WILD BEES AND AGROECOSYSTEMS

by

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ABSTRACT

Research in agriculture often focuses on development of new technologies rather than on potential environmental impacts. Pollinators, primarily bees, are essential to agriculture, providing significant yield benefit in over 66% of crop species. Currently, dramatic losses of managed honey bee pollinators in North America along with suspected world-wide losses of wild pollinators are focusing research attention on an impending but still poorly documented pollination crisis. Essential questions include: How important are wild bees to crop production? Are current agricultural practices harming pollinator populations? Can agricultural methods be modified in ways that promote pollinators and food production? In this thesis I examine the interaction between modern agriculture and wild bees through 1) laboratory experiments on effects of new genetically modified (GM), systemic, and bio-pest control products on bumble bee (*Bombus* spp.) health and foraging ability, and 2) field experiments on the impacts of agricultural landscapes on wild bee abundance, diversity, and pollination efficacy.

I developed a new method of assessing bee foraging after exposure to pesticides that is a useful and sensitive test for sub-lethal impacts on pollinators. The GM pesticidal proteins Bt Cry1Ac and chitinase did not negatively affect bumble bee colony or individual health or foraging ability. However, the pesticide imidacloprid in the new chloronicotinoid family of pesticides impaired bee foraging when bees were exposed to elevated doses during larval development. The new biopesticide spinosad, which is widely marketed and approved as an organic insecticide, rapidly killed bumble bee colonies at elevated doses and impaired foraging ability at realistic exposure rates.

In field studies, herbicide-tolerant genetically modified canola agroecosystems had fewer wild bees than organic fields, and there were an intermediate number of bees in conventional fields. Low bee abundance in GM fields and to a lesser extent, conventional fields was associated with low seed set and reduced yields. Weed cover in

fields and amount of uncultivated land around fields were positively related to bee abundance in fields. We determined that crop landscapes with uncultivated areas could have greater yield than homogenously tilled landscapes. These data can be used to design agroecosystems that benefit both conservation and crop production.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
GENERAL INTRODUCTION

Agricultural technology is advancing at a swift pace and food production has never been as efficient as it is today. Yet, a lack of information concerning the interaction between agricultural practices and the environment continues to hamper the development of productive, but sustainable agriculture. Concepts and practices of sustainable agriculture are developing simultaneously with concerns about our ability to maintain long-term intensive systems of crop production (Matson et al. 1997). Increased mechanization, conversion of marginal land to food-producing acreage, new pest control products, and most recently the development of genetically modified organisms can present novel problems for one of the most important components of sustainable agriculture, pollinators.

1.1 Agriculture

The total area of cultivated land world-wide has increased 466% since 1700 (Meyer & Turner 1992). With the 'green revolution' in the 1960's, intensification of agriculture resulted in large yield increases (Grigg 1993); yield increases have continued with more recent developments in high-yielding cultivars, GM crops, mechanization, and chemical application. Yet, agricultural intensification has serious, negative impacts on the environment such as increased soil erosion, lower soil fertility, reduced biodiversity, and pollution of ground water and the atmosphere (Matson et al. 1997). In addition, we

now have the technology to convert marginal areas into productive agricultural systems (Kearns & Inouye 1997). Global change resulting from agriculture is estimated to rival climate change in environmental impacts (Vitousek et al. 1997; Tilman et al. 2002). With growing world population and food distribution problems, agricultural intensification remains a priority (Matson et al. 1997) and we continue to lose natural, uncultivated fragments in agroecosystems (Daily 1997).

1.2 Pollination and bees

Pollination is a necessary ecosystem service that is being threatened by conversion of natural land to agriculture and pesticide use (Allen-Wardell et al. 1998). Pollination is essential for the production of many crops, but the interaction between wild pollinators and modern agricultural systems and practices has not been well-studied. Many pollination biologists believe that we may be facing a pollination crisis, in which both wild and managed pollinators are disappearing at alarming rates due to habitat fragmentation, disease, intensive monoculture, and pesticide use, resulting in serious threats to biodiversity and agricultural stability (e.g., Corbet et al. 1991; Kearns & Inouye 1997; Allen-Wardell et al. 1998). At least one cultivar of over 60% of the world's crop species requires insects, primarily bees, for pollination (Roubik 1995). In developed countries over 30% of food results from insect-mediated pollen transfer (O'Toole 1993). Most bee-pollinated crops depend upon managed honey bee (*Apis mellifera* L.) colonies, and in the United States two million colonies are rented out annually to growers during bloom (Free 1993). Recently, managed honey bee populations have experienced serious declines due to newly introduced varroa and tracheal mite parasites, diseases, and the encroachment of Africanized honey bees (Watanabe 1994; Matheson et al. 1996). In

addition, honey bees are not the most effective pollinators of many crops (Kevan et al. 1990).

Adding to the honey bee shortage has been a suspected decline of native pollinators (Torchio 1990; Matheson et al. 1996; Allen-Wardell et al. 1998). Wild insects are responsible for 20 to 80% of commercial pollination (Southwick & Southwick 1992; Ingram et al. 1996), with an annual crop value of $4—$16 billion US. Declines of wild bee pollinators have been documented world-wide as a result of habitat loss and pesticide use (Kearns & Inouye 1997; Allen-Wardell et al. 1998). Further, insect pollinators are of incalculable value for pollination of natural vegetation.

Little effort currently is being made to ensure that wild pollinator populations in agroecosystems are diverse and abundant (Corbet et al. 1991). Effects of this neglect have been evident in poor crop pollination and yields (Kevan 1977; Kevan et al. 1990; Williams 1995; Ingram et al. 1996; Matheson et al. 1996; Allen-Wardell et al. 1998; Kremen et al. 2002; Morandin & Winston 2005(Chapter 4)). One of the most critical priorities for future research in sustainable agriculture and conservation of pollinators is "multi-year assessments of the lethal and sub-lethal effects of pesticides, herbicides, and habitat fragmentation on wild pollinator populations in and near croplands" (Allen-Wardell et al. 1998). Because information on the role of wild pollinators in agriculture and the effects of agricultural methods on pollinators is largely speculative, research is critical to provide an understanding of this interaction.

In this thesis I examine the interaction between modern agriculture, wild bees, and crop production. I focus on two major mechanisms in which modern agriculture can

have impact on wild bees, lethal or sub-lethal toxicity of pesticides used in agriculture, and indirect effects from agricultural management practices.

1.3 Toxic effects

Pesticides may inadvertently cause harm to bees through lethal or sub-lethal toxicity (Johansen & Mayer 1990). Instances of poisoning of managed honey bees from use and misuse of pesticides have been well-documented and associated losses in crop pollination and yield are estimated in the billions in North America alone (Johansen & Mayer 1990). But lethal and sub-lethal impacts on wild bees are largely unstudied, and pesticide spray recommendations rarely protect wild bee health. In addition, modern pest control technologies are introducing potentially new problems for wild bees.

1.3.1 Systemic Pesticides

New chloronicotinoid pesticides that are applied to seeds, such as imidacloprid, disperse throughout plant tissues, potentially exposing bees orally through residues in nectar or pollen (Schmuck et al. 2001). Imidacloprid is manufactured by Bayer CropScience, registered in about 120 countries and used on over 140 crops against sucking insects (Bayer CropScience 2005).

1.3.2 Genetic Modification

As of 2004, 17 countries around the world were using at least one transgenic crop. The leaders in genetically modified (GM) crop production by area grown (millions of ha, percent of total) are the United States (47.6, 59%), Argentina (16.2, 20%), Canada (5.4, 6%), Brazil (5, 6%), and China (3.7, 5%). The rate of growth of the GM crop industry has increased since commercialization of GM crops in 1996, and between 2003 and 2004

there was a 20% increase in area used for genetically modified crop production. Twenty-nine per cent of corn, cotton, soybean, and canola are now GM globally. Herbicide tolerance has been the dominant trait introduced in crop species (72% of GM acreage) followed by insect resistance (Bt) (19% of GM acreage). Herbicide resistant crops are not thought to pose a direct risk to pollinators through toxicity; but because insect-resistant crops produce Bt pesticidal proteins, pollen produced by these crops may be toxic to bees. In Canada, the major commercially grown GM crops (% of acreage that is GM as opposed to standard breed varieties) are corn (76), soybean (85), and canola (80) (All GM statistics are from James 2004).

1.3.3 Biopesticides

Newer generations of pesticides, such as microbial biopesticides, are thought to be less harmful to humans and the environment than older, synthetic organophosphate, carbamate, and pyrethroid insecticides (Koul & Dhaliwal 2002). Because pesticides derived from natural organisms are thought to be less harmful to non-target organisms, use regulations generally are less stringent than for older classes of pesticides. Yet in some of the few studies conducted to date, exposure to these newer, environmentally safer, pesticides has resulted in significant bee mortality in laboratory experiments (Miles et al. 2002).

Spinosad (Dow AgroSciences) is a broad spectrum, novel microbial biopesticide made from a mixture of spinosyn A and D, two of the main metabolites formed from fermentation of the actinomycete bacterium, *Saccharopolyspora spinosa* (Salgado 1998; Sparks et al. 1998) and is classified as a reduced-risk pesticide by the U.S. EPA (Cleveland et al. 2002). As of 2001, spinosad was registered in 37 countries for use on

150 crops (Cleveland et al. 2002). Acute oral and contact toxicity studies have shown spinosad to be highly toxic to honey bees, bumble bees, alfalfa leafcutter bees, and alkali bees (Mayes et al. 2003).

1.4 Local and landscape-level effects of agroecosystems

Agricultural intensification and new cropping systems associated with herbicide tolerant GM crop varieties may alter agroecosystems in ways that negatively impact wild bee populations.

1.4.1 Herbicide tolerant crops

Extremely effective weed control is possible with crops that are genetically modified to resist broad spectrum herbicides. Depending on the degree of weed control, there is the potential of having fields that are virtual monocultures, with no flowering plants other than the crop. While weed reduction benefits crop yields (Harker 2001) it reduces forage for wild bees, before, during, and after crop bloom. Wild bees require nectar and pollen resources throughout their lives. Many annual crops only bloom for a couple of weeks in any one field, and therefore do not provide sustained forage for the lifespan of most bees. Bees with larger foraging ranges such as bumble bees may be able to take advantage of successive crop blooms on a spatial scale larger than a single crop field (Westphal et al. 2003) but most other bees have smaller foraging ranges, making it unlikely that they could benefit from resources that occur over agroecosystem landscapes. Without locally available resources, wild bee populations may be so low that pollination of crops will be greatly reduced.

