	Wheat	Canola	Wheat
Barrhead	4	A	54.07452 N 114.37685 W	Canola	Wheat	Canola
		B	54.05627 N 114.34988 W	Wheat	Canola	Wheat
	5	A	54.30392 N 114.47681 W	Canola	Wheat	Canola
		B	54.34530 N 114.47697 W	Wheat	Canola	Wheat
Wainwright	6	A	52.95971 N 111.43202 W	Canola	Wheat	Canola
		B	52.95963 N 111.43922 W	Wheat	Canola	Wheat
	7	A	52.90159 N 110.56340 W	Canola	Wheat	Canola
		B	52.88453 N 110.60859 W	Wheat	Canola	Wheat

Table 2-2. Lure composition and deployment schedule for each year of the study. All lures were deployed in non-saturating green Unitraps in the margin of canola and wheat fields in central Alberta, Canada.

Year	Lure	Components	Ratio	Amount
2014	FB	Acetic acid, 3-methyl-1-butanol	1:1	10 mL
	Redbacked cutworm (RBC; <i>Euxoa ochrogaster</i>)	Z5-12Ac, Z7-12Ac, Z9-12Ac, Z5-10Ac	200:2:1:1	1000 µg
	Bertha armyworm (BAW; <i>Mamestra configurata</i>)	Z11-16Ac, Z9-14Ac	95:5	500 µg
	True armyworm (TAW; <i>Mythimna unipuncta</i>)	Z11-16Ac	1	500 µg
	Unbaited control	-	-	-
2015	FB	Acetic acid, 3-methyl-1-butanol	1:1	10 mL
	FBMP	Acetic acid, 3-methyl-1-butanol, 2-methyl-1-propanol	1:1:1	10 mL
	FBPAA	Acetic acid, 3-methyl-1-butanol, phenylacetaldehyde	1:1:1	10 mL
	FB4	Acetic acid, 3-methyl-1-butanol, 2-methyl-1-propanol, phenylacetaldehyde	1:1:1:1	10 mL
	RBC	Z5-12Ac, Z7-12Ac, Z9-12Ac, Z5-10Ac	200:2:1:1	1000 µg
	BAW	Z11-16Ac, Z9-14Ac	95:5	500 µg
	TAW	Z11-16Ac	1	500 µg
	Pale western cutworm (PWC; <i>Agrotis orthogonia</i>)	Z7-12Ac, Z5-12Ac	2:1	500 µg
	Unbaited control	-	-	-
2016	FB	Acetic acid, 3-methyl-1-butanol	1:1	10 mL
	PAA	Phenylacetaldehyde	1	10 mL
	Moth	Dead moth pests	-	10 g
	RBC	Z5-12Ac, Z7-12Ac, Z9-12Ac, Z5-10Ac	200:2:1:1	1000 µg
	BAW	Z11-16Ac, Z9-14Ac	95:5	500 µg
	Unbaited control	-	-	-

Table 2-3. Abundance of bumble bee species captured as bycatch in monitoring traps positioned in agroecosystems in central Alberta, Canada.

Year	Species	Abundance
2014	<i>Bombus rufocinctus</i>	463
	<i>Bombus ternarius</i>	64
	<i>Bombus flavifrons</i>	25
	<i>Bombus borealis</i>	17
	<i>Bombus cryptarum</i>	16
	<i>Bombus vagans</i>	13
	<i>Bombus nevadensis</i>	8
	<i>Bombus insularis</i>	7
	<i>Bombus mixtus</i>	3
	<i>Bombus fervidus</i>	1
	<i>Bombus frigidus</i>	1
	<i>Bombus huntii</i>	1
	<i>Bombus perplexus</i>	1
	<i>Bombus sandersoni</i>	1
	<i>Bombus</i> spp. males	55
2015	<i>Bombus rufocinctus</i>	548
	<i>Bombus borealis</i>	72
	<i>Bombus ternarius</i>	36
	<i>Bombus vagans</i>	12
	<i>Bombus terricola</i>	8
	<i>Bombus centralis</i>	5
	<i>Bombus flavifrons</i>	3
	<i>Bombus cryptarum</i>	2
	<i>Bombus fervidus</i>	1
	<i>Bombus frigidus</i>	1

Table 2-3. (Continued).

Year	Species	Abundance
	<i>Bombus insularis</i>	1
	<i>Bombus mixtus</i>	1
	<i>Bombus nevadensis</i>	1
	<i>Bombus perplexus</i>	1
	<i>Bombus sandersoni</i>	1
	<i>Bombus</i> spp. males	4
2016	<i>Bombus rufocinctus</i>	418
	<i>Bombus borealis</i>	9
	<i>Bombus ternarius</i>	6
	<i>Bombus insularis</i>	4
	<i>Bombus terricola</i>	4
	<i>Bombus centralis</i>	3
	<i>Bombus huntii</i>	3
	<i>Bombus vagans</i>	2
	<i>Bombus flavifrons</i>	1

Table 2-4. Abundance of non-bumble bee hymenopteran pollinator bycatch in monitoring traps positioned in agricultural settings in central Alberta, Canada.

Year	Family	Abundance
2014	Andrenidae	9
	Apidae	25
	<i>Apis mellifera</i>	6
	Megachilidae	36
2015	Andrenidae	7
	Apidae	18
	<i>Apis mellifera</i>	5
	Halictidae	3
2016	Megachilidae	50
	Andrenidae	20
	Apidae	13
	<i>Apis mellifera</i>	6
	Halictidae	1
	Megachilidae	30

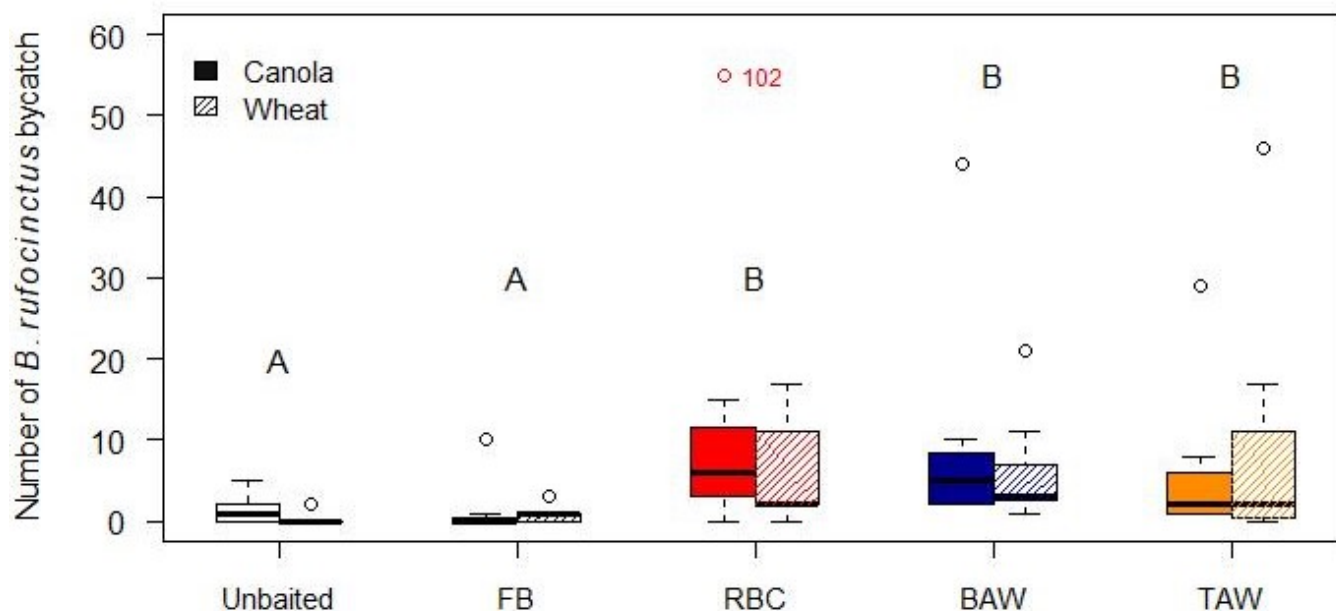


Figure 2-1. Boxplots of the season long capture of *Bombus rufocinctus* in Unitraps baited with the original food bait lure (FB=acetic acid + 3-methyl-1-butanol) and pheromone lures targeting different noctuid moth pest species (RBC=Redbacked cutworm; BAW=Bertha armyworm; TAW= true armyworm) in canola and wheat crops during the 2014 field season. The midline indicates the median and the top and bottom of the box indicates the first and third quartiles, respectively. Vertical line or whiskers represent the 1.5 interquartile range of the data or the maximum value. Open circles represent points more than 1.5 times the interquartile range (repositioned points are shown in red and original location indicated to allow for easier comparison between treatments). Different letters indicate significant differences (Tukey's HSD: $P < 0.05$).

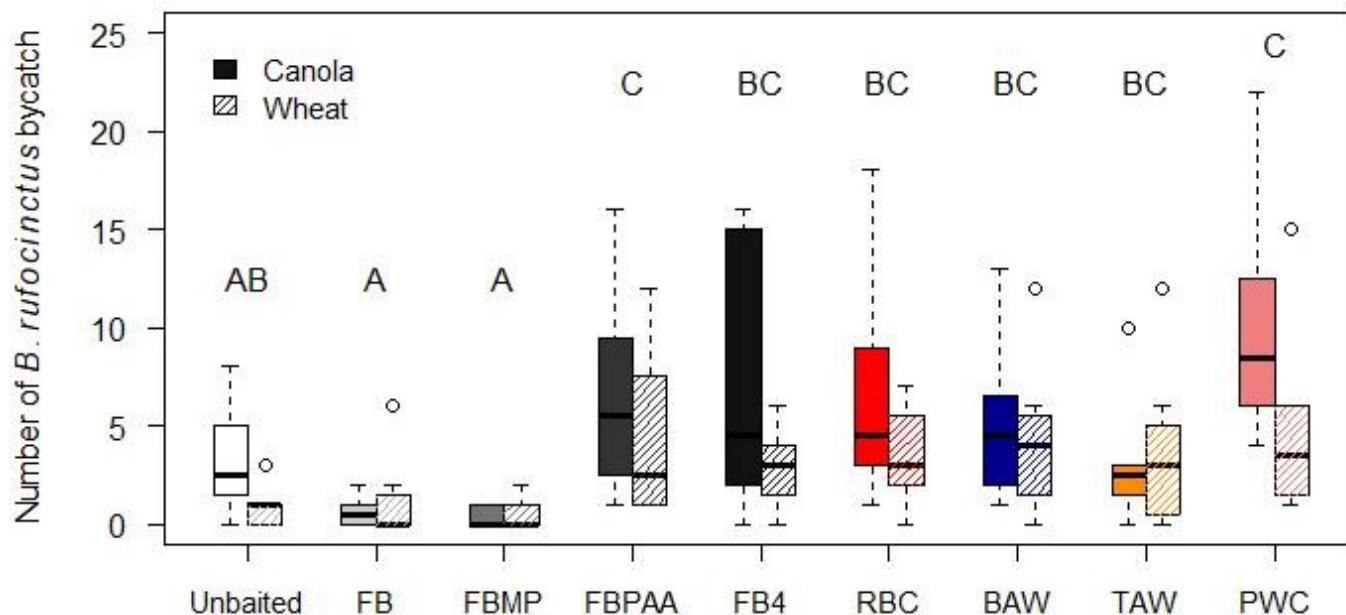


Figure 2-2. Boxplots of the season long capture of *Bombus rufocinctus* in Unitraps baited with feeding attractant and pheromone lures targeting different noctuid moth pest species (FB= acetic acid + 3-methyl-1-butanol; FBMP=FB + 2-methyl-1-propanol; FBPAA=FB + phenylacetaldehyde; FB4=FB + 2-methyl-1-propanol + phenylacetaldehyde; RBC=Redbacked cutworm; BAW=Bertha armyworm; TAW= true armyworm) in canola and wheat crops during the 2015 field season. The midline indicates the median and the top and bottom of the box indicates the first and third quartiles, respectively. Vertical line or whiskers represent the 1.5 interquartile range of the data or the maximum value. Open circles represent points more than 1.5 times the interquartile range. Different letters indicate significant differences (Tukey's HSD: $P < 0.05$).

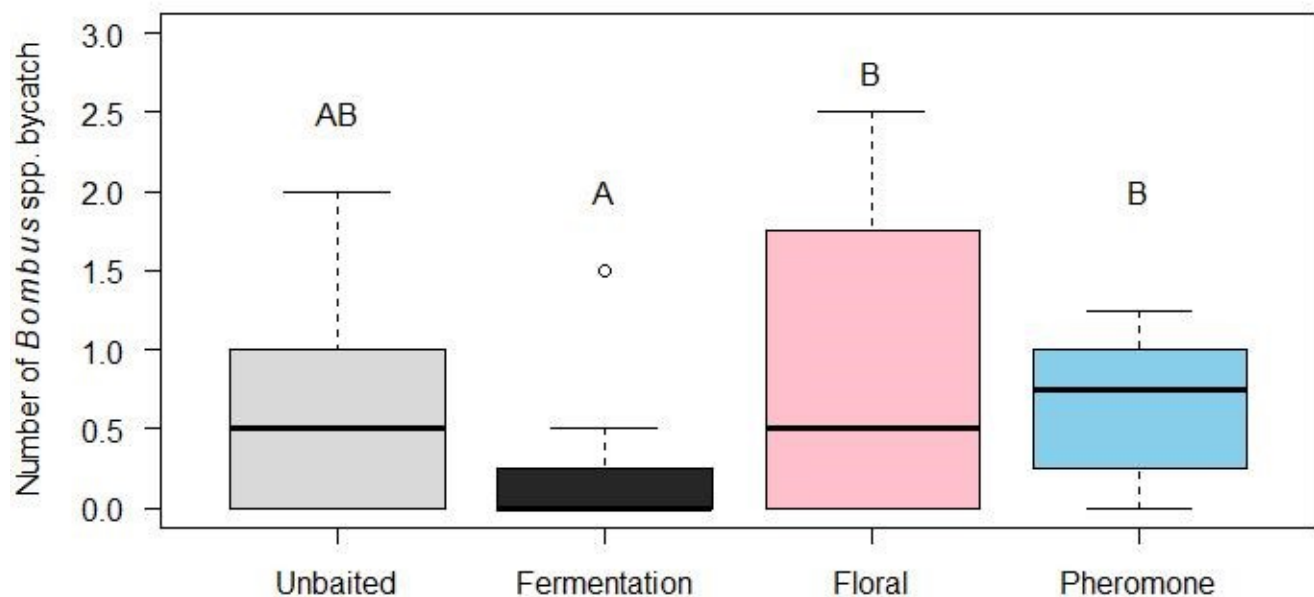


Figure 2-3. Boxplots of the season long capture of bumble bees (*Bombus* spp.), excluding *B. rufocinctus*, in Unitraps baited with feeding attractant and pheromone lures targeting different noctuid moth pest species during the 2015 field season. Bumble bee abundances were grouped according into lure classifications (i.e. Fermentation=FB + FBMP; Floral=FBPAA + FB4; Pheromone=RBC + BAW + TAW + PWC) and were standardized based on the number of traps in each category. The midline indicates the median and the top and bottom of the box indicates the first and third quartiles, respectively. Vertical line or whiskers represent the 1.5 interquartile range of the data or the maximum value. Open circles represent points more than 1.5 times the interquartile range. Different letters indicate significant differences (Tukey's HSD: $P < 0.05$).