1.4.2 Agricultural Intensification

Natural ecosystems provide services that are crucial to sustaining a biosphere suitable for human populations (Costanza et al. 1997; Matson et al. 1997), yet humans have transformed over half of the earths surface and no ecosystems remain unaffected by human influence (Vitousek et al. 1997). Agricultural expansion into natural areas and intensification of agricultural land-use has resulted in major disruptions to ecosystem function (Matson et al. 1997; Swift et al. 2004), threatening the continuance of high-productivity agroecosystems (Matson et al. 1997). Most studies in agricultural systems focus on environmental and biotic interactions at a local scale, neglecting larger scale effects such as those caused by variation in surrounding habitat at the landscape level. Understanding variation at the landscape-level scale is important for understanding species patterns (Swift et al. 2004). Bees require nesting and foraging habitat throughout their life, and areas of extremely intense agriculture, with little or no natural or semi-natural areas, may not provide sufficient mixture of habitats to support a large diversity and abundance of bees.

1.5 General Methods and Study Organisms

1.5.1 Bumble bees

Testing effects of pesticides on at least a few species from genera other than *Apis* would provide some knowledge of the sensitivity of wild bees to commonly used insecticides. Most toxicity studies of new pesticides on beneficial pollinators only assess direct toxic effects on adult honey bees. Bees (Superfamily: Apoidea) are a very diverse group, with 20 000 to 30 000 species from approximately seven families world-wide, and

range from solitary, to colonial, to primitively social species and the highly social honey bee (Michener et al. 1994).

During my thesis research I tested lethal and sub-lethal effects of a new systemic pesticide (imidacloprid), genetically modified pesticides (Bt and chitinase), and a naturally derived biopesticide (spinosad) on colonies and individuals of two bumble bee species, *Bombus impatiens* and *B. occidentalis*. There are approximately 54 species of bumble bees in North and Central America (Michener et al. 1994), and in Canada bumble bee individuals often represent 50% or more of all wild bees sampled (personal observation).

Bumble bees are eusocial, with a queen, sterile workers, and reproductive castes. Bumble bee queens emerge from hibernation in the spring and locate a nest site. They forage for pollen and nectar in which they lay their first brood of eggs. The first brood is generally all worker bees and once they begin to forage, the queen remains in the nest for the rest of the season, laying and incubating brood. Males and reproductive females are reared towards the end of the season, then leave the nest and mate with conspecifics from other colonies. Males and the rest of the colony's workers then die off and the mated queens hibernate over the winter (Heinrich 1979; Cnaani et al. 2002).

Like other bee species, developing bumble bee larvae feed on pollen and some nectar, potentially resulting in exposure to pesticides during development. Bumble bees tend to be generalist foragers and forage on both 'simple' flowers with easily accessible nectar and/or pollen, and also 'complex' flowers that require learning and motor coordination to access nectar and/or pollen (Heinrich 1979). Because of their life history

traits and foraging modes, bumble bees are good study organisms for investigating potential lethal, sub-lethal, and learning effects of pesticide use.

1.5.2 Canola

Examination of indirect effects of modern agriculture on wild bees requires field investigations at both local and landscape scales. For the purposes of this research I categorized farming into three general types, organic farming, farming with conventionally bred crop varieties, and farming with GM cultivars.

Canola was developed in Canada in the 1970's through traditional plant breeding of rapeseed, and the word canola is a combination of "Canada" and "oil." Canola was bred for reduced erucic acid and glucosynolates making it safe for human and animal consumption (CCC 2005). In Canada, Canola (*Brassica* spp.) is the fourth most important crop by acreage seeded (Statistics Canada 2003a) and the most important oilseed crop (Statistics Canada 2003b). Currently, Canada's annual exports of canola seed, oil, and meal alone are valued at over two billion Canadian dollars (CCC 2005). Approximately 50% of canola crops worldwide and over 80% of canola crops in North America are GM herbicide resistant (James 2004). Organic canola constitutes 0.07% of the canola grown in Canada (Brooks & Barfoot 2004). Canola is an ideal crop for study of the effects of modern agriculture on wild bees because it is attractive to many wild bees, yield is increased by insect pollination (Free 1993; Delaplane & Mayer 2000), canola oil and meal are produced from the result of pollination (seeds), and canola is produced using a variety of cropping methods including organic, conventional, and herbicide-tolerant GM systems.

1.6 Thesis Outline

This thesis examines the interaction between current agricultural practices and wild bees. In Chapters 2 and 3 I report on laboratory studies on toxicological effects of some modern pest control products on bumble bee (*Bombus* spp.) colony health and foraging ability. In Chapter 2, I examine the pesticidal proteins Bt Cry1Ac and chitinase and the systemic chloronicotinoid pesticide, imidacloprid. In Chapter 3, I investigate potential lethal, sub-lethal, and foraging effects of the biopesticide spinosad on bumble bees. I hypothesized that levels of these pesticides realistically encountered by wild bees in the field would not result in overt colony or individual toxicity but could cause negative sub-lethal effects or foraging impairment.

In Chapters 4, 5, 6, and 7, I report on field studies on local- and landscape-level effects of modern agriculture on wild bee populations and potential negative impacts on crop production. In Chapter 4, I examine the relationship between wild bee abundance in organic, conventional, and GM herbicide tolerant canola fields, and seed production. Data are from studies conducted during the summer of 2002. I expected that herbicide tolerant GM canola fields would have fewer wild bees than conventional and organic fields, resulting in poor seed set in GM fields. I also hypothesized that within agricultural systems, areas with greater bee abundance would show better seed set than areas with fewer wild bees.

In Chapter 5, I examine how local and landscape factors influence wild bee abundance and diversity in organic, conventional, and genetically modified canola fields. Data were collected in the same northern Alberta region as Chapter 4 during the summers of 2002 and 2003. Local factors that were analysed in relation to bee abundance and

diversity included edge flowering plant species richness and cover, field flowering weed species richness and cover, and crop type (organic, conventional, and GM). At the landscape scale, surrounding habitat was assessed in six increasing scale buffer zones around fields using satellite imagery, from 250 m to 1500 m distances from field edges. Land in each size buffer was quantified as either crop or semi-natural. Northern Alberta, where this study was conducted, is currently undergoing conversion to agriculture and still contains natural and semi-natural, non-agricultural areas, providing much variation among fields in surrounding landscape. Little is known concerning local and landscape characteristics and how they affect bee populations in agroecosystems but I expected that both local weed cover in and on the edges of fields, and amount of semi-natural land around fields would influence wild bee abundance and diversity in fields.

In Chapter 6, using the landscape scale that was most predictive of field bee abundance (Chapter 5), and data on bee abundance and seed production collected in 2002 and 2003 in Northern Alberta, I developed an economic model of canola profit in relation to area of semi-natural land around fields. Based on data presented in Chapter 5 and new data presented in Chapter 6, I proposed that there would be an optimal ratio of uncultivated land in agricultural landscapes in which pollination requirements from the 'free' pollinator service provided by wild bees could be balanced with agricultural acreage. Essential ecosystem services provided by natural ecosystems such as pollination by wild bees could give economic incentive to preserve natural land in agricultural areas, potentially benefiting both conservation and agriculture.

In Chapter 7, I report on a study conducted in GM herbicide tolerant canola fields in an agriculturally intense area of southern Alberta. Unmanaged natural land is virtually

non-existent in this area, but mosaics of land use types might better promote ecosystem services than homogenous landscapes (Sanderson et al. 2002). I assessed the relationship between amount of grazed pastureland versus tilled crop land around fields, and wild bee abundance in fields. I expected that fields with more pastureland as opposed to tilled crop land would have greater wild bee abundance potentially resulting in better crop pollination and yield.

In Chapter 8, I summarize the major results from the six data chapters. I formulate general conclusions, recommendations, and suggest areas for future research.

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CHAPTER 2
EFFECTS OF NOVEL PESTICIDES ON BUMBLE BEE (HYMENOPTERA: APIDAE) COLONY HEALTH AND FORAGING ABILITY

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2.1 Abstract

Two experiments were conducted testing for lethal and sub-lethal effects of the transgenic proteins Cry1Ac and chitinase, and the chemical seed and soil treatment imidacloprid on bumble bees (*Bombus occidentalis* Greene and *B. impatiens* Cresson, Hymenoptera: Apidae). In the first experiment, *B. occidentalis* colonies were exposed to realistic residue levels of Cry1Ac, chitinase, and imidacloprid found in pollen. There were no effects on pollen consumption, bumble bee worker weights, colony size, amount of brood, or the number of queens and males produced. In the second experiment, using *B. impatiens*, we tested the effects of Cry1Ac and two levels of imidacloprid. Similar colony health measures were collected as in the first experiment, but in addition foraging ability of individual bees was tested on complex artificial flowers. There were no differences in colony characteristics among treatments. However, bees in the high-imidacloprid treatment had longer handling times on the complex flowers than bees in the other treatments. No lethal, sub-lethal colony, or individual foraging effects of these novel pesticides were found at residue levels found in the field, suggesting that bumble

bee colonies will not be harmed by proper use of these pesticides. Use of an artificial flower foraging array proved to be a sensitive method for detecting sub-lethal response of bees to pesticides.

2.2 Introduction

Pesticides used on agricultural crops can be harmful to pollinators (Johansen & Mayer 1990), and sprayed applications generally are restricted to night-time or when the crop is not in bloom to minimize pollinator exposure. Recently developed insect control techniques, such as genetically modified crops with insecticidal proteins and systemic chemical seed and soil treatments, are often safer for non-target species than broad-spectrum insecticidal sprays (Betz et al. 2000). However, potential harm could come to pollinators if the insecticide is expressed in or transported to pollen or nectar.

Bees are important pollinators of many crop species (e.g., Delaplane & Mayer 2000). Research concerning pesticide impact on non-*Apis* pollinators is scarce, in spite of a growing concern over suspected declines of wild pollinators and its effect on agricultural production and biodiversity (Allen-Wardell et al. 1998). Until recently, studies conducted on the effects of new insect control treatments on bees have focused almost exclusively on honey bees (*Apis mellifera* L.), despite data indicating that bee species differ in their tolerance to pesticides (Johansen & Mayer 1990).

We tested the effects of three new pesticides; Bt and chitinase proteins transferred into crop plants (i.e., genetically modified (GM)), and the chloronicotinoid seed treatment imidacloprid on bumble bees (*Bombus* spp. Hymenoptera: Apidae). These pesticides were chosen because they are either widely used or have potential to harm pollinators

(see below). Bumble bees were chosen because they are ubiquitous wild non-*Apis* pollinators and also are increasingly managed for crop pollination (Delaplane & Mayer 2000).

Genetically modified crops do have human and environmental benefits, but this new technology also presents potential risks (Winston 2002). Nectar contains insignificant amounts of protein and is unlikely to contain transgenic products, but pollen is 8–40% protein and often expresses transgenic products dependent on plant species and variety, location of the inserted gene, and type of promoter (Wilkinson et al. 1997). More than 99% of commercialized, transgenic, insect-resistant crops have been transformed with genes coding for crystalline (Cry) proteins from the soil bacterium *Bacillus thurigiensis* (Bt) (ISAAA 2001). Transgenic cotton plants containing the Cry1Ac gene (Bollgard) from Bt express the protein in pollen at a concentration of 11.5 ng/g fresh weight (EPA 2001), whereas concentrations in nectar are below detectable levels of 1.6 ng/g (EPA 2001). Studies on the effects of Bt Cry proteins on honey bees, using test doses ranging from 20 μg/ml to 625 μg/g, showed no effect on survival or feeding behaviour (Sims 1995; Malone et al. 1999). However, few studies have investigated colony health or sub-lethal effects on adults, and none have examined lethal or sub-lethal effects on other managed and wild pollinators in either laboratory or field studies.