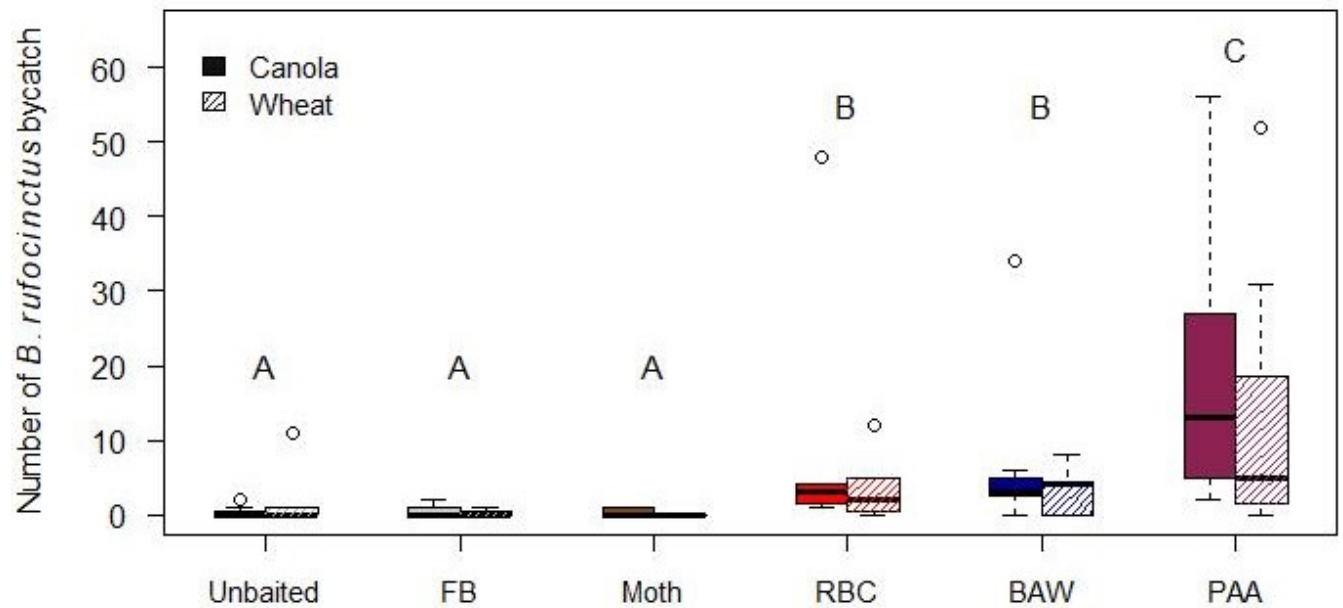


Figure 2-4. Boxplots of the season long capture of *Bombus rufocinctus* in Unitraps baited with the original food bait (FB=acetic acid + 3-methyl-1-butanol), phenylacetaldehyde, dead moth (Moth=10 g previously captured dead moth pests), and pheromone lures targeting different noctuid moth pest species (RBC=Redbacked cutworm; BAW=Bertha armyworm) in canola and wheat crops during the 2016 field season. The midline indicates the median and the top and bottom of the box indicates the first and third quartiles, respectively. Vertical line or whiskers represent the 1.5 interquartile range of the data or the maximum value. Open circles represent points more than 1.5 times the interquartile range. Different letters indicate significant differences (Tukey's HSD: $P < 0.05$).

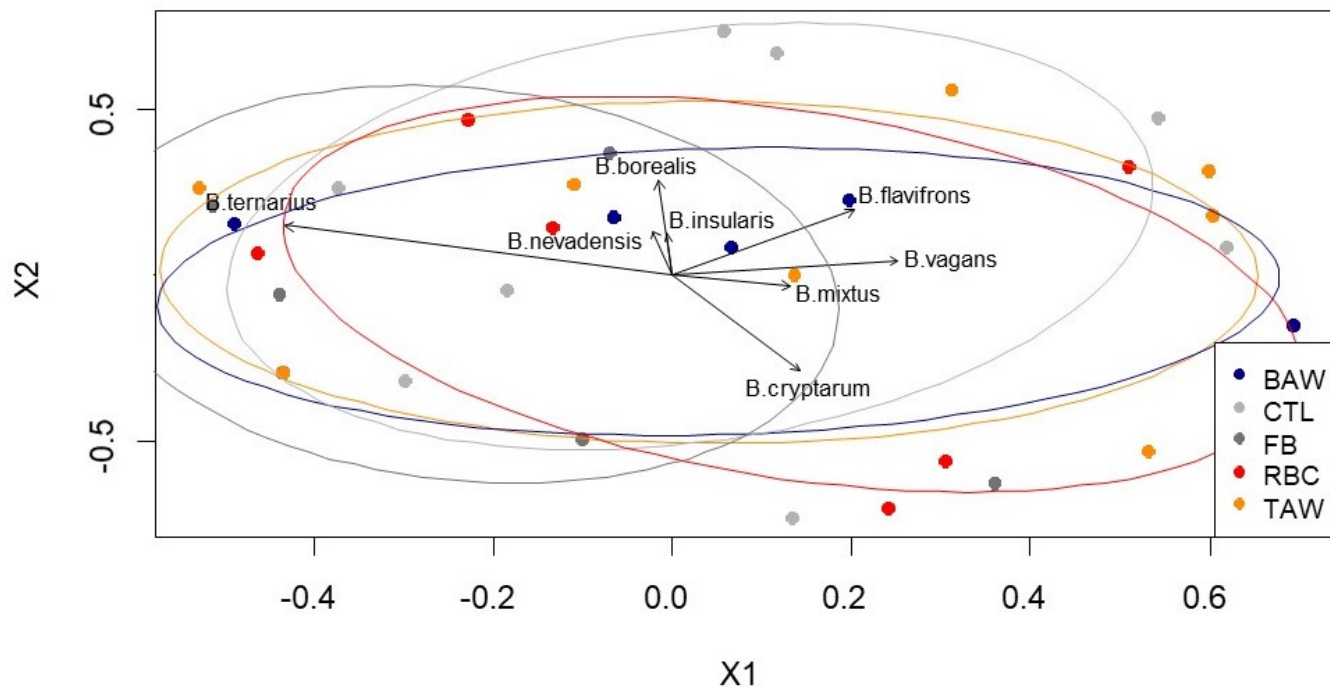


Figure 2-5. Non-metric multidimensional scaling (NMDS) ordination for bumble bee (*Bombus* spp.) species, excluding *B. rufocinctus*, captured as bycatch in non-saturating green-coloured Unitraps during the 2014 field season (Stress=0.224, $R^2=0.721$). Only *Bombus* spp. with ≥ 1 individual captured were included. Differences were assessed across five lure types: food bait (FB=acetic acid + 3-methyl-1-butanol), redbacked cutworm (RBC), bertha armyworm (BAW), true armyworm (TAW), and an unbaited control (CTL). Similar patterns emerge from data collected in 2015 and 2016.

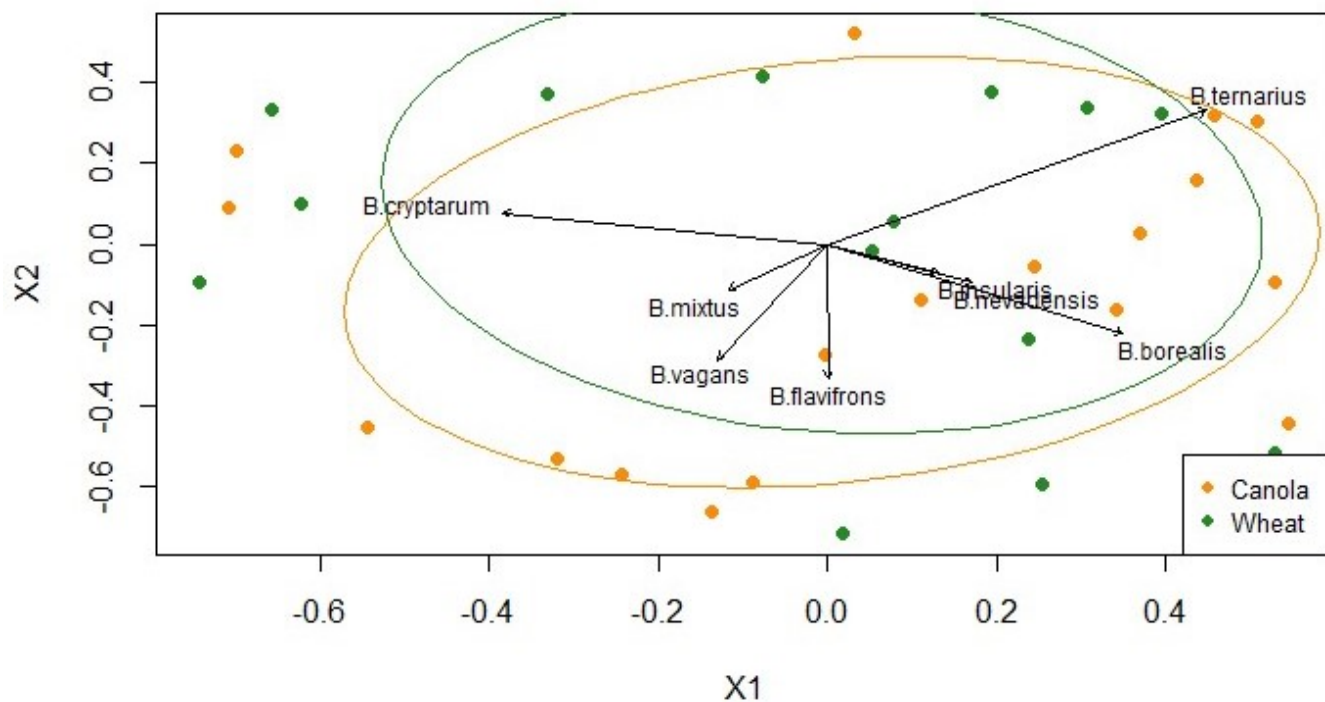


Figure 2-6. Non-metric multidimensional scaling (NMDS) ordination for bumble bee (*Bombus* spp.) species, excluding *B. rufocinctus*, captured as bycatch in non-saturating green-coloured Unitraps during the 2014 field season (Stress=0.224, $R^2=0.721$). Only *Bombus* spp. with ≥ 1 individual captured were included. Differences were assessed across two crop types: canola and wheat. Similar patterns emerge from data collected in 2015 and 2016.

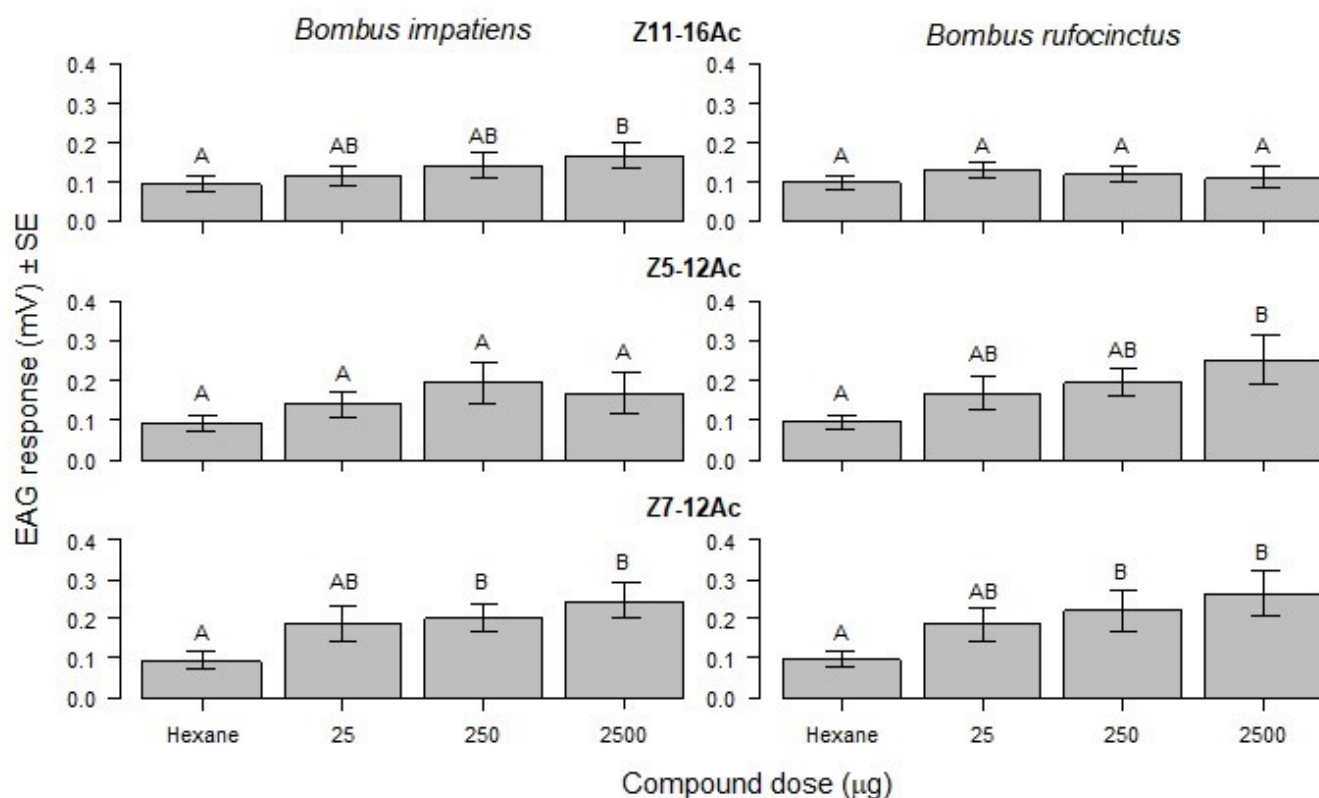


Figure 2-7. Mean ($\pm$ SE) electroantennogram (EAG) response (mV) generated from excised antennae of *Bombus impatiens* (n=10) and *B. rufocinctus* (n=10) workers stimulated with a hexane control and various doses of moth pest pheromone components: Z11-16Ac, Z5-12Ac, and Z7-12Ac. Different letters indicate significant differences after means comparison (Tukey's HSD: $P < 0.05$).

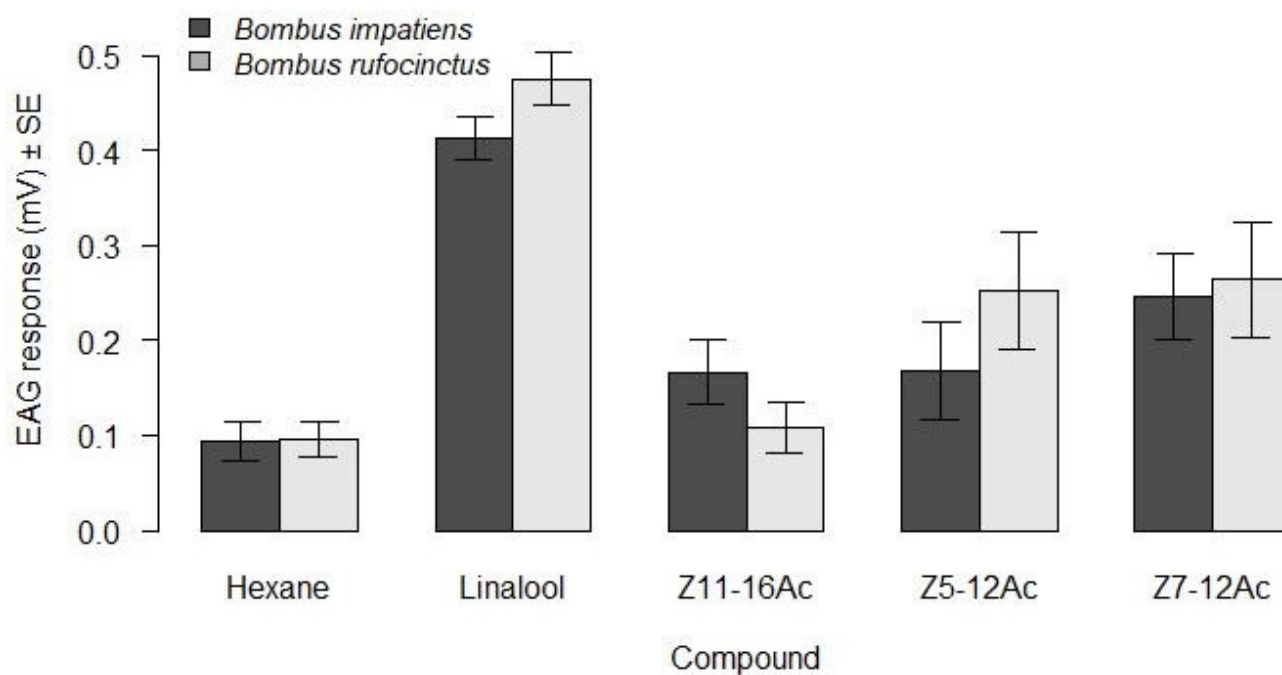


Figure 2-8. Mean ($\pm$ SE) electroantennogram (EAG) response (mV) generated from excised antennae of *Bombus impatiens* (n=10) and *B. rufocinctus* (n=10) workers stimulated with hexane, 25 μ L linalool (10 μ g/ μ L), or moth pest pheromone components (100 μ g/ μ L): Z11-16Ac, Z5-12Ac, and Z7-12Ac.

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Chapter 3: Local and landscape-scale features influence bumble bee (Hymenoptera: Apidae) bycatch in bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), pheromone-baited monitoring traps in Alberta, Canada

Abstract

The bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a significant pest of canola (*Brassica napus* L.) in western Canada. Its activity is monitored through a large network of pheromone-baited monitoring traps as a part of the Prairie Pest Monitoring Network (PPMN) across the Canadian Prairies. The unintentional bycatch of hymenopteran pollinators in pheromone-baited traps targeting moth pests occurs in many agroecosystems. Bumble bees (Hymenoptera: Apidae) are particularly vulnerable to capture in monitoring traps, which may have repercussions for biodiversity and pollination services of wild plants and managed crops. We conducted field experiments to determine the abundance and diversity of bee pollinators attracted to green-coloured BAW pheromone-baited traps across the canola growing regions of Alberta, Canada. A higher species diversity and more bumble bees were captured in BAW pheromone-baited than in unbaited control traps. *Bombus rufocinctus* Cresson was the most commonly captured species. Few other wild bees or honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae), were captured during this study. Additionally, we evaluated the influence of local and landscape-level habitat features on bee bycatch. This study provides a unique look at how habitat can influence pollinator bycatch in monitoring traps. Local flowering plant abundance was influential for the overall model fit but did not have a direct impact on bee bycatch. The proportion of natural and semi-natural habitat, and especially forested area, in the area around monitoring traps affected bee bycatch. Both local and landscape-scale factors were important in this study, and often have combined effects on bee communities. This study provides recommendations to reduce the bycatch of beneficial insect pollinators in a large-scale pheromone-baited monitoring network.

Introduction

The bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a generalist herbivore that is native to North America (Mason et al. 2001; Evenden et al. 2017). BAW goes through cyclical changes in population density and can be a serious pest in western Canada (Mason et al. 1998; Erlandson 2013; Evenden et al. 2017). With the development and adoption of canola, *Brassica napus* L. (Brassicaceae), cultivation across the Canadian Prairies (Shahidi 1990), BAW has become a significant pest of canola (Mason et al. 1998; Evenden et al. 2017). Traps baited with synthetic pheromone lures (Chisholm et al. 1975; Struble et al. 1975) are used to monitor BAW populations from year to year (Underhill et al. 1977). The Prairie Pest Monitoring Network (PPMN) has operated a coordinated monitoring program for BAW, throughout the canola growing region of the Prairies since 1995 (Olfert et al. 1996). A network of pheromone-baited traps is erected each year and counts of male moths are measured to generate risk assessment maps and to indicate the distribution and density of BAW to trigger subsequent larval sampling before control measures are implemented (Mason et al. 1998). Although moth sex pheromone signals are species-specific, pheromone-baited monitoring traps still capture non-target insects, or bycatch, in many agroecosystems (Spears & Ramirez 2015).

Monitoring traps that target lepidopteran pests frequently capture wild bees as bycatch in a variety of different agroecosystems (Meagher & Mitchell 1999; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016). Wild bee pollinators and especially bumble bees (Hymenoptera: Apidae) are frequently captured as bycatch in traps baited with moth pheromones, which consist of unsaturated carbon-12-18 acetates, aldehydes, and alcohols (Gross & Carpenter 1991; Meagher & Mitchell 1999; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016). In addition, members of the PPMN have documented large numbers of bumble bees captured in BAW pheromone-baited traps across the Prairies. There is a high diversity of bees in the Prairie Ecozone of Canada (Sheffield et al. 2014). Many bee species occur only in the Prairie habitat and are not found in other regions of the country (Sheffield et al. 2014). Although the impact of bee removal from agroecosystems in monitoring traps targeting moth pests is unknown (Meagher & Mitchell 1999), it may negatively influence local bee abundance and species diversity, which could alter pollination services for both wild plants and

managed crops (Goulson 2003; Potts et al. 2010) and potentially reduce seed set and yield of canola (Morandin & Winston 2006). As the global decline of wild bee populations is now well documented (Winfree et al. 2009; Potts et al. 2010; Goulson et al. 2015), monitoring practices that remove bees from agricultural landscapes should be altered to minimize the impact on beneficial insects.

Landcover, land-use intensity, and resource availability can impact pollinator communities (Senapathi et al. 2017). Both natural and semi-natural habitat areas are important resources to support diverse bee communities and habitat loss from agricultural conversion is commonly linked with the decline of wild bees (Potts et al. 2010; Goulson et al. 2015; Senapathi et al. 2017). This is likely because semi-natural and natural habitats provide nesting sites and stable floral resources for many bees in agriculturally dominated landscapes (Bailey et al. 2014; Bennett & Isaacs 2014; Hopfenmüller et al. 2014; Mallinger et al. 2016; Kammerer et al. 2016a). Flower abundance at both local and landscape scales can also influence bee diversity and abundance (Potts et al. 2009; Scheper et al. 2015). While the effect of landscape features on pollinator communities has been extensively investigated (Senapathi et al. 2017), to our knowledge the influence of landscape factors on the capture of wild bee bycatch in pheromone-baited traps has yet to be investigated. There is a need for this evaluation as many traps are positioned across the Prairie Provinces each year and have the potential to negatively influence these beneficial populations at a large scale.

Here, we assess the abundance and diversity of *Bombus* spp. and other wild bees captured in green non-saturating Unitraps baited with synthetic BAW pheromone lures positioned in canola fields across the growing regions of Alberta, Canada. Additionally, we provide the first evaluation of the influence of landscape features on the capture of bee pollinators in BAW pheromone-baited traps. This study produces recommendations to alter moth monitoring systems to minimize capture of beneficial non-target pollinators.

Materials and methods

Bycatch sampling methods

Field experiments were performed in 2016 and 2017 to determine the prevalence and species richness of bee bycatch in BAW pheromone-baited traps across much of Alberta's canola growing regions (Figure 3-1). Sites (n=20 in 2016; n=43 in 2017) were distributed throughout 24 counties across Alberta (Table B1). All experimental sites were separated by at least 10 km. At each site, three non-saturating green bucket traps (Unitraps; Contech Enterprises Inc., Delta BC) were positioned on rebar stands 1.5 m above ground and 50 m apart in a transect along the headland of a canola field following pre-established PPMN protocol (Otani 2013). Two of the three traps were baited with commercially available BAW pheromone lures (Contech Enterprises Inc., Delta BC) consisting of pre-extracted red rubber septa loaded with synthetic BAW pheromone (95:5 ratio of Z11-hexadecenyl acetate [Z11-16Ac] and Z9-tetradecenyl acetate [Z9-14Ac]; Underhill et al. 1977). Septa were transported to the field in a refrigerated container and positioned individually inside a basket at the top of each baited trap. A third unbaited trap acted as a control. An insecticidal strip of Hercon Vaportape II (10% dichlorvos; Hercon Environmental, Emigsville, PA) was placed inside the bucket of each trap to kill captured insects. As high bumble bee bycatch occurs in BAW traps in the Peace region of northwestern Alberta, only one baited trap per site was erected in this region to reduce the impact on bumble bee populations. Traps were positioned at canola fields in early June and were monitored for 7 weeks at each site.

Trap contents were collected weekly and were stored at -20 °C until sorting and identification of specimens could take place. All individual bees were pinned and dried for identification. Bumble bees and honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) were identified to species using identification guides (Packer et al. 2007; Williams et al. 2014). All other wild bees were identified to family (Packer & Ratti n.d.; Packer et al. 2007). Identifications were verified using comparisons with our own reference collections and specimens housed in the E. H. Strickland Entomological Museum, University of Alberta, Edmonton, Canada.

Environmental assessment

We assessed local site and landscape-scale landcover variables to determine how environmental factors influence bumble bee bycatch in BAW pheromone-baited traps. Local site variables were assessed using a transect sampling approach that started in late July of each study year when canola crops were in full flower. The sampling transect was oriented along the field edge and overlapped the trapping line, extending 25 m on each side (150 m in total). In 2016, the percentage of the headland covered by flowering plants (flower coverage) was estimated along the transect and categorized as low (0-10%; n=10) or high (>10%; n=9). Field margin width was measured from the crop edge to the road at three locations along the sampling transect. In 2017, we altered the methods for assessing floral resources to increase the accuracy of estimation. We established a 100 m sampling transect along the trapping line and used a 1 m² quadrat to estimate flower coverage every 10 m (Figure B1). We also collected flowering plant specimens at each site, which were identified to the lowest possible ‘morphospecies’ using identification guides (Royer & Dickinson 2007; Wheatland County 2017). Both species diversity and abundance estimates can be important for evaluation of the floral resources available for pollinators (Szigeti et al. 2016). We selected flower coverage for future analyses as both metrics provided similar information.

To assess landscape-scale landcover variables, we used the ‘Wall-to-wall Land Cover Map 2010’ (version 1.0) landcover data obtained from Alberta Biodiversity Monitoring Institute (ABMI). The data are a polygon-based representation of Alberta’s land cover generated from Landsat satellite imagery (30m spatial resolution) and classified into 11 landcover classes (minimum size of 0.5 ha for water and 2 ha for other classes) (Castilla et al. 2014). We reclassified the landcover types into biologically relevant categories including: agricultural, forested (coniferous, broadleaf, mixed forest, and shrubland combined), grassland, human developed, and water (Figure 3-1; Table B2). We used ArcGIS (version 10.3.1) to calculate the proportion of each landcover type inside a circular buffer with a radius of 5 km around each site (Figure B2), which encompasses the foraging distance of many bumble bee species (Rao & Strange 2012).

Principal component analysis

All statistical analyses were conducted using the statistical software R in ‘RStudio v1.1.447’ (R v3.5.0; R Core Team 2018). We tested for collinearity between landscape variables using the ‘rcorr’ function (package ‘Hmisc’; Harrell & Dupont 2008) to generate a Pearson’s correlation matrix. We considered a Pearson’s correlation coefficient of $|r| \geq 0.60$ as the threshold indicating collinearity that could influence model outcomes. The proportion of agricultural and forested habitat were significantly correlated in both field seasons (2016: $r = -0.68$, $df = 20$, $p = 0.001$; 2017: $r = -0.72$, $df = 43$, $p < 0.0001$) and were separated for all analyses. As the proportion of land covered by water was typically less than 5%, water cover was not included in further analyses. We used principal component analysis (PCA) with the ‘prcomp’ function to determine which covariates explained the majority of the variance in the data. In 2016, two principal components explained the majority of the variance in the data for the PCA including agricultural (94.6%) and forested areas (94.9%) (Table 3-1). Adding the third component increased the proportion of explained variance to 99.5% for both analyses (Table 3-1). In 2017, the first two components explained 95.7% and 94.6% of the variance for the PCA that included agricultural and forested areas, respectively (Table 3-1). This was increased to 98.2% and 98.0% with the addition of the third component (Table 3-1). Eigenvector coefficients with a value of ± 0.70 were considered important for explaining the principal components. For both years of the study, the first component was loaded strongly by flower coverage at the site, the second by agricultural or forested area in each of the respective analyses, and the third by grassland area (Table 3-1). These factors were included in base statistical models prior to model simplification.