In addition to Bt proteins, plants are being engineered with chitinases that naturally play a role in plant antifungal defence (Hou et al. 1998), including crops such as corn, grape, apple, strawberry, soybean, tomato, rapeseed, onion, alfalfa, potato, and tobacco (APHIS 2001). Chitin is present in the epithelial gut cells of insects (Kramer & Koga 1986) and in the exoskeleton (Boller 1988). Therefore, chitinases may have

insecticidal activity and potentially could harm pollinators. No data are available on expression levels of chitinase in transformed plants, but based on pollen protein content, chitinase could be present at concentrations of 0.6 μg/g fresh weight (Picard-Nizou et al. 1997). There is a lack of information on the effects of chitinase on honey bees and other pollinators except Picard-Nizou et al. (1997) who found no acute toxicity to honey bees when fed 11 μg per bee.

Foliar treatments of pesticides can be restricted to application only when a crop is not in bloom, minimizing pollinator exposure. However, new chloronicotinyl compounds used as seed and soil treatments, such as imidacloprid, are systemic, dispersing throughout the plant and potentially exposing bees orally through residues in nectar or pollen. In 1999, the French Ministry of Agriculture suspended use of the imidacloprid product Gaucho on sunflower crops because of a suspected relationship between honey bee losses and imidacloprid use (CDADF 2000; Suchail et al. 2000). A number of laboratory and field studies by Bayer and independent researchers have shown no adverse effect on honey bees at levels of imidacloprid <20 ppb (Schmuck 1999; Schmuck et al. 2001). Analyses of residue levels of imidacloprid in canola and sunflower pollen indicate levels consistantly <8 ppb, and usually at undetectable quantities below one ppb (Schmuck 1999; Rogers & Kemp 2003). Above 20 ppb, honey bees exhibit a decreased ability to recruit foragers to food sources (Schmuck 1999). Although field residue levels of imidacloprid in nectar and pollen have not demonstrated harm to honey bees, only one study has been published on the effects of imidacloprid on non-*Apis* pollinators. Tasei et al. (2001) exposed bumble bee (*Bombus terrestris* L.) colonies to

imidacloprid-treated sunflowers in the field and concluded that proper application of imidacloprid would not affect worker behaviour or colony development.

The purpose of the current experiments was to test for lethal and sub-lethal effects of novel pesticides on bumble bee colonies, and to assess a new method of testing sub-lethal foraging effects of pesticides on individual bees. Two experiments were conducted. First, the effects of Cry1Ac, chitinase, and imidacloprid on colony health in the bumble bee *B. occidentalis* Greene, at levels that could be found in pollen of field crops, were examined. In the second experiment, the effects of Cry1Ac and two concentrations of imidacloprid on *B. impatiens* Cresson colony health and individual bee foraging ability were tested. In this experiment, the higher concentration of imidacloprid tested was above the no-effect level established for honey bees. Our hypothesis was that this treatment would result in detrimental effects to colony health and bee foraging.

2.3 Materials and Methods

2.3.1 Experiment 1: Colony Health

Twenty-four *B. occidentalis* colonies were obtained from Biobest Canada Ltd (Leamington, Ontario, Canada). Upon delivery, each colony contained a queen and approximately 5 to 10 workers ("first brood" stage). Colonies were housed in plastic containers 20 × 28 × 18 cm, surrounded by an outer cardboard casing and equipped with a bag containing a nectar substitute that bees could access freely.

The isolated proteins and insecticide were added to non-GM pollen at levels that realistically could be found in transgenic pollen or imidacloprid-treated plants (Picard-Nizou et al. 1997; EPA 2001). Colonies were divided into four treatment groups with six

colonies per treatment: (1) Control: pollen and 30% sucrose solution; (2) Imidacloprid: control plus technical imidacloprid (98%) from Bayer AG (Leverkusen, Germany) at 7 ng ([AI])/g fresh pollen; (3) Chitinase: control plus chitinase (30%) from Sigma-Aldrich (Oakville, Ontario) at 0.6 μg ([AI])/g pollen; and (4) Cry1Ac: control plus Cry1Ac (19%) from Monsanto (St. Louis, MO) at 11 ng ([AI])/g pollen. All pesticide concentrations represent the level of active ingredient that likely would be found in field collected pollen.

Pollen was collected from pollen traps on honey bee colonies in British Columbia, Canada, cleaned of dead insects and debris, and frozen for later use. The packed pollen lumps collected by honey bees were ground using an electric food processor before being mixed with the sucrose solution. Purified protein powders and imidacloprid were added to pollen by first being dissolved in distilled water, then added to 30% sucrose solution in distilled water and stirred for 5 min. The sucrose solution was then added to the pollen in a 2:1 pollen to sucrose solution mixture calculated by weight. Bees were fed pollen from the appropriate treatment twice weekly, ad libitum. At each feeding time, old pollen was removed and weighed, and weight of fresh pollen added was recorded.

Colonies were received on 18 May 2001 and monitored until 8 August 2001. At the beginning of the experiment, all bees were removed from colonies, cooled at 4°C for 10 min, weighed on an Ohaus Explorer electronic balance (Ohaus Company, Florham Park, NJ) to 0.01 g, and marked with a standard colour pattern using Fast Drying Liquid Paper of various colours. Each week, all newly emerged bees were removed, cooled, weighed, and marked with a Liquid Paper colour pattern unique to their emergence week. The numbers of workers, amount of brood (defined as number of egg masses, larval

masses, larval cells, and pupae), number of queens, and number of males were assessed weekly in each colony.

Data Analysis

For all analyses, bumble bee colony was treated as the replicate. The amount of pollen consumed by each colony, from feeding to removal was calculated twice weekly and divided by the estimated number of adult bees in the colony. The mean difference in pollen weight per bee for each treatment was used to estimate pollen use and consumption, and was compared among treatments using analysis of variance (ANOVA) (SPSS 1999). Weights of newly emerged workers each week were compared using a repeated-measures ANOVA (SAS 1999). The number of bees that emerged from each colony each week was highly variable, ranging from 0 to more than 60 and this variation was included in the model. Weekly mean number of workers, eggs, larval masses, larval cells, pupae, queens, and males were $\log_{10}$ transformed to meet the assumptions of ANOVA and were compared among treatments using a multivariate repeated-measures ANOVA (SPSS 1999).

2.3.2 Experiment 2: Colony Health and Foraging Ability

In the second experiment, similar colony health variables were monitored as in the first experiment, although worker weights were not measured. In addition, individual bees were assessed for their ability to forage on complex artificial flowers. Preliminary experiments with *B. occidentalis* suggested that this species did not forage well in an artificial array, so 24 *B. impatiens* colonies were obtained from Biobest Canada (Leamington, Ontario), and we began a new experiment began on 27 September 2001.

All colonies were at the first brood or early second brood stage at hive receipt. Hive design was the same as for the *B. occidentalis* colonies. As soon as the colonies were received and throughout the entire experiment, they were fed pollen from one of the following treatment groups, prepared in the same manner as the first experiment: (1) Control: pollen and 30% sucrose solution; (2) Cry1Ac: control plus Cry1Ac (19%) from Monsanto (St. Louis, MO) at 11 ng ([AI])/g pollen; (3) Imidacloprid low: control plus technical imidacloprid (98%) from Bayer AG (Leverkusen, Germany) at 7 ng ([AI])/g fresh pollen; and (4) Imidacloprid high: control plus technical imidacloprid (98%) from Bayer AG (Leverkusen, Germany) at 30 ng ([AI])/g fresh pollen.

Pollen was replaced biweekly and the amount of pollen consumed was calculated for each colony. The number of worker bees, males, queens, egg masses, larval masses, larval cells, and pupae in each hive were counted weekly. All adult bees were marked with Liquid Paper on the abdomen 20 d after the experiment began. Marked individuals were not used in the foraging experiment, ensuring that all tested bees were of similar age and had consumed treated pollen throughout their developmental stages and as adults.

Bees were tested for their ability to access complex artificial flowers. The simple artificial flowers were designed from 1.5-ml clear micro tubes (Sarstedt, Newton, NC) with the caps removed. An artificial foraging array was created by embedding 30 tubes into a 60-cm × 60-cm green Styrofoam base. Flowers were in rows, with each flower 10 cm apart. Rows were staggered, 5 cm between each, resulting in flowers 7 cm from their nearest neighbour. Hives were connected to a 1.2 × 1.2 × 1-m mesh flight cage by a 20-cm gated mesh tunnel. Each flight cage contained one foraging array. Throughout the experiment, two flight cages were used, each with only one colony connected at a time.

Because only two colonies could be connected to flight cages simultaneously, four of the six colonies from each treatment were chosen for testing. To ensure that test colonies would have enough foragers for the experiment, colonies tested from each treatment were selected because they were judged the healthiest in each group based on worker number and amount of brood.

Collection of foraging data began on 14 November 2001, 6 wk after the colonies began receiving treated pollen. Testing of bees in a colony began by disconnecting the colony's nectar supply. The hive was then connected to a flight cage and bees were allowed to forage on the artificial flowers containing 30% sucrose solution. Ten to 15 bees making regular foraging trips were marked with a unique Liquid Paper colour combination. All bees then were returned to the hive and bee access gates to the flight cage were closed. The array of centrifuge tubes was removed and replaced with a similar array containing 17 complex artificial flowers designed using the method of Gegear and Laverty (1998). Complex flowers were constructed using clear centrifuge tubes with caps bent over the top, creating a 4-mm opening. Two microliters of 30% sucrose solution were put into each flower using a 100-μl syringe with a PB600 2-μl repeating dispenser (Hamilton Company, Reno, NV). One marked forager was released into the cage and videotaped for the duration of 40 successful flower visits. A flower visit was determined to be successful if the entire bee entered the tube and accessed the solution at the base of the flower. From initial observations, it was determined that bees completely drained the 2 μl of solution on each successful visit. Immediately after a bee had successfully accessed a flower, it was refilled with 2 μl sucrose solution. If a bee

returned to the colony before completing at least 30 flowers, it was let back into the cage after voiding its sucrose solution into the colony.

Five bees from each colony were tested in the following treatment order: control, imidacloprid 7 ppb, Cry1Ac, and imidacloprid 30 ppb. The order was repeated four times with new colonies each round, resulting in a potential total of 20 bees from four colonies for each treatment. At times it was not possible to get a complete test for all five bees from a colony, and, thus, the actual numbers of bees included in analyses were 20, 14, 17, and 20 in the control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb, respectively. Each colony took 3 to 6 d to test, so the foraging experiment was conducted over a 6-wk period. Consequently, colonies tested later in the experiment were older and therefore we included round (i.e., the set of four colonies, one from each treatment) as a factor in the statistical tests.