Statistical analysis

Models were conducted independently for each field season. All models included an offset (‘offset’ function; ‘R Stats’ package; R Core Team 2018) to account for the different number of pheromone-baited traps at sites in different regions. An offset can be used to account for a known source of variation in a dataset (Kéry 2010), in this case the different number of baited traps at sites in the Peace region compared to the rest of the province. We used generalized linear models with the negative binomial distribution (‘glmer.nb’ function; package ‘lme4’; Bates et al. 2015) to assess if bumble bees were attracted to BAW pheromone-baited traps. Models initially included all possible interactions, which were removed if they were not

statistically significant ($\alpha = 0.05$). The response variable was bumble bee catch per trap (summed for the entire field season), the predictor variable was lure type, and site was used as a random term. In the 2016 field season, bumble bee bycatch was compared among different regions across Alberta using the 'glm.nb' function (package 'lme4'). Sites were categorized into three regions according to spatial position: north (n=7), central (n=7), and south (n=6) (Figure 3-1). The response variable was bumble bee catch per trap and the predictor variable was region. Date was not included as a predictor variable due to low capture rates, which prevented models from running when bycatch was not pooled across the season. We also assessed differences in the proportion of forest cover between the regions in both years of the study using models constructed with the 'lm' function followed by 'anova'. We performed Wald chi-square analysis using the 'Anova' function (package 'car'; Fox 2012) to test for significance for all models. If there were significant differences among treatments, post hoc means separation was performed using the Tukey method ($\alpha=0.05$; package 'lsmeans'; Lenth & Hervé 2015).

We created generalized linear models with the 'glm.nb' function to assess the influence of landscape variables on the capture of *Bombus* spp. in BAW pheromone-baited traps. Model simplification was performed by removing the least influential variable until the best model was determined according to Akaike Information Criterion corrected for small sample sizes (AICc; package 'AICcmodavg'; Mazerolle 2016). Models with $\Delta\text{AICc} \leq 2$ were considered equally competitive models. Wald chi-square tests were used to determine the significance of explanatory variables in the best models. Significant relationships ($\alpha=0.05$) were visualized using the 'effect_plot' function (package 'jtools'; Long 2018), which generated the line of best fit and confidence bands.

Results

Bycatch sampling

Bees captured in 2016 included a total of 182 female and 3 male bumble bees from 9 species, but males were not identified to species (Table 3-2). *Bombus rufocinctus* Cresson was the most abundant species captured and represented ~90.1% of the bumble bee bycatch, excluding males. We also captured a total of 92 other bees from 3 families: Andrenidae, Apidae,

and Megachilidae (Table 3-3). There were significantly more bumble bees captured per trap in BAW pheromone-baited Unitraps than in unbaited control traps (Wald $\chi^2=12.843$, $df=1$, $p=0.0003$; Figure 3-2). *Bombus* spp. capture in BAW traps was also influenced by region (Wald $\chi^2=11.812$, $df=2$, $p=0.0027$). Traps at sites in the northern region captured significantly more bumble bees per trap than traps in the central and southern regions (Figure 3-3).

A total of 206 female bumble bees from 12 species as well as 5 males that were not identified to species were captured in 2017 (Table 3-2). *Bombus rufocinctus* was the most abundant species captured and represented ~71.6% of the bumble bee bycatch, excluding males. We also captured a total of 175 other bees from 5 families: Andrenidae, Apidae, Colletidae, Halictidae and Megachilidae (Table 3-3). As in the previous field season, significantly more bumble bees were captured in BAW pheromone-baited traps than in unbaited control traps (Wald $\chi^2=15.719$, $df=1$, $p<0.0001$; Figure 3-2).

Environmental variables

The proportion of landcover ranged from near 0% for less common classes up to complete domination of the landscape, in the case of agricultural landcover. Agriculture and forested habitat were the dominant landcover types surrounding our study sites with a mean proportion of ~70% and ~10%, respectively in both years of the study (Table 3-4). Water, grassland, and human developed areas covered less than ~10% of the landscape on average (Table 3-4). For the 2016 field season, the average proportion of agricultural land cover was lowest in the north (65.5 ± 5.2), intermediate in the central region (71.6 ± 4.1), and highest in the south (79.4 ± 3.2). The average proportion of forest cover showed the opposite trend and was highest in the north (18 ± 4.8), intermediate in the central region (12.4 ± 4.2), and extremely low in the south (0.76 ± 0.38). In the 2017 field season, there was a lower proportion of agriculture and higher forest cover in the north (63.8 ± 7.2 and 18 ± 7.7 , respectively) compared to the central region (73.3 ± 1.6 and 9.6 ± 1.1 , respectively). The northern region had significantly more forest cover than the rest of the province, however the difference was only marginally significant in the second year of this study (2016: $F_{1, 18}=4.55$, $p=0.047$; 2017: $F_{1, 41}=4.04$, $p=0.051$).

The two best models in the 2016 field season had similar AICc values and included flower coverage, and agricultural or forested area (Table 3-5). Both agricultural and forested area

were significant predictors of bumble bee abundance in the respective models (Ag. 6-1: Wald $\chi^2=4.707$, $df=1$, $p=0.030$; For. 6-1: Wald $\chi^2=5.114$, $df=1$, $p=0.024$). Bumble bee bycatch was significantly lower at sites with a higher proportion of agricultural area (Figure 3-4). Whereas, for sites with a high proportion of forested area more bumble bee bycatch was detected (Figure 3-5). Flower coverage at each site did not significantly impact the number of bees captured in pheromone-baited traps in the various models (Ag. 6-1: Wald $\chi^2=0.793$, $df=1$, $p=0.373$; For. 6-1: Wald $\chi^2=0.067$, $df=1$, $p=0.795$). Models that included flower coverage, however, better fit the data as the AICc value increased substantially when this variable was removed (Table 3-5: Ag. 7-1, For. 7-1).

For the 2017 field season, model simplification resulted in two competing models with flower coverage and grassland area as predictor variables (Table 3-6). As in the previous year, flower coverage was not a significant predictor of bumble bee bycatch (AF. 2-2: Wald $\chi^2=2.038$, $df=1$, $p=0.154$; AF. 1-2: Wald $\chi^2=1.986$, $df=1$, $p=0.159$). Similarly, the proportion of grassland area did not significantly influence bumble bee bycatch (AF. 1-2: Wald $\chi^2=1.796$, $df=1$, $p=0.180$). Interestingly, agricultural and forested area did not affect bumble bee bycatch in 2017 (Ag. 5-2: Wald $\chi^2=0.271$, $df=1$, $p=0.603$; For. 5-2: Wald $\chi^2=0.082$, $df=1$, $p=0.774$). There was, however, a trend for the proportion of agricultural area around a site to negatively affect bumble bee bycatch. The negative effect of agricultural landcover surrounding the site was similar to the previous year, whereas the proportion of forested area showed the opposite trend.

Discussion

Traps baited with BAW pheromone lures attracted and captured bumble bees at sites across Alberta. The capture of wild bee pollinators and especially bumble bees is common in monitoring traps baited with lepidopteran pheromones in many agroecosystems (Gross & Carpenter 1991; Meagher & Mitchell 1999; Mori & Evenden 2013; Spears et al. 2016). *Bombus rufocinctus* was the most commonly captured species in this study. Other studies in Alberta found a similar diversity of *Bombus* spp. bycatch using pan traps and active netting (Kohler 2017; Sturm 2017). *Bombus rufocinctus*, *B. ternarius* Say, and *B. borealis* Kirby were the most frequently captured bumble bee species in studies using these sampling methods and in

pheromone-baited traps in this study (Kohler 2017; Sturm 2017). The proportion of captures of different bumble bee species in pheromone-baited traps was similar to that recovered with the less biased sampling techniques (Westphal et al. 2008; Kohler 2017; Sturm 2017), however a higher proportion of *B. rufocinctus* was captured in this study. As green non-saturating Unitraps are not visually attractive for bumble bees (Gross & Carpenter 1991; Mori & Evenden 2013; Spears et al. 2016) and fewer bees were captured in unbaited control traps, the orientation of *B. rufocinctus* to pheromone-baited traps must be due to the recognition and response to BAW pheromone components. *Bombus rufocinctus* is attracted to the pheromone signals of multiple noctuid moths, including BAW, in wheat and canola agroecosystems (Chapter 2). Although *B. rufocinctus* responded electrophysiologically to several noctuid pheromone components, it did not display a significant response to Z11-16Ac, the main component of the BAW pheromone lure (Chapter 2). This suggests that *B. rufocinctus* is responding to the minor component of the BAW lure (Z9-14Ac) in the field.

Flower coverage was an important variable that influenced model fit of the data in both years of this study. There were more bumble bees captured at sites that had more than 10% flower coverage along the header strip of the canola field in the first year of the study. Flower abundance along the canola fields did not influence bumble bee bycatch in the second year of the study. In other studies, more floral resources in the vicinity of agricultural land relates to more bumble bee species and more individuals (Bäckman & Tiainen 2002; Pywell et al. 2006; Potts et al. 2009; Wood et al. 2015). Many agri-environment schemes provide pollinators with additional floral resources (e.g. wildflower strips, rotational flowering crops, sowing flowers in field margins, etc.), to attract pollinators to forage in or near the crop, but it is unclear if these resources promote pollinator population growth (Potts et al. 2009; Scheper et al. 2015; Wood et al. 2015). The presence of mass flowering crops such as canola can also provide plentiful floral resources for bumble bees and other wild bees (Westphal et al. 2003; Westphal et al. 2009). Canola was flowering during the majority of the monitoring period in this study, which likely reduced the importance of local wild flower abundance for foraging bees. Bumble bees are capable of long-distance foraging trips to access quality food resources in agroecosystems (Greenleaf et al. 2007; Stephen & Rao 2012). They are also typically more responsive to landscape scale than local habitat features (Kennedy et al. 2013; Hopfenmüller et al. 2014;

Steckel et al. 2014), which further explains the lack of response to local wildflower abundance in this study.

Bumble bee abundance was low at sites with a high proportion of agricultural area surrounding monitoring traps in the first year of this study. The loss of natural and semi-natural habitat as a result of increasing agricultural intensity is one of most commonly cited reasons for the decline of wild bees (Potts et al. 2010; Goulson et al. 2015; Senapathi et al. 2017). This trend was also evident in the second year of this study; however, it was not statistically significant. Variability in precipitation between years can influence flowering plant abundance and diversity on the landscape, which consequently impacts some bee communities (Minckley et al. 2013; Thomson 2016). The second year of our study had lower than average precipitation across much of the province (Figure B3), which likely corresponds with a reduction in wildflower availability and increased pollinator reliance on mass flowering canola crops. More reliance on canola during dry years may explain the weakened negative response between bee bycatch and agricultural area in the second year of the study. Alternatively, less food resources at a landscape scale could explain the lower abundance of *Bombus* spp. captured in the second year of this study.

The amount of natural and semi-natural habitat on the landscape influences pollinator abundance and species diversity (Kennedy et al. 2013; Steckel et al. 2014; Senapathi et al. 2017). Bumble bee bycatch was higher at sites with a greater proportion of forested area surrounding monitoring traps in the first but not the second year of this study. Forest and forest edge habitat provide quality nesting sites and stable floral resources for many bees in agriculturally dominated landscapes (Bailey et al. 2014; Bennett & Isaacs 2014; Kammerer et al. 2016a). Additionally, pollinator abundance and diversity in agroecosystems decreases with distance from forest habitat (Bailey et al. 2014; Kammerer et al. 2016b). Interestingly, the most abundant species captured in our study, *B. rufocinctus*, prefer nesting in wooded areas in Alberta (Hobbs 1965). The preference for forested habitat by *B. rufocinctus* is likely driving the higher bycatch numbers in the northern part of the province. The Peace region in northwestern Alberta has more forested area and a high abundance of bumble bee bycatch compared to the rest of the province. This region was sampled more intensively in the first year of this study, which could be driving the significant effect of forest in that year.

Grasslands are another important semi-natural habitat that can provide long-term floral resources and nesting sites for diverse bee communities (Bennett & Isaacs 2014; Hopfenmüller et al. 2014; Mallinger et al. 2016). There was a minor trend for greater numbers of bumble bees captured as the proportion of grassland habitat in the landscape surrounding trapping sites increased. This trend, however, was not significant in either year of the study. In another study conducted in Alberta, grassland cover influenced overall wild bee abundance and species diversity, but had no influence on bumble bees (Kohler 2017). Although as little as 2% semi-natural habitat on agricultural landscapes in Germany can provide sufficient nesting and floral resources for bumble bees, more quality habitat should enhance pollinator abundance and diversity (Westphal et al. 2003). The response of bee taxa to landscape features is influenced by both foraging distance (Rao & Strange 2012; Jha & Kremen 2013) and the degree of diet specialization of the bees (Steffan-Dewenter et al. 2002; Steffan-Dewenter 2003). Bumble bees are generalist pollinators that can use floral and nesting resources in a wide range of habitats at a large scale (Rollin et al. 2013), however preferential habitat use still occurs. For example, in an intensively farmed area of western France, bumble bees are most commonly encountered in mass flowering oilseed rape crops and wooded habitat, both of which represent less of the landscape than grassland or cereal crops (Rollin et al. 2013). As previously discussed, canola was in the flowering stage for much of the trapping period during this study, and this could explain why grassland habitat did not influence bumble bee abundance in this study.

This study demonstrated that bumble bees are attracted to BAW pheromone lures on a wide scale across Alberta. We confirmed that more bumble bee bycatch in pheromone-baited Unitraps occurs in the Peace region of Alberta compared to other areas of the province. Similarly, more *Bombus* spp. and other large-bodied bees were captured by pan traps and netting in the northern Boreal region of Alberta, especially in canola fields (Kohler 2017). This trend may indicate high landscape heterogeneity in this region (Kohler 2017), which is generally linked with the proportion of semi-natural and natural habitat on the landscape (Gustafson 1998). The higher prevalence of bumble bee bycatch in northern Alberta is likely due to an increase in the proportion of forested area compared to the rest of the canola growing regions across Alberta.

Five other families of hymenopteran pollinators (Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae) were captured in relatively low numbers in this study. The number

of wild bee families captured was similar to that collected using pan traps and active netting of bees in Alberta (Kohler 2017; Sturm 2017). The proportion of non-*Bombus* bees captured using these relatively unbiased sampling techniques was much higher compared to findings of the current study. This indicates that although the non-*Bombus* bees were likely present in the agroecosystems where we conducted our study, they were not frequently captured in the monitoring traps. In the second year of the study, more Apidae specimens, excluding bumble bees and honey bees, were captured in pheromone-baited Unitraps (Table 3-3) than in the first year. Previous studies have reported capture of other Apidae in noctuid pheromone-baited traps (Meagher & Mitchell 1999; Spears et al. 2016). For example, large numbers of *Melissodes* spp. (Hymenoptera: Apidae) and *Anthophora* spp. (Hymenoptera: Apidae) were captured in moth pheromone traps positioned in alfalfa and corn fields in Utah (Spears et al. 2016). These species, however, also use visual cues as more bees were captured in pheromone-baited yellow and white Unitraps compared to green traps baited with the same lures (Spears et al. 2016). Green-coloured traps are less attractive than white, yellow, and blue to bee pollinators (Clare et al. 2005; Stephen & Rao 2005; Mori & Evenden 2013; Spears et al. 2016). It is also possible that lower precipitation in the second year of this study resulted in an increased reliance of wild bees on mass flowering crops. Bees that oriented to canola may have resulted in increased encounters with the monitoring traps in the second year of the study. Research in the Prairies should further test the factors influencing non-*Bombus* pollinator bycatch in pheromone-baited traps.

This study demonstrates that BAW pheromone-baited Unitraps attract bumble bees across the canola growing regions of Alberta, Canada. *Bombus rufocinctus* was the most commonly captured species across both years of the study. Capture of this species is driving the significant trap capture in pheromone-baited traps, as relatively low numbers of other *Bombus* spp. and other wild bees were captured. This attraction is not driven by visual cues of moth traps, as more *B. rufocinctus* are captured in pheromone-baited traps than in unbaited control traps. We also confirmed that bee bycatch in BAW pheromone-baited traps is highest in the Peace region of Alberta as compared to other canola growing regions in the province. This finding supports the decision of the PPMN to use only one BAW pheromone monitoring trap per field in the Peace Region in an attempt to minimize the impact on beneficial insect populations.

Environmental variables assessed both locally and at the landscape-scale contributed to bumble bee bycatch in monitoring traps positioned at canola fields. Local floral resources influenced the overall model fit of the data but did not directly impact the bumble bee bycatch. Whereas, the proportion of natural and semi-natural habitat, and especially forested area, surrounding the trapping location was highly important. Variability between years in our findings could also indicate that climactic conditions (e.g. precipitation) influence landscape variables and ultimately drive bee bycatch, however future research in this area is needed. In this and other studies, the influence of local and landscape level habitat characteristics are often linked (Nayak et al. 2015; Scheper et al. 2015; Quistberg et al. 2016). Large bodied bumble bees are more responsive to landscape-scale than local features (Steffan-Dewenter et al. 2002; Kennedy et al. 2013; Hopfenmüller et al. 2014; Steckel et al. 2014) as they can forage over large distances (Greenleaf et al. 2007; Stephen & Rao 2012). The level of bee bycatch in this study may not pose a significant threat to pollinator populations, however any reduction in bycatch would be beneficial as monitoring is essential for control of BAW in the Prairie Provinces. Producers should avoid placing monitoring traps near or within large patches of flowering plants as these local blooms could still increase capture of bee pollinators. This recommendation is especially important if traps are positioned in field margins pre or post canola flowering as wild flowers are necessary for sustaining pollinators throughout the growing season (Westphal et al. 2009). Similarly, monitoring trap placement should be delayed until canola reaches the flowering stage in each region, but especially in the Peace Region. This may reduce the number of *Bombus* spp. and other wild bees captured in pheromone-baited traps as foraging in field margins would be lower when the crop is in bloom. This recommendation may be especially important for the Peace region as it may reduce queen capture in early-stage canola, as compared to the rest of the province. A final recommendation is to position traps in the field margin as far from forest edges as possible, which may minimize the number of pollinators that encounter monitoring traps (Bailey et al. 2014). These suggestions are not likely to impact pest monitoring efficiency, however this should be verified prior to adopting these strategies. The impact of monitoring traps on other beneficial insects such as vespid wasps (Landolt et al. 2007), lady beetles (Spears et al. 2016), and parasitic wasps and flies should also be considered before recommendations are implemented.

Table 3-1. Summary of principal component analysis (PCA) results for environmental variables assessed in 2016 and 2017. The variance explained by each principle component is included. Eigenvector coefficients with a value of ± 0.70 are shown in bold.

PCA	Variable	PC1	PC2	PC3
2016: Agriculture	<i>Variance Explained</i>	51.6%	43.0%	4.9%
	Agriculture	-0.419	0.908	0.002
	Human Developed	-0.045	-0.017	-0.081
	Flower Coverage	0.906	0.418	0.039
	Grassland	-0.039	-0.019	0.996
2016: Forest	<i>Variance Explained</i>	60.9%	34.0%	4.6%
	Forest	0.557	0.822	0.116
	Human Developed	-0.044	-0.018	-0.100
	Flower Coverage	0.826	0.561	0.007
	Grassland	-0.076	0.090	0.988
2017: Agriculture	<i>Variance Explained</i>	70.4%	25.3%	2.5%
	Agriculture	-0.085	-0.981	-0.153
	Human Developed	-0.005	0.086	-0.032
	Flower Coverage	0.996	-0.087	0.010
	Grassland	0.024	0.148	-0.988
2017: Forest	<i>Variance Explained</i>	77.6%	17.0%	3.4%
	Forest	0.067	0.998	-0.019
	Human Developed	-0.007	-0.006	-0.259
	Flower Coverage	0.998	-0.067	0.018
	Grassland	0.019	-0.020	-0.966

Table 3-2. Abundance of bumble bee species captured as bycatch in monitoring traps positioned at canola fields in Alberta, Canada.

Year	Species	Abundance
2016	<i>Bombus rufocinctus</i>	164
	<i>Bombus ternarius</i>	5
	<i>Bombus borealis</i>	4
	<i>Bombus flavifrons</i>	2
	<i>Bombus nevadensis</i>	2
	<i>Bombus perplexus</i>	2
	<i>Bombus centralis</i>	1
	<i>Bombus occidentalis</i>	1
	<i>Bombus terricola</i>	1
	<i>Bombus</i> spp. males	3
2017	<i>Bombus rufocinctus</i>	151
	<i>Bombus ternarius</i>	27
	<i>Bombus vagans</i>	12
	<i>Bombus centralis</i>	3
	<i>Bombus flavifrons</i>	3
	<i>Bombus borealis</i>	2
	<i>Bombus nevadensis</i>	2
	<i>Bombus terricola</i>	2
	<i>Bombus cryptarum</i>	1
	<i>Bombus mixtus</i>	1
	<i>Bombus suckleyi</i>	1
	<i>Bombus sylvicola</i>	1
	<i>Bombus</i> spp. males	5

Table 3-3. Abundance of non-bumble bee hymenopteran pollinator bycatch in monitoring traps positioned in canola fields in Alberta, Canada.

Year	Family	Abundance
2016	Andrenidae	35
	Apidae	23
	<i>Apis mellifera</i>	7
	Megachilidae	27
2017	Andrenidae	9
	Apidae	121
	<i>Apis mellifera</i>	3
	Colletidae	1
	Halictidae	1
	Megachilidae	40

Table 3-4. Summary of environmental variables assessed in 2016 and 2017. Values provided include the range (minimum and maximum values) and mean ($\pm$ SE) proportion of land covered by each type of environmental variable.

Year	Environmental Variable	Minimum Proportion	Maximum Proportion	Mean Proportion ($\pm$ SE)
2016	Agriculture	49.8%	86.7%	71.8% $\pm$ 2.7%
	Human Developed	4.2%	23.5%	7.9% $\pm$ 0.9%
	Forest	0%	37.4%	10.9% $\pm$ 2.7%
	Grassland	0.3%	17.5%	5.7% $\pm$ 0.9%
	Water	0%	35.6%	3.5% $\pm$ 1.7%
2017	Agriculture	50.8%	89.0%	72.5% $\pm$ 1.6%
	Human Developed	5.3%	20.5%	8.6% $\pm$ 0.4%
	Forest	0.2%	38.2%	10.3% $\pm$ 1.3%
	Grassland	0%	13.7%	5.3% $\pm$ 0.6%
	Water	0%	33.3%	3.1% $\pm$ 0.8%
	Flower Coverage	0%	69.9%	14.6% $\pm$ 2.7%

Table 3-5. Summary of generalized linear models testing the influence of environmental variables on *Bombus* spp. bycatch in monitoring traps baited with bertha armyworm pheromone lures in Alberta, Canada for the 2016 field season. All models were constructed with a negative binomial distribution and included an ‘offset’ to account for the different number of traps deployed in different regions of the province. The environmental variables included in each model, Akaike Information Criterion adjusted for small sample sizes (AICc), and the overall change in AICc values (Δ AICc) are presented. Competitive models are shown in bold.

Model ID	Variables	AICc	Δ AICc
For. 6-1	Forest + Flower Coverage	128.18	0
Ag. 6-1	Agriculture + Flower Coverage	128.48	0.30
For. 5-1	Forest + Flower Coverage + Grassland	131.91	3.73
Ag. 5-1	Agriculture + Flower Coverage + Grassland	132.23	4.05
For. 4-1	Forest + Flower Coverage + Grassland + Forest:Grassland	133.32	5.14
For. 7-1	Forest	134.58	6.4
Ag. 4-1	Agriculture + Flower Coverage + Grassland + Agriculture:Flower Coverage	136.42	8.24
Ag. 7-1	Agriculture	136.77	8.59
For. 3-1	Forest + Flower Coverage + Grassland + Forest:Grassland + Flower Coverage:Grassland	137.69	9.51
Ag. 3-1	Agriculture + Flower Coverage + Grassland + Agriculture:Flower Coverage + Flower Coverage:Grassland	141.32	13.14
For. 2-1	Forest + Flower Coverage + Grassland + Forest:Grassland + Flower Coverage:Grassland + Flower Coverage:Forest	143.47	15.29
Ag. 2-1	Agriculture + Flower Coverage + Grassland + Agriculture:Flower Coverage + Flower Coverage:Grassland + Agri:Grassland	147.53	19.35
For. 1-1	Forest * Flower Coverage * Grassland	150.08	21.9
Ag. 1-1	Agriculture * Flower Coverage * Grassland	155.09	26.91

Table 3-6. Summary of generalized linear models testing the influence of environmental variables on *Bombus* spp. bycatch in monitoring traps baited with bertha armyworm pheromone lures in Alberta, Canada for the 2017 field season. All models were constructed with a negative binomial distribution and included an ‘offset’ to account for the different number of traps deployed in different regions of the province. The environmental variables included in each model, Akaike Information Criterion adjusted for small sample sizes (AICc), and the overall change in AICc values (Δ AICc) are presented. Competitive models are shown in bold.

Model ID	Variables	AICc	Δ AICc
AF. 2-2	Flower Coverage	234.55	0
AF. 1-2	Flower Coverage + Grassland	235.25	0.7
Ag. 5-2	Agriculture + Flower Coverage + Grassland	237.54	2.99
For. 5-2	Forest + Flower Coverage + Grassland	237.73	3.18
Ag. 4-2	Agriculture + Flower Coverage + Grassland + Flower Coverage:Grassland	238.40	3.85
For. 4-2	Forest + Flower Coverage + Grassland + Flower Coverage:Grassland	239.08	4.53
Ag. 3-2	Agriculture + Flower Coverage + Grassland + Flower Coverage:Grassland + Agriculture:Grassland	240.58	6.03
For. 3-2	Forest + Flower Coverage + Grassland + Flower Coverage:Grassland + Flower Coverage:Forest	241.01	6.46
Ag. 2-2	Agriculture + Flower Coverage + Grassland + Flower Coverage:Grassland + Agriculture:Grassland + Flower Coverage:Agriculture	243.46	8.91
For. 2-2	Forest + Flower Coverage + Grassland + Flower Coverage:Grassland + Flower Coverage:Forest + Forest:Grassland	243.98	9.43
For. 1-2	Forest * Flower Coverage * Grassland	245.97	11.42
Ag. 1-2	Agriculture * Flower Coverage * Grassland	246.53	11.98