Access time for each of the 40 visits was calculated for each bee from videotape data using a handheld stopwatch accurate to 0.01 s. Access time was measured as the total amount of time that a bee spent touching any of the flowers until it reached the nectar at the bottom of a tube (successful access). Time spent between flowers was not included in access time estimates. Foraging rates were estimated for each bee by the total time taken to access 10 flowers, including inter-flower time, from the 21st to 30th flowers. Access times generally did not decrease substantially after the 15th flower accessed, therefore foraging rate estimates taken from flowers 21–30 were considered to be rates of experienced foragers. Foraging rates are expressed as the number of flowers accessed per minute.

Data Analysis

Colony health variables were analyzed using repeated-measures ANOVA and multivariate repeated-measures ANOVA (SPSS 1999) with colony as the replicate. Data were $\log_{10}$ transformed to meet the assumptions of ANOVA. All reported means and graphs are from the non-transformed data.

Access times were compared among treatments by repeated-measures ANOVA with flower number as the repeated measure (SPSS 1999). Variation in foraging rates among treatments was tested using univariate ANOVA followed by Tukey's pair-wise comparison test (SPSS 1999).

2.4 Results

2.4.1 Experiment 1: Colony Health

Mean estimated daily pollen consumption per bee (±SE) was 0.042 ± 0.006, 0.047 ± 0.008, 0.046 ± 0.008, and 0.043 ± 0.005 g in the control, chitinase, Cry1Ac, and imidacloprid treatments, respectively, and was not different among treatments ($F = 0.11$; $df = 3, 20$; $P = 0.95$). Repeated-measures ANOVA on mean weights of newly emerged workers over time indicated no differences among treatments ($F = 0.52$; $df = 3, 20$; $P = 0.68$; Figure 2.1). There was no effect of treatment on number of workers, amount of brood (eggs, larval cells, larvae, and pupae) (Figure 2.2), number of queens, or number of males (multivariate repeated-measures ANOVA; $F = 1.17$; $df = 12, 57$; $P = 0.362$; Figure 2.3).

2.4.2 Experiment 2: Colony Health and Foraging Ability

The number of workers and amount of brood (eggs, larval cells, larvae, and pupae) (Figure 2.4) were not different among treatments (multivariate repeated-measures ANOVA; $F = 0.695$; $df = 12, 57$; $P = 0.75$). When presented with an artificial array of complex flowers, bees in all treatments combined successfully accessed a mean ± SE of 46.8 ± 1.0 flowers before returning to the colony. There was no difference in the number of successful flowers accessed per foraging trip among treatments ($F = 1.28$; $df = 3, 67$; $P = 0.290$).

There were missing values in access times for some bees after 30 flowers, hence the analysis included only flowers 1–30 for each bee. The interaction between the repeated measure of flower access time (1–30), round (1–4), and treatment was not significant ($F = 0.979$; $df = 243, 1485$; $P = 0.576$; $1\text{-}\beta = 1.00$). There was an interaction between repeated access times of flowers and treatment ($F = 1.531$; $df = 81, 129$; $P = 0.015$; Figure 2.5). Pair-wise comparisons of repeated access times over the 30 flowers indicated that foragers in the imidacloprid 30 ppb treatment took longer to access the flowers than in the other three treatments (control: $P < 0.001$, Cry1Ac: $P = 0.012$, imidacloprid 7 ppb: $P = 0.011$). No other pair-wise comparisons were significant. Foragers in the imidacloprid 30 ppb treatment spent a mean ± SE of 6.59 ± 0.37 s accessing flowers, 42.6% more time than control, Cry1Ac-, and imidacloprid 7 ppb-treated bees (4.27 ± 0.37, 4.76 ± 0.44, and 4.84 ± 0.40 s, respectively, overall mean ± SE for these three treatments = 4.62 ± 0.18 s).

Access times rapidly decreased over the first 10 flowers, and foragers were considered "experienced" after they had successfully accessed 20 flowers. Access times

of experienced foragers (flowers 21–30) were different among treatments (repeated-measures ANOVA flower number*treatment: $F = 1.649$; $df = 27, 183$; $P = 0.029$). Pair-wise comparisons among treatments showed that access times of foragers in the imidacloprid 30 ppb treatment were significantly greater than in each of the other treatment groups ($P < 0.001$; Figure 2.6).

Foraging rates of experienced foragers also were different among treatments ($F = 10.94$; $df = 3, 69$; $P < 0.001$). Foraging rate for bees in the imidacloprid 30 ppb treatment ± SE was 3.07 ± 0.14 flowers per minute, less than the foraging rates for control (4.04 ± 0.14 flowers per minute), Cry1Ac (3.75 ± 0.16 flowers per minute), and imidacloprid 7 ppb (3.98 ± 0.14 flowers per minute) individuals (Tukey's pair-wise comparison test, $P < 0.01$). Foragers in the imidacloprid 30 ppb treatment were 27.7% slower than foragers in the other three treatments, successfully accessing approximately one flower less per minute than bees in the other treatments.

Using the above data, bees in the control treatment took 14.8 s from exiting one flower to exiting the next flower (one flower cycle). Flower access time for bees in the control group averaged 3.0 s, leaving 11.8 s during which the bees were engaged in other activities such as flying above the array, walking on the array, uptake of sucrose solution, and exiting the flower. Bees in the imidacloprid 30 ppb treatment took an average of 19.5 s between successive flower exits, and using the average access time from the treatment of 4.6 s, the bee was engaged in activities other than flower handling for an average of 14.9 s of each flower cycle.

2.5 Discussion

There were no measurable effects on bumble bee colony or individual bee health from exposure to Cry1Ac, chitinase, or imidacloprid at concentrations similar to and above the highest residue levels found in pollen, consistent with previously published results for honey bees (Sims 1995; Picard-Nizou et al. 1997; Schmuck 1999; Schmuck et al. 2001; Scott-Dupree & Spivak 2001). The pesticide concentrations that we tested on *B. occidentalis* and *B. impatiens* colonies were chosen to reflect levels present in or higher than pollen of treated or modified commercially grown crops. Results suggest that genetically modified crops and imidacloprid seed treatments, expressing field levels of the proteins and pesticide as tested, will not harm wild bumble bee colonies.

The Bt protein Cry1Ac did not cause any lethal or sub-lethal effects to *B. impatiens* colonies. Access times and foraging rates did not differ from those of control bees, indicating that plants transformed with the Cry1Ac gene should be safe for bumble bees in the field. Previous studies on honey bees have found no acute toxic effects or colony health effects when individuals or colonies were exposed to the Bt proteins Cry1Ac, Cry1Ab, Cry9C, Cry3A, Cry3B, and Cry1Ba (summarized in Malone & Pham-Delegue 2001). The current study provides the first evidence that Bt proteins fed to bees throughout their development and as adults will not disrupt colony health or foraging ability.

Picard-Nizou et al. (1995; 1997) conducted a series of studies on the effects of chitinase on honey bees including acute toxicity tests, standard conditioned proboscis extension assays, and foraging trials on control and transformed oilseed rape. Similar to

the current study, Picard-Nizou et al. (1995; 1997) found no detrimental health or other effects on bees exposed to chitinase proteins.

In the current two experiments, *B. occidentalis* and *B. impatiens* colonies exposed throughout colony life to purified imidacloprid at 7 ppb did not exhibit detrimental effects. In addition, access times and foraging rates of individual *B. impatiens* bees on artificial complex flower arrays were not affected by long-term exposure to the pesticide at that concentration. However, when *B. impatiens* colonies were exposed to imidacloprid at 30 ppb, access times and foraging rates of individual bees were slower than bees exposed to 7 ppb imidacloprid or controls. Bees in the imidacloprid 30 ppb treatment may have spent longer in activities such as flying above the array and uptake of sucrose solution, in addition to spending more time handling flowers. Additional testing would be required to determine what, in addition to longer access times, caused bees in the imidacloprid 30 ppb treatment to have lower foraging rates than bees in the other treatments. Lower foraging rates for bees in the imidacloprid 30 ppb treatment of almost one less flower accessed per minute could mean that wild bumble bees if exposed to this level of pesticide may either take longer for each foraging trip, or possibly collect less pollen or nectar each trip, potentially affecting colony health.

Analysis of imidacloprid residue levels in nectar and pollen of plants grown from treated seeds, or plants grown in fields after soil treatments, have shown low, and, in most cases, undetectable levels of imidacloprid. C. Scott-Dupree (personal communication) analyzed levels of imidacloprid and its metabolites in honey bee pollen collected from treated plants and found detectable levels (limit of detection: 0.3 ppb) in two of eight samples; 7.6 and 4.4 ppb. Schmuck et al. (2001) tested nectar and pollen of

sunflowers grown in greenhouses from seeds treated with imidacloprid and found no detectable levels (limit of detection: 1 ppb) of imidacloprid or its metabolites. Rogers and Kemp (2003) analyzed nectar and pollen from wild flowers and clover in years after soil application of the imidacloprid product Admire. They found no detectable residues of imidacloprid or its metabolites in clover and wild flowers or in honey bee collected pollen and nectar (limit of detection: 2 ppb). The conclusion of our study suggests that levels of imidacloprid at or below 7 ppb in pollen will not harm bumble bee colony health or foraging ability, whereas concentrations of 30 ppb, approximately four times the highest residue level recorded in any study to date, may have sub-lethal effects on foraging.

Use of complex flower artificial arrays was found to be a sensitive method for testing for sub-lethal impacts of pesticides. Negative impacts of pesticides that might not be observed in acute toxicity tests may be detectable on artificial foraging arrays. For example, no measurable impact of 30 ppb imidacloprid on colony characteristics was found, yet the foraging array revealed a sub-lethal behavioural effect at that higher dose. This method provides a practical and useful measure of foraging ability that could supplement or replace more expensive and logistically difficult field experiments. By altering flower design or tasks required to access a reward, artificial arrays could be modified to test for negative effects of pesticides on different aspects of foraging behaviour and on different types of bees.