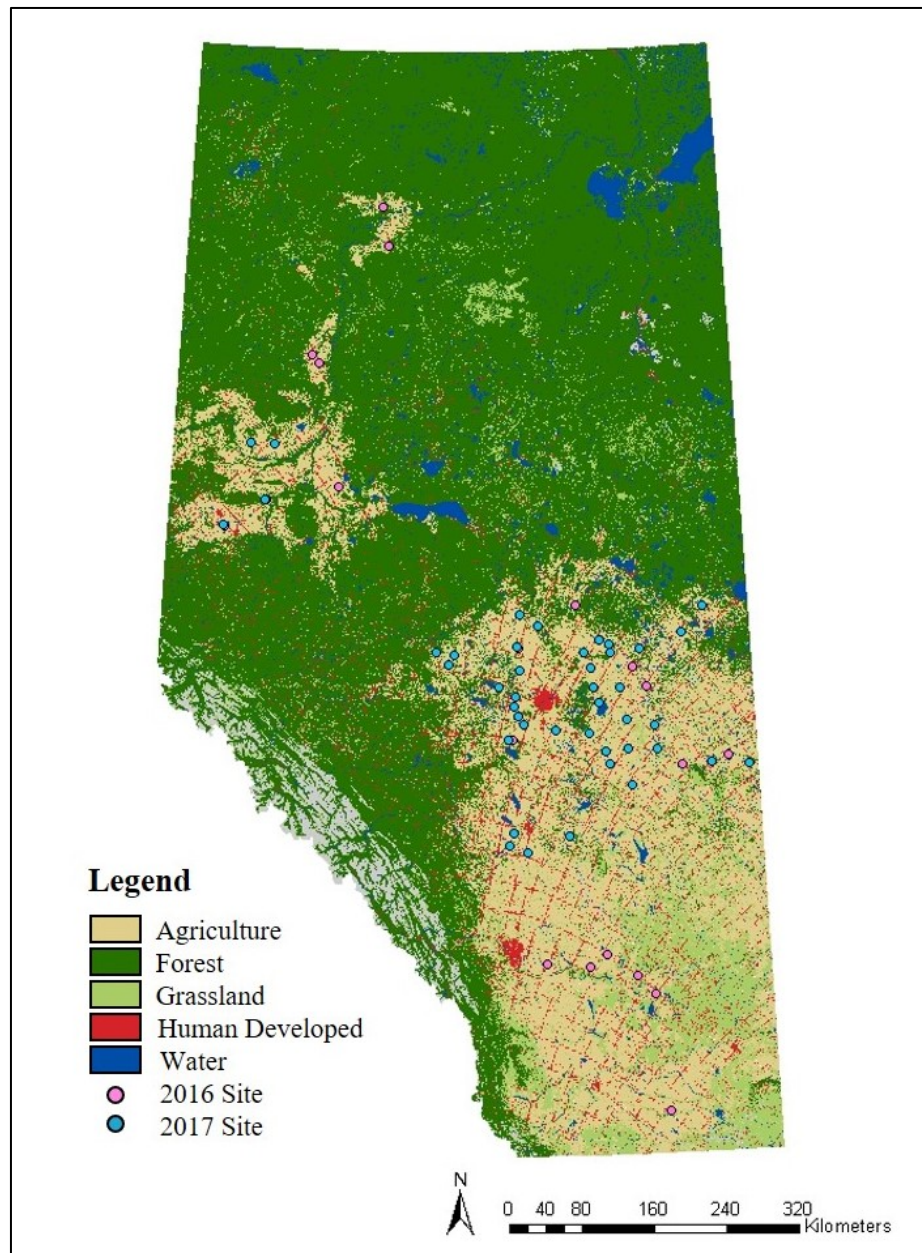


Figure 3-1. Locations of study sites across the canola growing regions of Alberta, Canada. This map was generated using ArcGIS (version 10.3.1) to reclassify Alberta Biodiversity Monitoring Institute ‘Wall-to-wall Land Cover Map 2010’ (version 1.0) data into biologically relevant classes for this study. Bertha armyworm pheromone-baited and unbaited control traps were positioned at each site for 7 weeks starting in early June of 2016 and 2017. Sites were surveyed once per year in late July, while canola was in bloom, to sample local environmental variables.

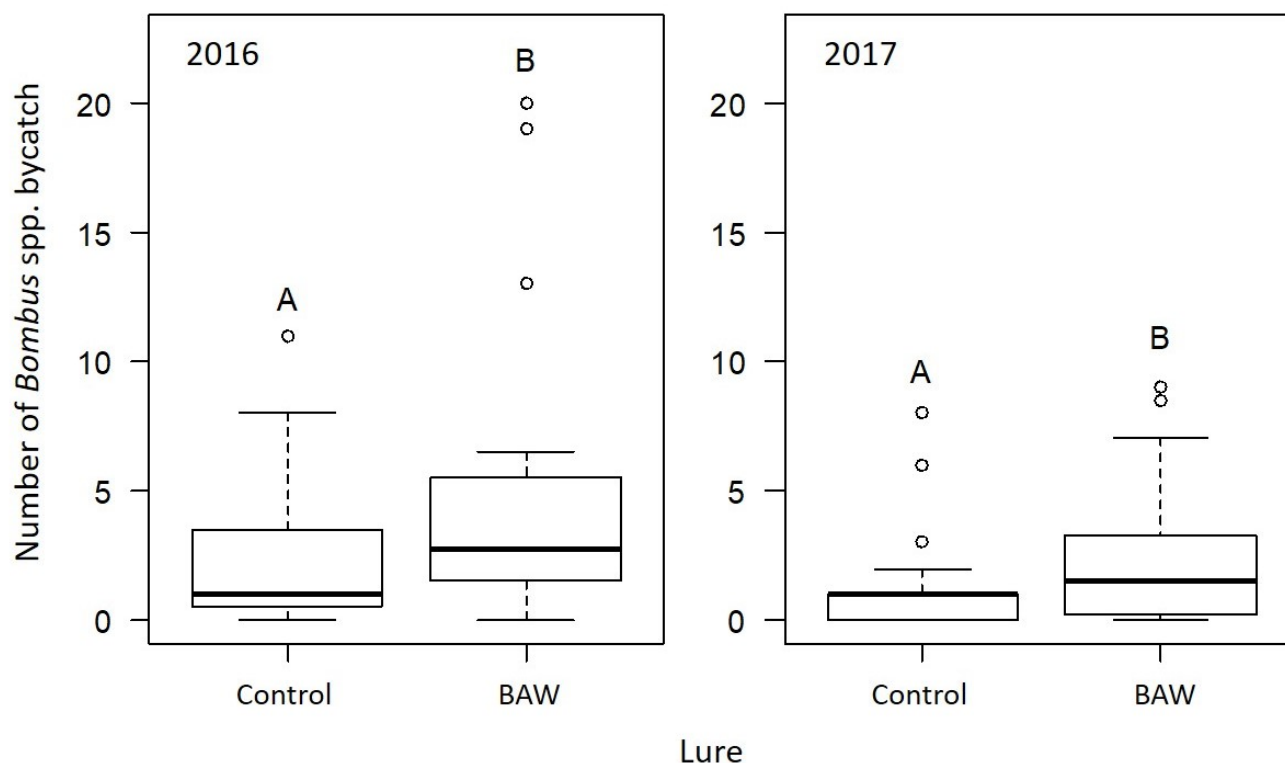


Figure 3-2. Boxplots of the season long capture of *Bombus* spp. in Unitraps baited with bertha armyworm (BAW) pheromone lures compared with capture in an unbaited control trap. All traps were positioned along canola crops for both the 2016 (left) and 2017 (right) field seasons. The midline indicates the median and the top and bottom of the box indicates the first and third quartiles, respectively. Vertical line or whiskers represent the 1.5 interquartile range of the data or the maximum value. Open circles represent points more than 1.5 times the interquartile range. Different letters indicate significant differences (Tukey's HSD: $P < 0.05$).

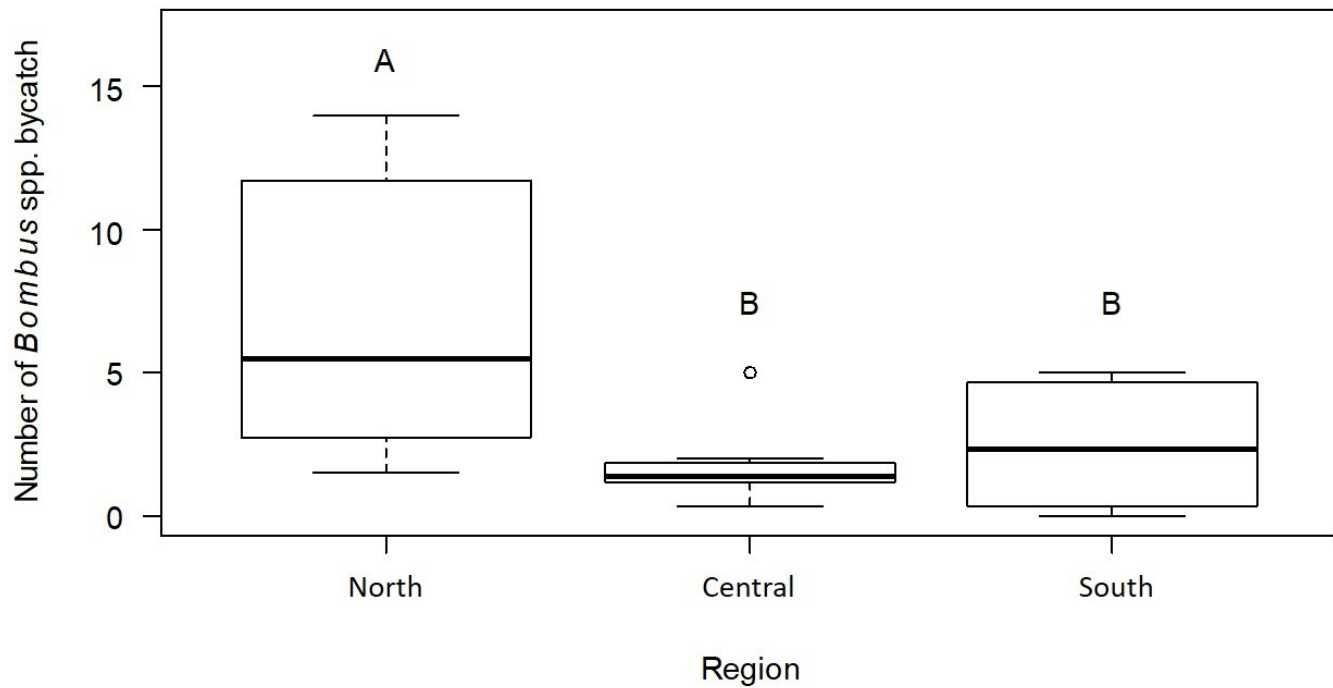


Figure 3-3. Boxplots of the 2016 field season capture of *Bombus* spp. in monitoring traps in three regions, separated according to spatial position: north (n=7), central (n=7), and south (n=6). All traps were positioned at canola crops. The midline indicates the median and the top and bottom of the box indicates the first and third quartiles, respectively. Vertical line or whiskers represent the 1.5 interquartile range of the data or the maximum value. Open circles represent points more than 1.5 times the interquartile range. Different letters indicate significant differences (Tukey's HSD: $P < 0.05$).

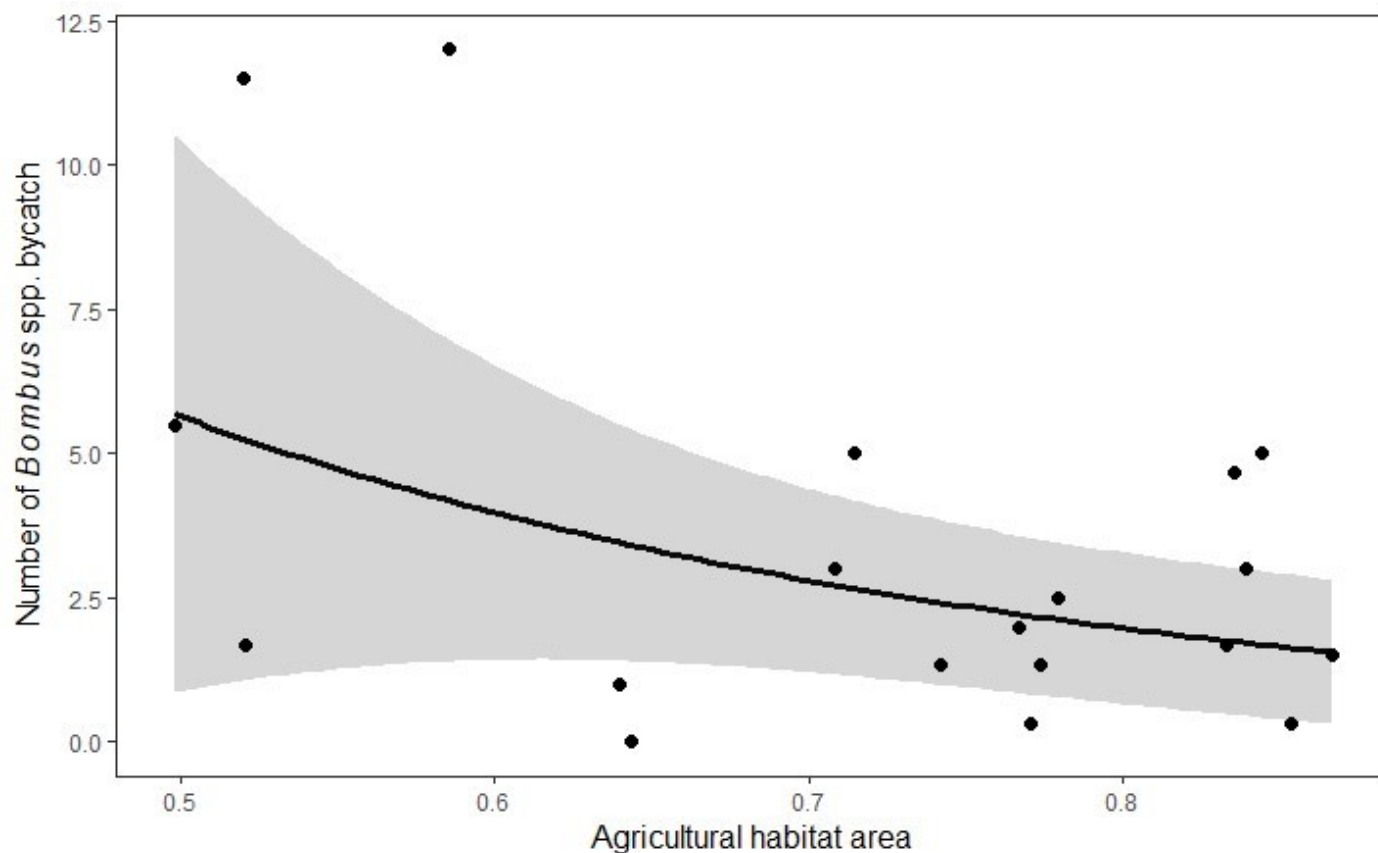


Figure 3-4. Relationship between the proportion of agricultural area in the landscape surrounding sites and *Bombus* spp. capture in monitoring traps during the 2016 field season. Each point represents a canola field, the line shows the best fit, and the grey area covers the confidence bands based on the generalized linear model.