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2.7 Figures

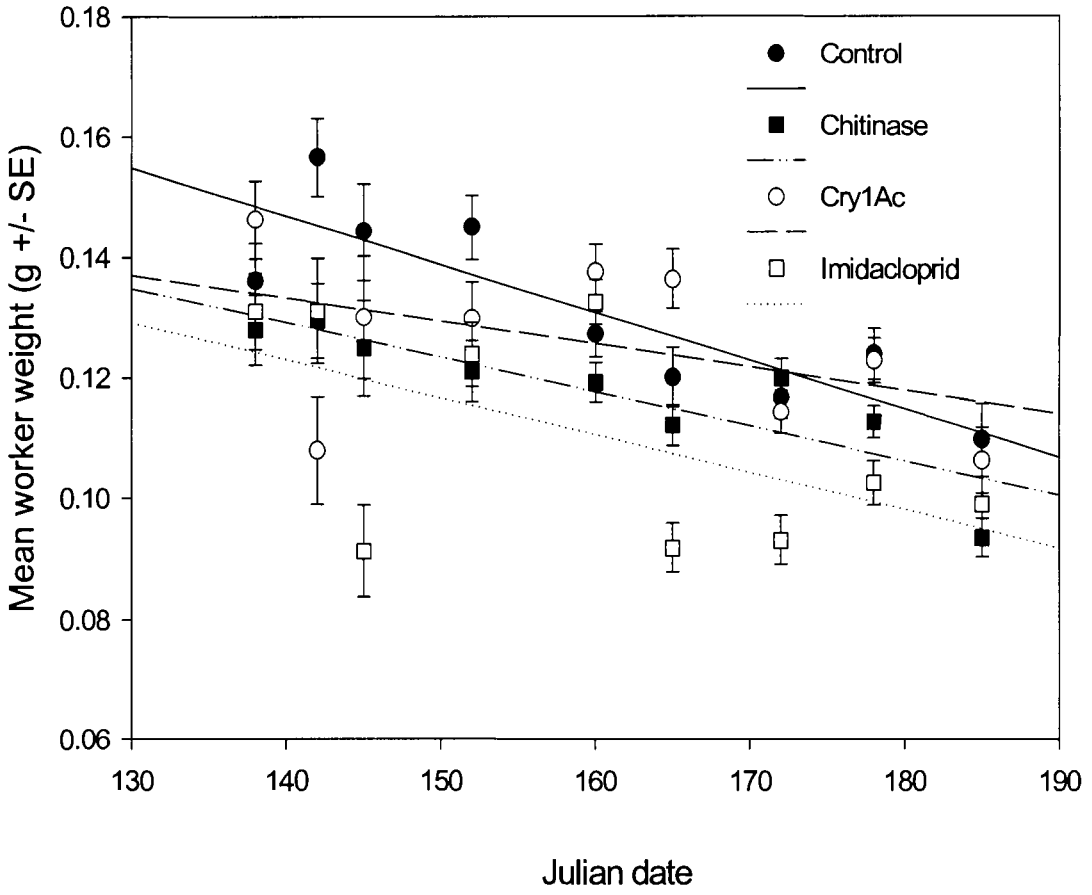

Figure 2.1 **Mean weights (± SE) of workers from six *B. occidentalis* colonies in each of four treatments; control, chitinase, Cry1Ac, and imidacloprid 7 ppb. Regression lines were generated by simple least squares regression.**

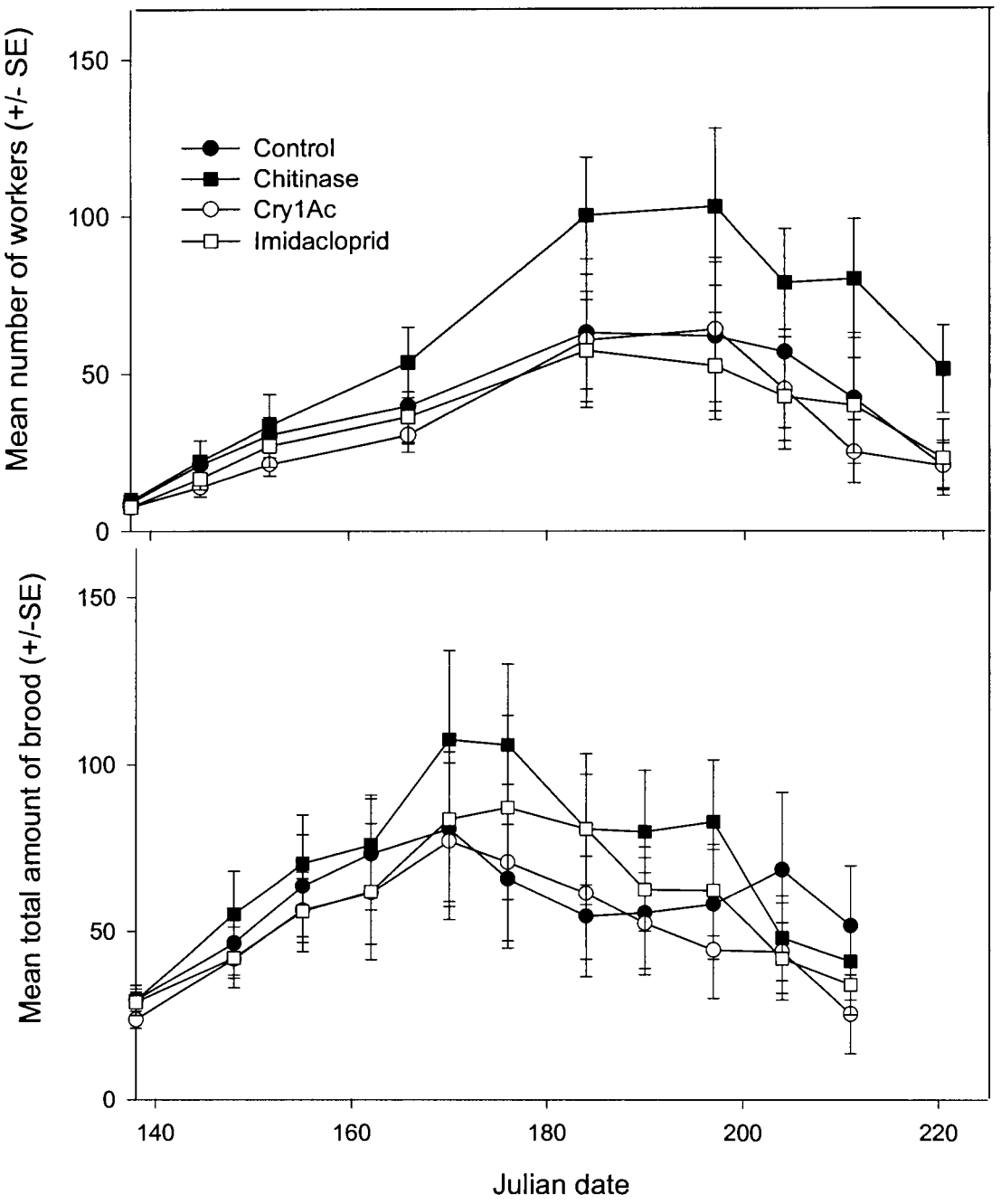

Figure 2.2 **Mean number of adult workers (± SE) and mean amount of brood (number of egg masses, larval masses, and pupae) (± SE) from six *B. occidentalis* colonies in each of four treatments; control, chitinase, Cry1Ac, and imidacloprid 7 ppb.**

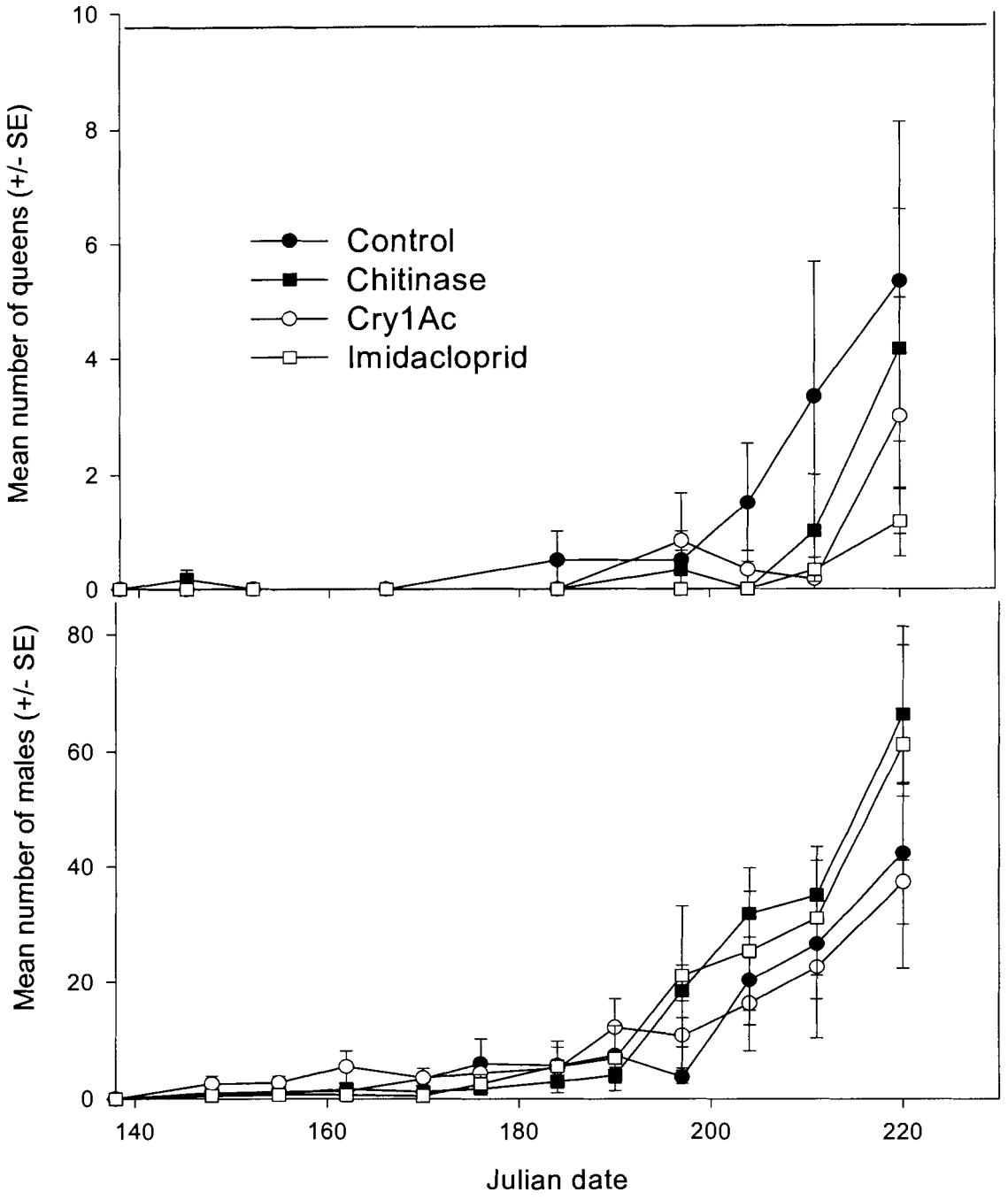

Figure 2.3 **Mean number of queens (± SE) and males (± SE) from six *B. occidentalis* colonies in each of four treatments; control, chitinase, Cry1Ac, and imidacloprid 7 ppb.**

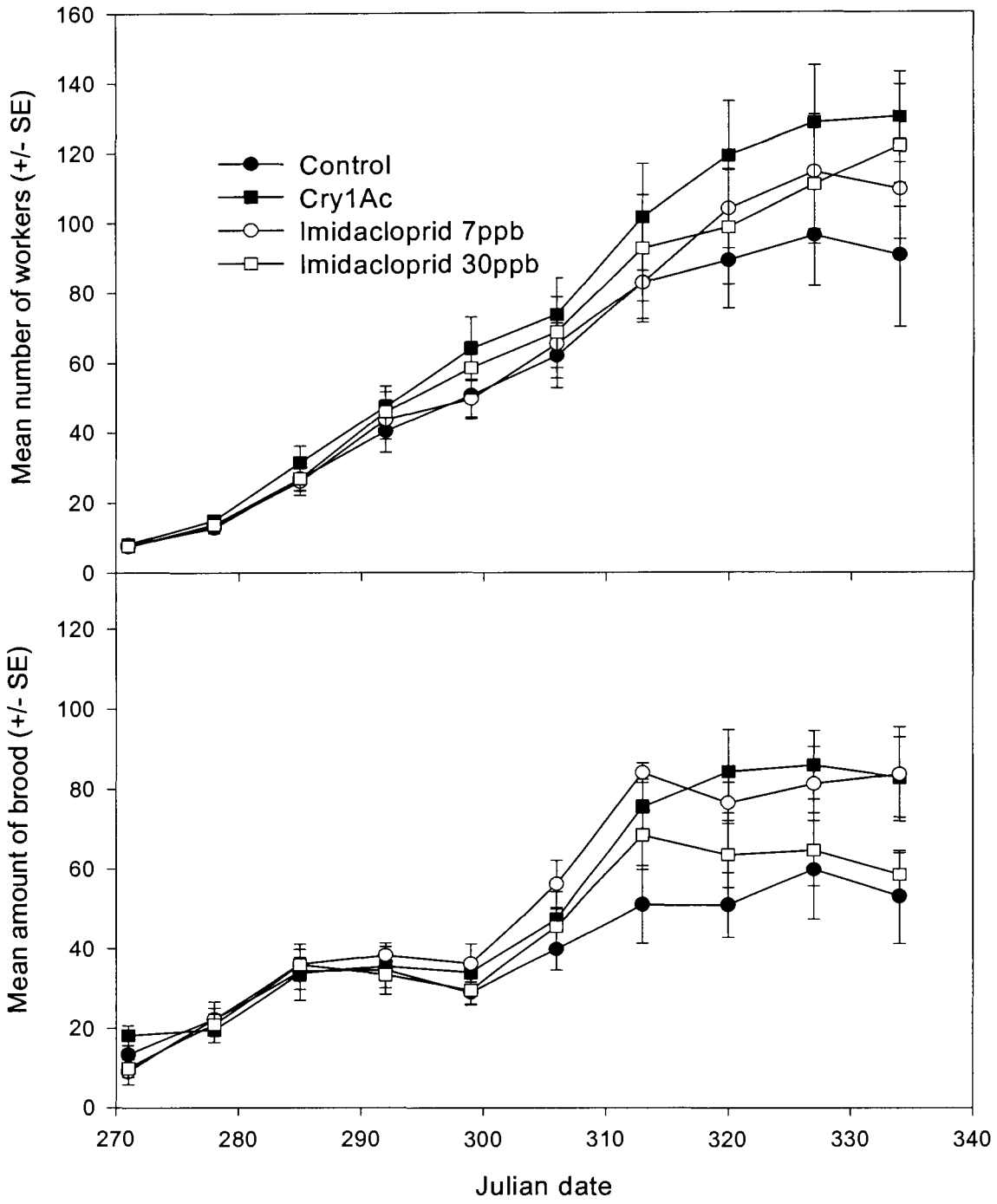

Figure 2.4 **Mean number of adult workers (± SE) and mean amount of brood (number of egg masses, larval masses, and pupae) (± SE) from six *B. impatiens* colonies in each of four treatments; control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb.**

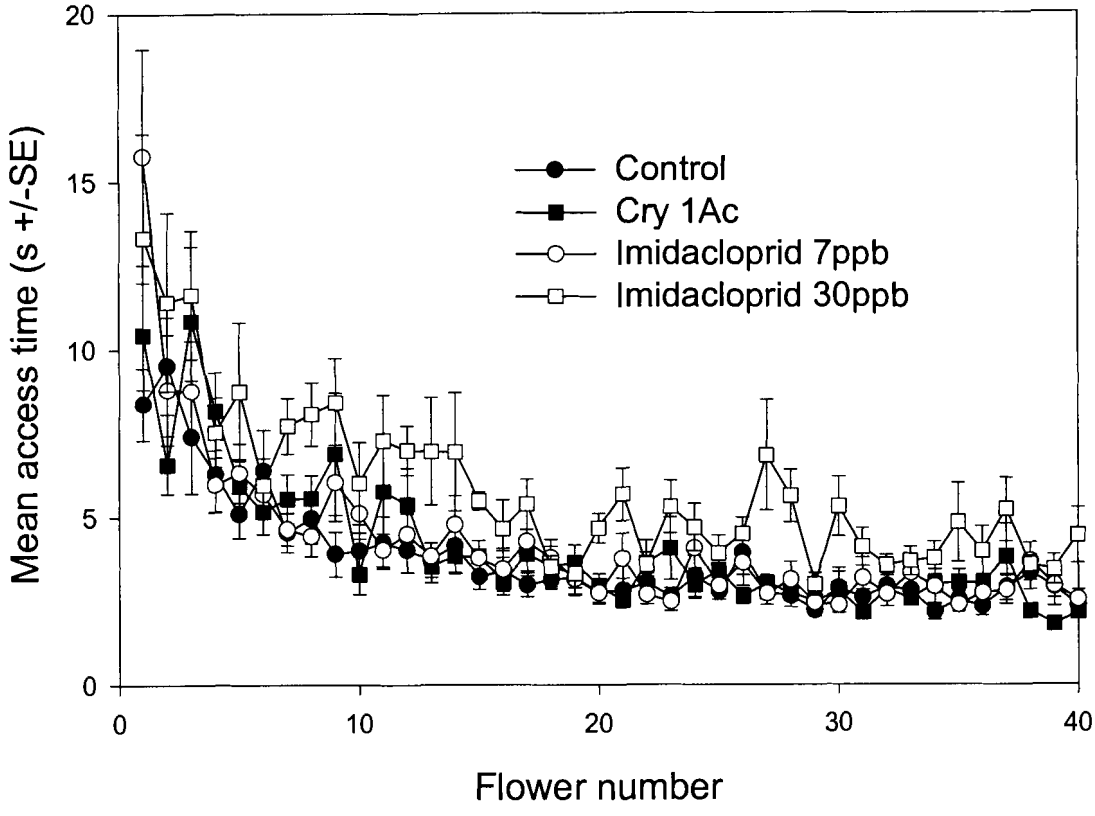

Figure 2.5 **Flower access times (± SE) for *B. impatiens* on artificial complex flowers from four colonies in each of four treatments; control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb. The number of bees tested from each treatment was 20, 14, 17, and 20 respectively. Access times for each flower were calculated as the total amount of time bees spent touching flowers prior to successfully entering a flower.**

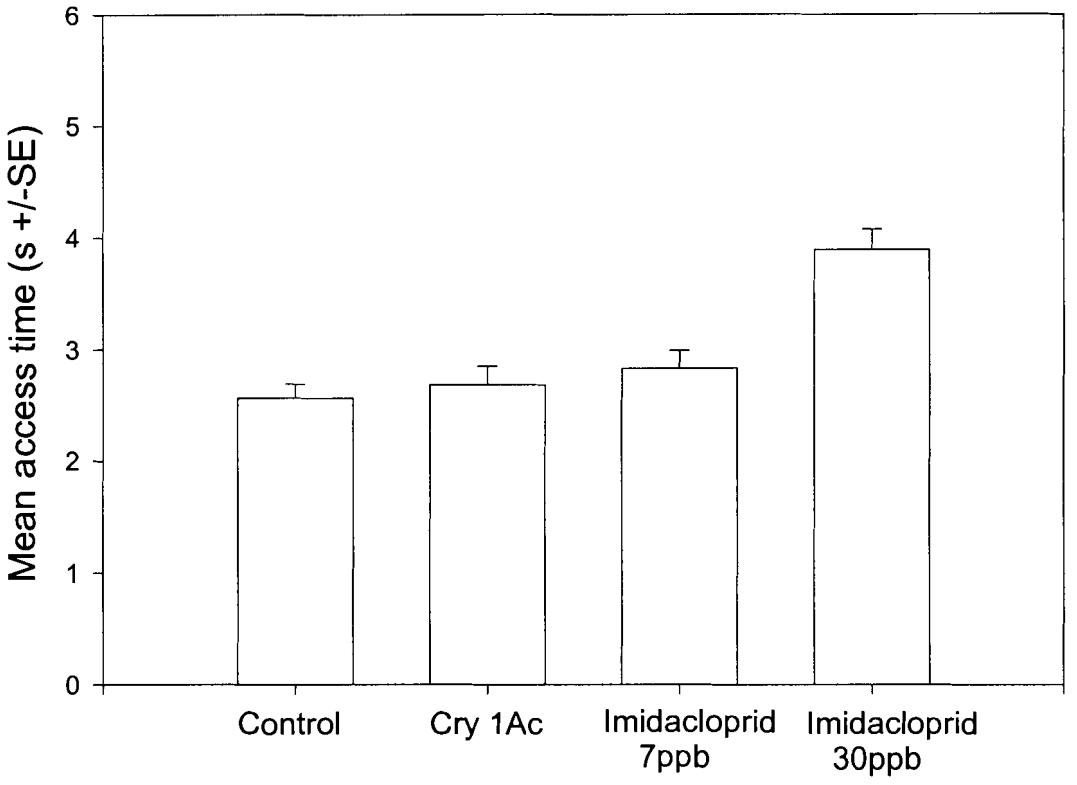

Figure 2.6 **Mean (+ SE) foraging rates of 'experienced' bees (see Materials and Methods) from four colonies in each of four treatments; control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb. The number of bees tested from each treatment was 20, 14, 17, and 20 respectively.**

CHAPTER 3
LETHAL AND SUB-LETHAL EFFECTS OF THE NOVEL PESTICIDE SPINOSAD ON BUMBLE BEES (*BOMBUS IMPATIENS* CRESSON)

The following chapter has been published in Pest Management Science with Mark L. Winston, Michelle T. Franklin, and Virginia A. Abbott as co-authors.