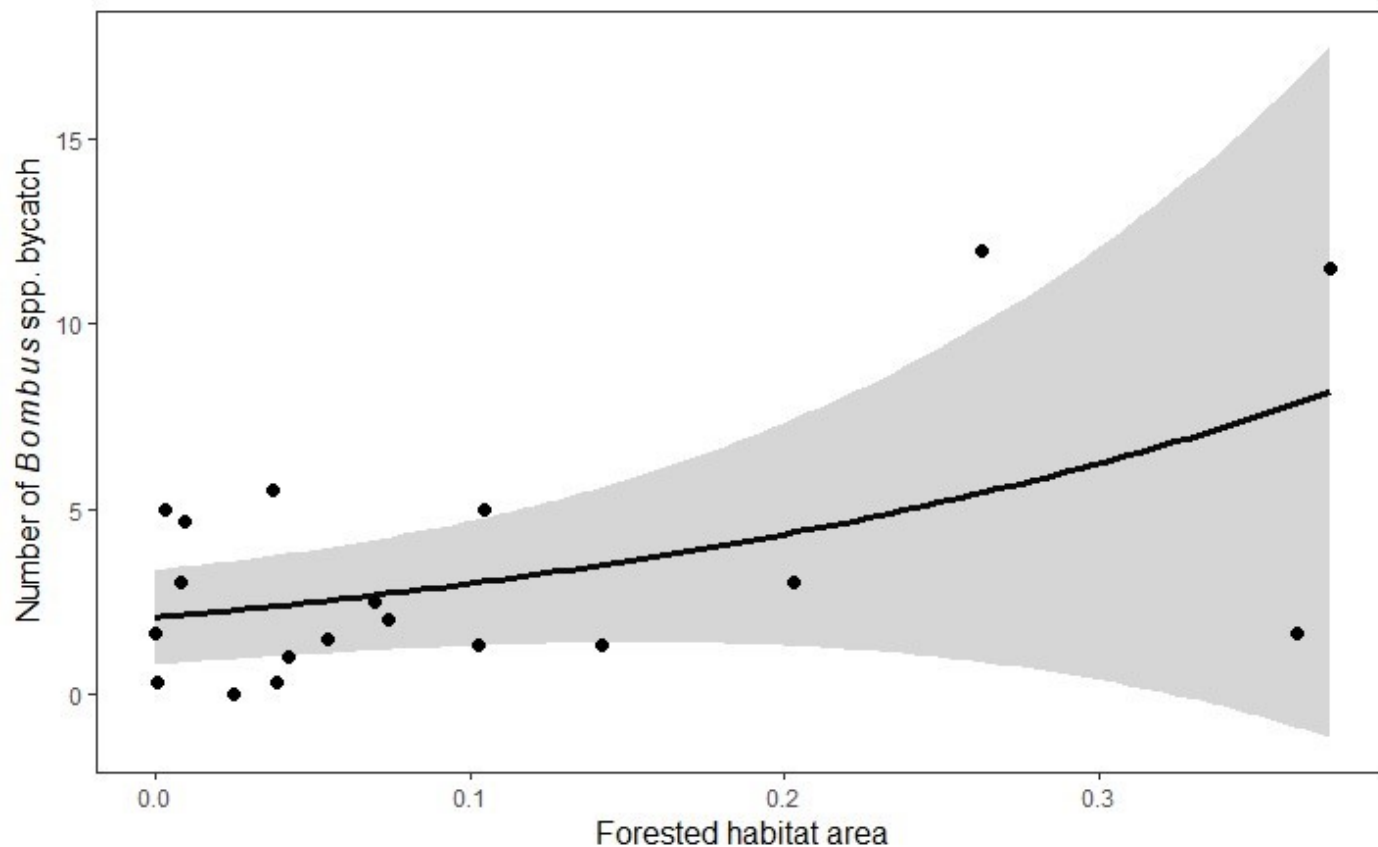


Figure 3-5. Relationship between the proportion of forested area in the landscape surrounding sites and *Bombus* spp. capture in monitoring traps during the 2016 field season. Each point represents a canola field, the line shows the best fit, and the grey area covers the confidence bands based on the generalized linear model.

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Chapter 4: Conclusion

Cutworms and armyworms (Lepidoptera: Noctuidae) can be economically important pests of many annual crops on the Canadian Prairies (Byers & Struble 1987; Spears et al. 2016; Floate 2017). As pest outbreaks are sporadic and unpredictable, it is necessary to have effective, continuous monitoring programs in the Prairies. Monitoring programs often rely on species-specific pheromone lures, however these lures may be less effective than feeding attractant lures for assessing the breeding population. Unfortunately, it has also been widely documented that many bee pollinators are captured as bycatch in a variety of monitoring programs targeting lepidopteran pests (Landolt et al. 2007; Meagher & Mitchell 1999; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016). While the ecological impact of pollinator removal as bycatch is unknown, alterations to monitoring programs to reduce unintentional capture of beneficial insects should be considered. Here, we used multiple approaches to explore the attraction of beneficial pollinators to semiochemical lures in Alberta, Canada.

In Chapter 2, we evaluated the attraction of wild bees to pheromone lures targeting four noctuid pest species: redbacked cutworm (*Euxoa ochrogaster* [Guenée]), bertha armyworm (*Mamestra configurata* Walker), true armyworm (*Mythimna unipuncta* [Haworth]), and pale western cutworm (*Agrotis orthogonia* [Morrison]). Additionally, we assessed pollinator attraction to feeding attractant lures consisting of both fermentation by-products and floral volatiles. All experiments were performed at paired canola, *Brassica napus* L. (Brassicaceae), and wheat, *Triticum aestivum* L. (Poaceae), fields to examine potential differences in bycatch between cropping systems. Bumble bees, and especially *Bombus rufocinctus* Cresson (Hymenoptera: Apidae), were captured as bycatch in green-coloured Unitraps baited with all tested noctuid pheromone lures. Bumble bees were highly attracted to the floral volatile phenylacetaldehyde, had an intermediate attraction to fermentation by-product lures that incorporated floral volatiles, and were not attracted to fermentation by-product lures. Other wild bees and honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), were captured in low numbers and do not appear to be attracted to the semiochemical lures tested here. Generally, there were more bees captured in traps positioned at canola field edges compared to wheat fields, however the community composition of bee bycatch did not vary with crop type. This increase at canola fields is consistent with the propensity for mass flowering crops to provide floral resources for

bees (Westphal et al. 2003; Westphal et al. 2009). Overall, this work corroborates previous research suggesting that both floral-based feeding attractant lures (Meagher & Mitchell 1999; Landolt et al. 2007) and moth pheromone lures (Meagher & Mitchell 1999; Mori & Evenden 2013; Aurelian et al. 2015; Spears et al. 2016) can attract *Bombus* spp.

We used electroantennogram (EAG) assays to determine if two bumble bee species, *B. rufocinctus* and *B. impatiens* Cresson, could perceive components of the field-tested noctuid pheromone lures. To our knowledge, this study provides the first evidence that bumble bees have antennal receptors to respond to these cues, which evolved as sexual signals in a different insect order. Here, we also present a hypothesis to explain one potential mechanism that could be driving this unusual attraction. Bumble bees are likely responding to moth pheromone lures because of similarities in the molecular structure of moth and male-produced bumble bee pheromones (De Meulemeester et al. 2011). Components of pheromones in both groups are straight chain hydrocarbons and can have acetate, alcohol, and aldehyde functional groups (Appelgren et al. 1991; Bergstrom et al. 1996). *Bombus rufocinctus* pheromones contain acetate components typical of bumble bee species that exhibit the male perching mating behaviour (O'Neill et al. 1991; Bertsch et al. 2008). Additionally, *B. rufocinctus* workers were able to perceive acetate pheromone components during EAG assays and were attracted to these semiochemicals in field studies. Bee attraction to monitoring traps is not due to visual elements as more bees are captured in pheromone-baited than tested controls.

Chapter 3 focused on assessing the attraction of bee pollinators to bertha armyworm (BAW) pheromone-baited traps across the canola producing regions of Alberta. The study sites selected for this study were a subset of the Prairie Pest Monitoring Network (PPMN), which operates annually to monitor BAW and other important pest populations in the Canadian Prairies. As in Chapter 2, *B. rufocinctus* was the most abundant species captured and bumble bees were more attracted to pheromone-baited Unitraps than unbaited controls. Similarly, we captured few other wild bees during this study. As the second component of this study, we evaluated how both local and landscape scale habitat features influence the bycatch of bees in monitoring traps. To our knowledge, this is the first study attempting to fill this knowledge gap. Overall, there was a greater importance of landscape composition than local floral resource availability. The results of this study agree with literature suggesting that the effect of local and

landscape habitat characteristics are often linked (Nayak et al. 2015; Scheper et al. 2015; Quistberg et al. 2016). Bumble bees and other large bodied bees typically have greater responses to landscape-scale features (Steffan-Dewenter et al. 2002; Kennedy et al. 2013; Hopfenmüller et al. 2014; Steckel et al. 2014) as they can fly large distances to access resources (Greenleaf et al. 2007; Rao & Strange 2012). Generally, the proportion of agricultural land surrounding monitoring traps had a negative influence on bee bycatch, whereas forested and grassland habitats positively influenced the number of bees captured in monitoring traps. These are well established patterns that are likely driven by the provisioning of floral and nesting resources in natural and seminatural habitats, both of which are scarce in landscapes that are dominated by intensive agriculture (Bailey et al. 2014; Bennett & Isaacs 2014; Hopfenmüller et al. 2014; Mallinger et al. 2016; Kammerer et al. 2016; Senapathi et al. 2017). It is important to note that the response of bees was not always consistent across both years of this study, which could be due to the interaction of environmental conditions and climatic factors (e.g. precipitation).

Several possibilities for improving existing monitoring protocols in the Prairie Provinces were identified throughout the course of this research. First, fermentation by-product (food bait) lures may be a valuable monitoring tool in the future as they are attractive to both sexes of multiple noctuid moth species (Landolt et al. 2007; Batallas 2018) but did not attract bumble bees or other wild bees. The efficacy of implementing food bait lures in widescale monitoring and the impact of food bait lures on other beneficial insect groups remain to be tested before these lures could be widely adopted. Second, altering the placement of pheromone-baited monitoring traps, both spatially and temporally, may help to reduce pollinator bycatch. One recommendation is to position traps in the field margin as far from forests as possible, as pollinator abundance decreases further from forest habitat (Bailey et al. 2014). Although we did not find a large influence of local flowering resources on bumble bee bycatch, it would likely still be beneficial to avoid placing traps in the vicinity of large flower patches, as they can be highly attractive to bees, especially in agricultural landscapes (Pywell et al. 2006; Potts et al. 2009; Scheper et al. 2015; Wood et al. 2015). Wild flower abundance may be less important once canola crops are in flower as the crop can provide plentiful resources for bees (Westphal et al. 2003; Westphal et al. 2009). As such, trap placement should be delayed until canola reaches the flowering stage in each region, which may be especially important for monitoring in the Peace region. These recommendations could decrease the frequency that bees “randomly”

encounter monitoring traps while foraging in field margins and would not likely diminish the effectiveness of pest monitoring, however this should be further researched prior to implementing these recommendations.

Overall, this study provides the first evaluation of pollinator bycatch in traps baited with pheromone and feeding attractant lures in the Canadian Prairies. This study also contributes to the scientific understanding of bumble bee attraction to unrelated pheromone cues. The ecological impact of pollinator bycatch in monitoring systems is poorly understood. Undoubtedly, monitoring protocol changes that reduce pollinator bycatch would be beneficial and several changes have been recommended here. Canada has limited historical information about pollinator population trends (Sheffield et al. 2014) and while some recent monitoring of pollinator communities in the Prairies has occurred (Sheffield et al. 2014; Kohler 2017; Sturm 2017), continued monitoring is essential to detect declines and support conservation of native pollinators. If established monitoring protocols cannot be modified and the use of pheromone lures continues, perhaps there can be an increased effort to make use of the information provided by bycatch, which is often discarded (Spears & Ramirez 2015).

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Appendix A

Table A1. Summary of generalized linear models testing the influence of different semiochemical lures and crop types on bumble bee (*Bombus* spp.) bycatch in monitoring traps in central Alberta, Canada. All models were constructed with a negative binomial distribution with site included as a random factor. The explanatory variables included in each model and the Akaike Information Criterion adjusted for small sample sizes are presented. Significant terms (Wald chi-square test, $\alpha = 0.05$) are shown in bold.

Year	Model ID	Model	AICc
2014	Rufo-14	Sum <i>Bombus rufocinctus</i> ~ Lure + Crop + (1 Site)	326.46
	Other-14	Sum Other <i>Bombus</i> spp. ~ Lure + Crop + (1 Site)	286.10
2015	Rufo-15	Sum <i>Bombus rufocinctus</i> ~ Lure + Crop + (1 Site)	659.10
	Pooled-15	Sum Other <i>Bombus</i> spp. ~ Lure Classification + Crop + (1 Site) + Offset(Number of Traps/Lure Classification)	206.28
2016	Rufo-16	Sum <i>Bombus rufocinctus</i> ~ Lure + Crop + (1 Site)	331.28

Appendix B

Table B1. Site locations for canola crops used in 2016 (n=20) and 2017 (n=43) field experiments.

Year	Site	County/M. D.	Latitude (°)	Longitude (°)
2016	1	Wainwright	52.872	-111.268
	2	Wainwright	52.934	-110.494
	3	Forty Mile	49.425	-111.709
	4	Newell	50.598	-111.856
	5	Newell	50.776	-112.134
	6	Wheatland	50.998	-112.609
	7	Wheatland	50.882	-112.873
	8	Wheatland	50.921	-113.546
	9	Two Hills	53.658	-111.796
	10	Two Hills	53.85	-112.016
	11	Westlock	54.076	-113.945
	12	Athabasca	54.489	-112.961
	13	Grand Prairie	55.225	-119.019
	14	Grand Prairie	55.509	-118.317
	15	Mackenzie	58.46	-116.38
	16	Mackenzie	58.065	-116.238
	17	Northern Lights	56.974	-117.618
	18	Northern Lights	56.883	-117.463
	19	Smoky River	55.669	-117.054
	20	Leduc	53.149	-114.057
2017	1	Red Deer	52.031472	-113.837455
	2	Red Deer	52.0999	-114.1206
	3	Red Deer	52.193615	-113.147592
	4	Red Deer	52.232383	-114.037373
	5	Flagstaff	52.6828	-112.1084

Table B1. (Continued).

Year	Site	County/M. D.	Latitude (°)	Longitude (°)
2017	6	Wainwright	52.833633	-110.172933
	7	Wainwright	52.87905	-110.77735
	8	Camrose	52.9015	-112.4472
	9	Flagstaff	53.0215	-112.514
	10	Beaver	53.0305	-111.668
	11	Beaver	53.0361	-112.148266
	12	Leduc	53.150215	-114.123596
	13	Camrose	53.2069	-112.7881
	14	Leduc	53.246903	-113.336108
	15	Beaver	53.2668	-111.693
	16	Leduc	53.30872	-113.87206
	17	Beaver	53.330497	-112.148013
	18	Parkland	53.3938	-113.9589
	19	Parkland	53.4829	-114.0179
	20	Beaver	53.5124	-112.607
	21	Parkland	53.5794	-113.9839
	22	Lamont	53.647192	-112.238148
	23	Lamont	53.658543	-112.68162
	24	Lac Ste. Anne	53.686683	-114.278717
	25	Sturgeon	53.849639	-113.9169708
	26	Lamont	53.857985	-112.728022
	27	Lac Ste. Anne	53.901	-115.117633
	28	Lamont	54.003268	-112.381338
	29	Lac Ste. Anne	54.006333	-115.011883
	30	Lamont	54.007177	-112.82541

Table B1. (Continued).