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3.1 Abstract

Recent developments of new families of pesticides and growing awareness of the importance of wild pollinators for crop pollination have stimulated interest in potential effects of novel pesticides on wild bees. Yet, pesticide toxicity studies on wild bees remain rare, and few studies have included long-term monitoring of bumble bee colonies or testing of foraging ability after pesticide exposure. Larval bees feed on exogenous pollen, and exposure to pesticides during development may result in lethal or sub-lethal effects during the adult stage. We tested the effects of a naturally derived biopesticide, spinosad, on bumble bee (*Bombus impatiens* Cresson) colony health, including adult mortality, brood development, weights of emerging bees, and foraging efficiency of adults that underwent larval development during exposure to spinosad. We monitored colonies from an early stage, over a 10 week period, and fed spinosad to colonies in pollen at 4 levels: control, 0.2 ppm, 0.8 ppm, and 8.0 ppm, during weeks 2 through 5 of the experiment. At concentrations that bees would likely encounter in pollen in the wild (0.2 to 0.8 ppm) we detected minimal negative effects to bumble bee colonies. Brood

and adult mortality was high at 8.0 ppm spinosad, about twice the level that bees would be exposed to in a 'worst case' field scenario, resulting in colony death two to four weeks after initial pesticide exposure. At more realistic concentrations there were potentially important sub-lethal effects. Adult worker bees exposed to spinosad during larval development at 0.8 ppm were slower foragers on artificial complex flower arrays than bees from low or no spinosad treated colonies. Inclusion of similar sub-lethal assays to detect effects of pesticides on pollinators would aid in development of environmentally responsible pest management strategies, and benefit crop productivity.

3.2 Introduction

Managed and wild bumble bees are important pollinators of crop plants and wild flowers (Corbet et al. 1991). However, few pesticides have been tested on bumble bees or other wild bees prior to commercial release. Wild bees are thought to contribute a substantial amount of pollination service to the approximately 30% of human food that results from bee pollination (O'Toole 1993). North American agriculture relies largely on imported, managed honey bees (*Apis melifera* L) for crop pollination (Kearns & Inouye 1997), and there has been little incentive to investigate the role of native, non-*Apis* pollinators. However, recent declines in feral and managed honey bee colonies due to parasites and disease have led to a growing concern over the state of potentially important unmanaged pollinators (Watanabe 1994; Allen-Wardell et al. 1998).

Populations of wild bees also may be declining in agricultural areas (Allen-Wardell et al. 1998), likely due to habitat loss, decreased plant diversity (Williams 1986; Banaszak 1996; Cane & Tepedino 2001) and increased pesticide use (Johansen & Mayer 1990). Newer generations of pesticides, such as microbial biopesticides, are thought to

be less harmful to humans and the environment than older, synthetic organophosphate, carbamate, and pyrethroid insecticides (Koul & Dhaliwal 2002). Yet in some of the few studies conducted to date, exposure to these newer, environmentally safer, pesticides has resulted in significant bee mortality in laboratory experiments (Miles et al. 2002).

Oral and acute toxicity tests on the domesticated honey bee are now commonly required prior to pesticide registration and commercial use in Canada and the United States. Yet, bees (Superfamily: Apoidea (Michener et al. 1994)) are a very diverse group, with 20 000 to 30 000 species from seven families world-wide, and range from solitary, to colonial, to primitively social species and the highly social honey bee. There have been few pesticide toxicity studies on any bees other than honey bees, yet bees from different genera and families likely differ widely in their vulnerability to pesticide exposure. Testing at least a few species from genera other than *Apis* would provide some knowledge of the sensitivity of other bees to commonly used insecticides.

Sub-lethal effects of pesticides may have significant impacts on bees and pollination in addition to more easily observable mortality, disrupting foraging and causing decreased pollination and/or bee reproduction. Adult bees perform complex behaviours to collect pollen and nectar and provision their offspring. Exposure in earlier life stages could affect development, resulting in negative impacts that would only be evident if studies were of long enough duration to monitor adult behaviour following larval exposure.

Spinosad (Dow AgroSciences) is a novel microbial biopesticide made from a mixture of spinosyn A and D, two of the main metabolites formed from fermentation of the actinomycete bacterium, *Saccharopolyspora spinosa*. Spinosyns are broad spectrum

insecticides, with activity against Diptera, Lepidoptera, Hymenoptera, Siphonaptera, and Thysanoptera (Salgado 1998; Sparks et al. 1998), yet have little effect on other insects, mammals, or other wildlife. Thus spinosad is classified as a reduced-risk pesticide by the U.S. EPA (Cleveland et al. 2002). Spinosad causes activation of the nicotinic acetylcholine receptors and alters the function of GABA-gated chlorine channels (Salgado 1998; Miles et al. 2002). Over-activation of the acetylcholine receptors is the primary cause of death, initially resulting in involuntary muscle contractions and tremors, and after long periods of exposure, paralysis and death (Salgado 1998). As of 2001, spinosad was registered in 37 countries for use on 150 crops (Cleveland et al. 2002). Application rates of spinosad range between 25 to 150 g AI/ ha to theoretical 'worst case', high volume sprays up to 540 g AI/ha (Miles et al. 2002).

Acute oral and contact toxicity studies have shown spinosad to be highly toxic to honey bees, bumble bees, alfalfa leafcutter bees, and alkali bees (Mayes et al. 2003). However, dried residues were not harmful to adult honey bees or larvae in laboratory studies, or to adults, brood, or foraging rates in field studies. Therefore, recommendations for spinosad application include allowing drying time before bee exposure. Greenhouse studies suggest that development of bumble bee brood may be impaired by spinosad (Kaneshi 2000; Mayes et al. 2003).

The purpose of our study was to assess the effects of spinosad on bumble bee (*Bombus impatiens* Cresson) colony health, adult bumble bee mortality, and foraging ability of adults exposed during larval development, mimicking realistic levels bees may be exposed to in the field in a controlled laboratory setting. We present a method for testing pesticide effects on bumble bees that could be applied to a wide range of

pesticides and, with modifications, on various bee species. We hypothesized that at low doses, bumble bee mortality and brood would not be affected by spinosad, but that larvae developing under exposure to spinosad might exhibit impaired foraging ability as adults. At high doses, we hypothesized that bee mortality would increase, and brood development and foraging ability would be negatively affected.

3.3 Materials and Methods

In the wild, mated bumble bee queens locate a nest site in the spring, collect nectar and pollen to provision the nest, and begin laying eggs. The first brood of eggs usually numbers five to ten, and develops into female worker bees. Once the first brood of worker bees begins foraging, the queen remains in the nest and continues to lay and incubate brood. Eggs generally are laid in or on a mixed mass of pollen and nectar, and, for *B. impatiens*, develop for approximately five days before they enter the larval or feeding stage (Cnaani et al. 2002). Worker larvae are fed pollen and nectar for approximately nine days, after which they enter the pupal stage which lasts for approximately ten days and receive no exogenous food. Adult bees consume little pollen, primarily collecting it to provision their brood.

The experiment was conducted from March to May 2004. A concentrated stock mixture of analytical grade spinosad (90.4%, Dow AgroSciences, Calgary, Alberta, Canada) and pollen from Planet Bee Apiaries, British Columbia, Canada (Food grade) was made using the following procedure. Because of the low solubility of spinosad in water, ground pollen and spinosad were mixed with HPLC grade acetone in a round bottomed flask. The flask was placed on a Rotovap for approximately 30 min to mix the contents and evaporate the acetone. The flask was then dried under vacuum suction at

room temperature for approximately 3 h to remove any remaining moisture. All handling of spinosad and mixing procedures involving spinosad were done in the dark or under red light due to a high rate of photodegredation. Complete drying of the pollen and spinosad in the stock mixture would mimic field residues that were dry prior to bee exposure (e.g., night time application). Because spinosad residues have been found to be much less toxic to bees if allowed to dry prior to bee exposure (Mayes et al. 2003), this aspect of our procedure was a 'best case' scenario. It is conceivable that wild bees could be exposed to wet residues if growers apply spinosad during daylight when the crop is in bloom, contrary to label recommendations, or if environmental conditions increase residue drying times.

Three treatment levels of spinosad were made by adding appropriate amounts of stock pollen mixed at 2 + 1 by weight with 30% sucrose solution mixture to achieve pollen patties containing 0.2, 0.8, and 8 mg/kg. Treatment control pollen patty was also made to feed to control colonies during times when treatment colonies were fed spinosad treated pollen. Treatment control pollen was mixed using the highest level of acetone (i.e., the same that was used for the 8 ppm treatment) created by the same methods as above, but with no spinosad. This was to ensure that any effect of possible acetone residues or some other aspect of stock pollen creation, other than spinosad addition, was mimicked in pollen fed to control colonies during treatment weeks.

We are aware of only one residue test examining spinosad levels in pollen post spraying. In sweet corn, with an application rate of 40 g AI/ha (Success 480SC formulation), residue levels of spinosad were 0.32 mg/kg (ppm) in pollen (Bailey 2004). Equivalent spray rates on different crops would likely result in highly different residue

levels in pollen. However, estimates using the available residue data, and spray rates (Miles et al. 2002), result in approximate realistic levels that could be found in pollen of field treated plants from 0.2 ppm (at 25 g AI/ha), 1.2 ppm (at 150 g AI/ha), to 4.3 ppm (at 540 g AI/ha: theoretical 'worse case' application rate) (Miles et al. 2002). New maximum use rates as of December 2004 are 216 g AI/ha (M. Miles, Dow AgroSciences, *personal communication*).

We obtained 28 bumble bee (*Bombus impatiens* Cresson) colonies from Biobest Canada Ltd (Leamington, Ontario, Canada) at the first brood stage (5 to 10 workers). Colonies were housed in plastic containers 20 x 28 x 18 cm, surrounded by an outer cardboard casing and equipped with a bag containing a nectar substitute that bees could access freely. The experiment was conducted for 10 weeks, beginning from initial receipt of colonies. Colonies were fed treatment pollen, *ad libidum*, during the second, third, fourth, and fifth weeks of the experiment. At all other times colonies were fed untreated pollen and sucrose solution *ad libidum*. At each feeding time, new pollen was weighed and recorded and old pollen was removed and weighed. We fed treated pollen for only four weeks of the colonies life in order to simulate a situation that wild bees could experience in an agricultural setting if foraging on a number of crops that were consecutively treated, and/or a single crop that received consecutive spinosad treatments. With this exposure schedule, we were able to mark and monitor a group of bees that we knew to have developed for their entire larval stage during treated pollen feeding. Weekly, visual estimates were made of the number of egg masses, larval cells, pupae, workers, queens, males, and dead bees in each colony. Colonies were monitored daily for newly emerged workers, conspicuous because of their white coloration in the first few

hours after emergence. Newly emerged workers were cooled at 4°C and weighed on an Ohaus Explorer electronic balance (Ohaus Company, Florham Park, New Jersey, USA) to 0.01 g.

Foraging ability of adult worker bees was tested on artificial arrays (Morandin & Winston 2003; Chapter 2) only if their entire larval stage overlapped with the pesticide feeding period (weeks 2 through 5). Colonies were connected to one of three mesh flight cages (1.2 x 1.2 x 1 m) and allowed to forage on 'simple' artificial flowers made from 1.5 ml clear micro tubes (Sarstedt, Newton, North Carolina, USA) with the caps removed. Tubes were filled with a 30% sucrose solution. On the second morning after colonies had been attached to the foraging cages, we conducted scan surveys, every 15 min for three hours, of the number of bees on the array, in flowers on the array, and flying within 30 cm above the array.