Year	Site	County/M. D.	Latitude (°)	Longitude (°)
2017	31	Lac Ste. Anne	54.0346	-115.314467
	32	Smoky Lake	54.035	-111.888611
	33	Westlock	54.07658	-113.95106
	34	Smoky Lake	54.093611	-112.397222
	35	Smoky Lake	54.138333	-112.575278
	36	Bonnyville	54.180967	-111.172383
	37	Westlock	54.286389	-113.600583
	38	Westlock	54.396558	-113.905376
	39	Bonnyville	54.424372	-110.786872
	40	Grande Prairie	55.233283	-119.0513
	41	Grande Prairie	55.512283	-118.330533
	42	Fairview	56.062776	-118.210211
	43	Fairview	56.075917	-118.627673

Table B2. Description of reclassified landcover variables. Original descriptions are reprinted from Castilla et al. (2014).

Landcover Variable	Original Landcover Class	Description
Agriculture	Agriculture	Annually cultivated cropland, tame pastures (fields planted or sown with non-native grasses/legumes where livestock is directly grazing on them), forage crops (same as tame pasture, but instead cut for hay) and woody perennial crops (fruit orchards and vineyards). Includes annual field crops, vegetables, summer fallow, orchards and vineyards. Bare agricultural soil (i.e., tilled) belongs to this class.
Human Developed	Developed	Urban and built-up areas (including industrial sites), impervious artificial surfaces (e.g. airport runways), railways and roads. Acreages and farmsteads are included in this class. Oil and gas well pads are included in this class if connected to a road and not abandoned or under reclamation. Urban terrain under development is included in this class, even if the land is exposed. Urban green areas are excluded from this class if larger than 2 ha and if they have less than 2 buildings per hectare.
Forest	Coniferous Forest	Treed areas with at least a 10% crown closure of trees, where coniferous trees (spruce, pine, fir, larch) are 75% or more of the crown closure. Providing crown closure is more than 10% and dominated by conifers, young plantations or regenerating cutblocks, and treed wetlands (e.g. black spruce bogs and fens) are included in this class providing mean tree height exceeds 2 m.
	Broadleaf Forest	Treed areas with at least a 10% crown closure of trees, where broadleaf trees (trembling aspen, balsam poplar and white birch) are 75% or more of the crown closure. Providing crown closure is more than 10% and dominated by broadleaf trees, young plantations or regenerating cutblocks, and treed swamps along floodplains or wetlands are included in this class providing mean tree height exceeds 2 m.

Table B2. (Continued).

Landcover Variable	Original Landcover Class	Description
	Mixed Forest	Treed areas with at least a 10% crown closure of trees, where neither coniferous nor broadleaf trees account for 75% or more of crown closure.
	Shrubland	At least 20% ground cover which is at least one-third shrub, with no or little presence of trees (< 2m height, and recently burned forest areas.
Grassland	Grassland	Predominantly native grasses and other herbaceous vegetation with a minimum of 20% ground cover; may include some shrub cover (but less than a third of the vegetated area) or a few trees (but the tree cover cannot exceed 10%). Land used for range or native unimproved pasture (e.g., rough fescue) is included in this class. Alpine meadows fall into this class. Marshes and other non-woody wetlands with at least 20% vegetation cover (sedges, cattails, or moss) belong to this class. Note: A forestry cutblock harvested more than a year ago containing seedlings with less than 10% cover, belongs to this class. If the cutblock had no successful regeneration and is covered by more than 20% shrubs, it would belong to the 'Shrubland' class.
Water	Water	Lakes, lagoons, rivers, canals, and artificial water bodies. Shallow open water is included in this category, unless there is more than 20% vegetation cover, in which case it belongs to the relevant vegetated class.



Figure B1. Sampling quadrat (1 m^2) used to sample flower coverage during the 2017 field season. Divisions were spaced every 0.10 m to create a grid with 100 individual spaces to ease estimation.

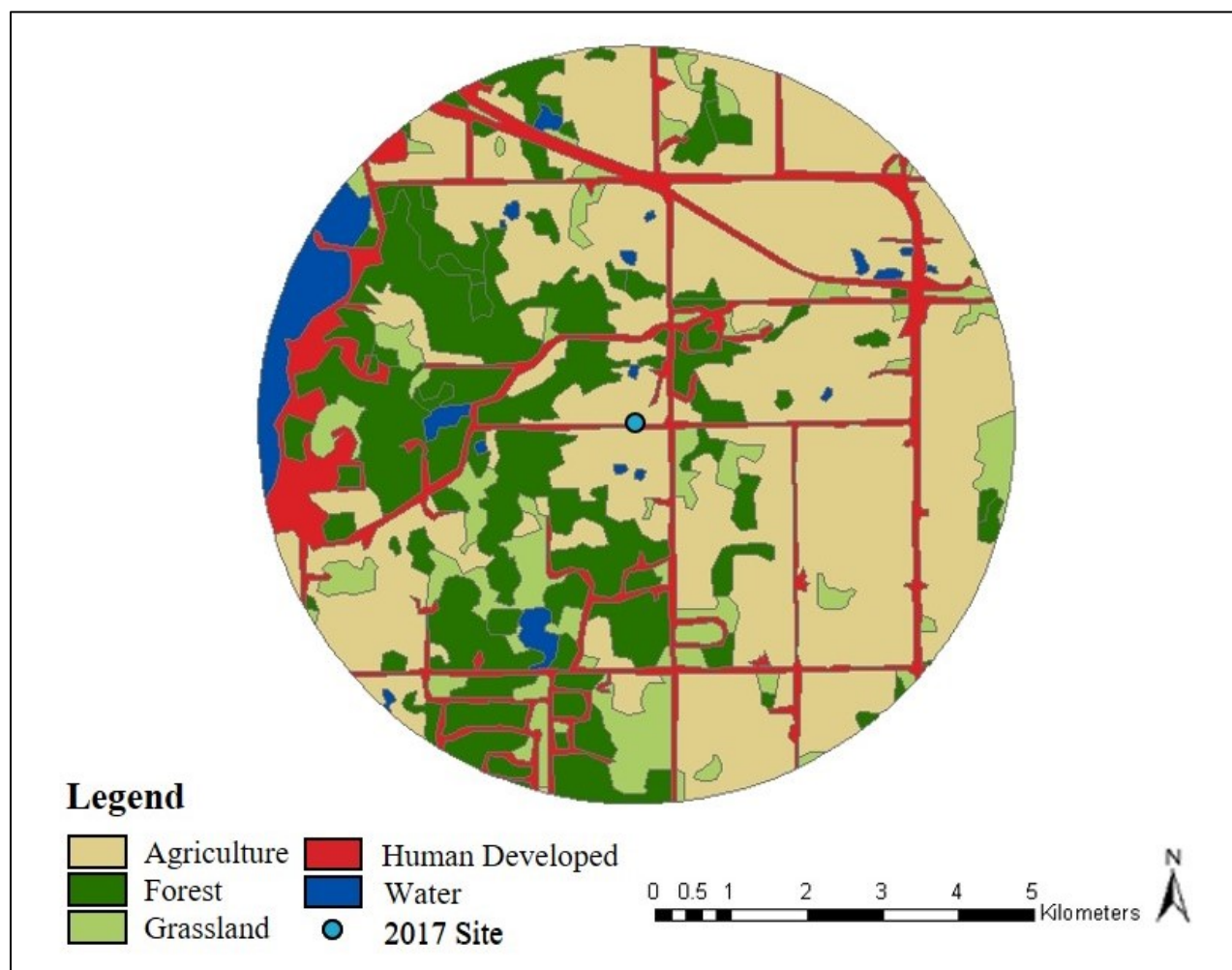


Figure B2. Example of circular buffer (5 km radius) surrounding a site (green point). This analysis was completed in ArcGIS (version 10.3.1).

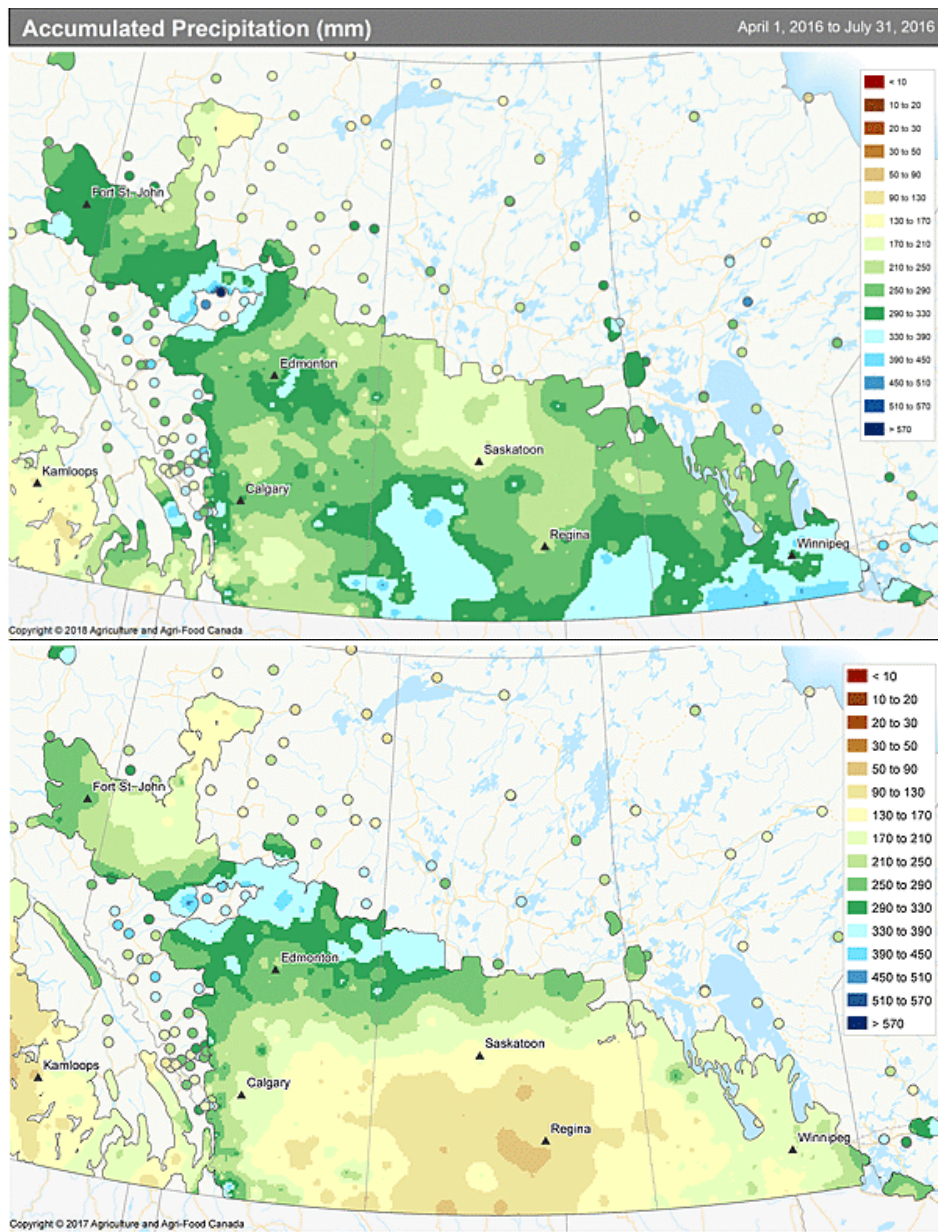


Figure B3. Accumulated precipitation from 1 April – 31 July during the 2016 (above) and 2017 (below) growing season. Figures were prepared by Agriculture and Agri-Food Canada’s Science and Technology Branch in partnership with Environment Canada and can be accessed from: <http://www.agr.gc.ca/DW-GS/historical-historiques.aspx?lang=eng&jsEnabled=true>.

Appendix C

During the 2017 field experiment (Chapter 3), we estimated the bee pollinator diversity at a subset of the sites ($n=16$), which were randomly selected across the study area. We used active netting and blue vane trapping to assess background bee diversity. Netting was performed by two individuals (N. Grocock and M. Russell) for a period of 30 minutes between 10am and 5pm (MST) on days with low wind speeds ($<30\text{km/hr}$) and temperatures between $15\text{ }^{\circ}\text{C}$ and $30\text{ }^{\circ}\text{C}$. Netting was performed along the sampling transect and all hymenopteran pollinators were targeted. Blue vane traps, which are attractive to diverse bee taxa (Stephen & Rao 2005), were positioned in the headland of the canola fields 50 m from the trapping line and contained an insecticide strip. The contents of blue vane traps were collected 48 ± 4 hrs later. Captured bumble bees and honeybees were identified to species and all other wild bees were identified to family (as described previously).

Table C1. Abundance of bumble bee species captured in blue vane traps and by active netting at canola fields in Alberta, Canada during the 2017 field season.

Method	Species	Abundance
Blue vane trap	<i>Bombus rufocinctus</i>	6
	<i>Bombus ternarius</i>	25
	<i>Bombus vagans</i>	12
	<i>Bombus borealis</i>	11
	<i>Bombus centralis</i>	7
	<i>Bombus terricola</i>	2
	<i>Bombus nevadensis</i>	2
	<i>Bombus mixtus</i>	1
	<i>Bombus sandersoni</i>	1
	<i>Bombus</i> spp. males	5
Netting	<i>Bombus rufocinctus</i>	21
	<i>Bombus ternarius</i>	28
	<i>Bombus vagans</i>	25
	<i>Bombus borealis</i>	10
	<i>Bombus centralis</i>	3
	<i>Bombus terricola</i>	0
	<i>Bombus nevadensis</i>	0
	<i>Bombus mixtus</i>	0
	<i>Bombus sandersoni</i>	0
	<i>Bombus</i> spp. males	9

Table C2. Abundance of non-bumble bee hymenopteran pollinators captured in blue vane traps and by active netting at canola fields in Alberta, Canada during the 2017 field season.

Method	Family	Abundance
Blue vane trap	Andrenidae	3
	Apidae	121
	<i>Apis mellifera</i>	12
	Colletidae	1
	Halictidae	26
	Megachilidae	5
Netting	Andrenidae	12
	Apidae	18
	<i>Apis mellifera</i>	48
	Colletidae	4
	Halictidae	6
	Megachilidae	12