Worker bees making regular foraging trips were cooled and marked with a unique paint combination. After 5 to 10 foragers had been marked from a colony, all bees were returned to the hive, and the simple flower array was replaced with an array of 'complex' flowers designed from the same micro tubes as the simple flowers but with the caps left attached and bent over the opening of the tube leaving about a 7 mm opening. 2 µl of sucrose solution was put into each complex flower immediately prior to testing. One marked forager was released into the cage at a time and recorded on a videotape for the duration of at least 30 successful flower visits, defined as the bee completely inside the complex flower and able to access the sucrose solution. We collected data on the amount of time taken until the sucrose in the first complex flower was successfully accessed, handling time (total flower contact time) to access flowers one through 35, and foraging

rate for flowers 11 to 20 (experienced forager). Because of time constraints on the life of colonies, we were able to test bees from four to five of the seven colonies from each group.

Data analysis

All data analyses were done using SAS (SAS 1999). Pollen consumption per week was compared among treatments using repeated measures ANOVA with pollen consumption as the response variable and week as the repeated measure. Colony health data were analyzed using repeated measures ANOVA with number of workers, amount of brood, and dead bees as the response variables, and week as the repeated measure. In all repeated measures analyses, the interaction between treatment and week was included in the model. Pairwise comparisons of least squares means were conducted among treatments, within weeks, for each response variable. As a measure of brood viability, the number of worker bees emerging ('emergers') was estimated, each week starting in week 2, by the equation: $(CS_t + DB_t) - CS_{t-1}$, where CS = colony size, DB = the number of dead bees, and t = time in weeks.

Number of worker weights recorded each week was highly variable among colonies and treatments, depending on the number of newly emerged workers that could be found, and we therefore analyzed these data separately from colony health data using repeated measures ANOVA with treatment as the main effect and week as the repeated unit. Because of the unbalanced design, the test statistics did not follow an exact F distribution, so P values were estimated using an F approximation with fractional degrees of freedom (Satterthwaite approximation).

Number of bees observed in each cage during scan samples was totalled for each colony and divided by the estimated total number of worker bees to obtain a measure of proportional foraging force. Proportional foraging force was arcsine square-root transformed and compared among treatments using univariate ANOVA. Flower access times of bees were compared among treatments using repeated measures ANOVA (Satterthwaite approximation) with flower number as the repeated measure and colony as a random factor. Foraging rates were calculated as the total amount of time for a bee to access flowers 11 to 20, not including time spent in the colony if the bee returned to deposit nectar. Rates were compared among bees in different treatments using a mixed model ANOVA with colony as a random factor. Analyses were followed by comparison of differences of least squares means among treatments when an overall effect of treatment was found.

3.4 Results

3.4.1 Colony health

There was a difference in the amount of pollen consumed among treatments ($F_{4,24}$ = 137.17, $P < 0.0001$), with colonies in the 8.0 ppm treatment consuming the least amount of pollen per week (only significant difference of least squares means 0.8 ppm treatment vs. 8.0 ppm treatment; $t_{24} = 2.12$, $P = 0.045$). However, when we controlled for differences in colony size by adding the number of worker bees per colony as a covariate, there was no difference in the amount of pollen consumed per week among treatments ($F_{3,85.9} = 0.13$, $P = 0.939$). For the amount of brood, number of workers, and the number of dead bees each week, the 8.0 ppm treatment was significantly different than the other three groups. All colony health measures began declining in week four or five in the 8.0

ppm treatment, and by weeks eight and nine, there were virtually no bees or brood left in any of the colonies in this treatment. In four out of seven colonies in the 8.0 ppm treatment, the queen died before the end of the experiment, which did not happen in any colonies from the other treatments.

There was a significant treatment by week interaction in the number of worker bees per colony ($F_{27,207} = 6.00$, $P < 0.0001$). Overall, there were fewer workers in colonies from the 8.0 ppm treatment than in the other treatments (control vs. 8.0 ppm $t_{23} = 5.26$, $P < 0.0001$; 0.2 ppm vs. 8.0 ppm $t_{23} = 3.80$, $P = 0.0009$; 0.8 ppm vs. 8.0 ppm $t_{23} = 4.67$, $P < 0.0001$; Figure 3.1). The number of workers was not different among treatments until week 5, after which the number of workers declined significantly in the 8.0 ppm treatment and was different in weeks 5 to 10 from all other treatments. There was no significant difference in the number of workers at any time between colonies in the control, 0.2 ppm, and 0.8 ppm treatments.

There was a greater proportion of dead workers (dead workers week t/colony size week t-1) in colonies from the 8.0 ppm treatment than in colonies from the other treatment groups (control vs. 8.0 ppm $t_{23} = -5.79$, $P < 0.0001$; 0.2 ppm vs. 8.0 ppm $t_{23} = -4.76$, $P < 0.0001$; 0.8 ppm vs. 8.0 ppm $t_{23} = -4.68$, $P < 0.0001$; Figure 3.2). There were proportionally more dead workers in the 8.0 ppm treatment in weeks five, six, seven, eight, and nine than in the other three treatments. We did not compare number of dead bees from the 8.0 ppm treatment to the other treatments in week 10 because only one 8.0 ppm colony had more than three workers, and most had no bees.

Worker weights declined over the first four weeks of the experiment and then increased in week five in all treatments except in the 8.0 ppm treatment (Figure 3.3).

Because of the low number of new bees after week four in 8.0 ppm treatment, we were only able to obtain newly emerged worker weights for the first four weeks. Worker bee weights in the 8.0 ppm treatment were comparable to weights of worker bees from the other treatments, and because of the missing values after week four, we removed the 8.0 ppm treatment from analyses of worker weights. Worker bees that had been fed treated pollen during their entire larval development began emerging as adults in week five and continued through week 8 of the experiment. Worker weights ● SE in the control, 0.2 ppm, and 0.8 ppm treatments during these four weeks were 0.147 ● 0.007, 0.143 ± 0.005, and 0.134 ● 0.004 g, respectively. There was an interaction between worker weights and treatment ($F_{6,232} = 5.44$, $P < 0.0001$). From week five to eight, the slope of the relationship between weight and week was lower in the 0.8 ppm treatment than in the control and 0.2 ppm treatments.

The total amount of brood increased in all treatments from weeks one to three but then decreased in all treatments in week four (Figure 3.4). Between weeks four and six, the amount of brood generally stayed the same or slightly decreased in all treatments, and then from weeks seven to ten the amount of brood in the 8.0 ppm treatment continued to decline while the amount of brood in colonies from the control, 0.2 ppm, and 0.8 ppm treatments increased. From weeks one to six there was no difference among treatments in the amount of brood (all pairwise comparisons of least squares means > 0.05). From weeks seven to ten there was a significant difference in the amount of brood between the 8.0 ppm treatment and the control, 0.2 ppm, and 0.8 ppm treatments (pairwise comparisons of least squares means < 0.05). There were no differences among the control, 0.2 ppm, and 0.8 ppm treatments in the amount of brood at any time.

Closer examination of the number of eggs, larval masses, distinct larval cells, pupal cells, worker bees, and dead workers found each week in colonies from the 8.0 ppm treatment can provide some indication at which stage the bumble bee life cycle was affected by exposure to 8.0 ppm spinosad (Figure 3.5). In the 8.0 ppm treatment, the number of egg masses declined slightly over the course of the experiment; however, the mean number of egg masses remained similar to that in other treatment groups until week eight when the control, 0.2 ppm, and 0.8 ppm treatments began to increase. Egg masses did not appear to develop into larval cells after week two. The mean number of larval cells declined in week three, only one week into spinosad feeding, and never went above approximately three per colony. The mean number of pupal cells declined sharply between weeks three and four. Most of these pupal cells must have developed into adult bees as evident by the mean increase in colony size and number of dead adult worker bees found. Between weeks three and four, few larval cells developed into new pupal cells. These data taken together indicate that the queen bees in the 8.0 ppm treatment were continuing to lay eggs for three to four weeks after spinosad feeding began. However, few eggs developed into larvae. After one week of exposure at levels of 8.0 ppm spinosad in pollen, larval cells were not developing into pupal cells.

3.4.2 Foraging experiment

There were not enough worker bees in the colonies in the 8 ppm treatment to be included in this part of the study. Scan samples of the number of bees on or above flower arrays showed no difference in the foraging force among colonies from the three remaining treatments included in experiments below ($F_{2,11} = 0.16$, $P = 0.852$).

Mean handling times ± SE for each treatment, for flowers 1 to 35, was 2.8 ± 0.6, 2.9 ± 0.5, and 5.5 ± 0.5 for control, 0.2 ppm, and 0.8 ppm respectively. Repeated measures ANOVA on flower handling time, with flower number as the repeated measure, showed no interaction between the treatment and the flower number ($F_{68,1044} = 1.14$, $P = 0.206$). There was a treatment effect on handling times ($F_{2,31} = 7.84$, $P = 0.0018$) with bees in the 0.8 ppm treatment having longer handling times than bees from the control and 0.2 ppm treatments (Differences of least squares means control vs. 0.2 ppm $t_{31} = -0.11$, $P = 0.914$, 0.2 ppm vs. 0.8 ppm $t_{31} = -3.29$, $P = 0.002$, control vs. 0.8 ppm $t_{31} = -3.50$, $P = 0.0014$; Figure 3.6). Handling times ± SE during the flower 'learning phase' (flowers 1 to 10) was 3.6 ± 0.9 s, 4.3 ± 0.7 s, and 7.3 ± 0.8 s for the control, 0.2 ppm, and 0.8 ppm treatments respectively. There was no treatment by flower number interaction ($F_{18,279} = 0.79$, $P = 0.716$) but there was an effect of treatment ($F_{2,31} = 5.85$, $P = 0.007$). Bees from the control and 0.2 ppm treatments did not differ (difference of least squares means; $t_{31} = -0.59$, $P = 0.561$) and were both faster than bees from the 0.8 ppm treatment (control vs. 0.8 ppm $t_{31} = -3.08$, $P = 0.004$, 0.2 ppm vs. 0.8 ppm $t_{31} = -2.76$, $P = 0.010$). Separate analysis of flowers 11 to 35 ('experienced foragers') showed an interaction between flower number and spinosad treatment ($F_{48,734} = 1.46$, $P = 0.025$). Mean handling times ± SE were 2.5 ± 0.2 s, 2.4 ± 0.1 s, and 4.7 ± 0.2 s for bees in the control, 0.2 ppm, and 0.8 ppm treatments respectively.

Foraging rates were calculated as the total time taken to access 10 flowers (flowers 11 to 20 for every bee) and were longer for bees in the 0.8 ppm treatment (5.2 ± 0.7 min; overall $F_{2,32} = 4.49$, $P = 0.019$) than bees from the control treatment (3.0 ± 0.3 min; $t_{32} = -2.54$, $P = 0.016$) and 0.2 ppm treatment (3.35 ± 0.5 min; $t_{32} = -2.57$, $P = 0.015$)

colonies. Foraging rates were not different between the control and 0.2 ppm treatments (t_{32} = -0.21, P = 0.835). On a per flower basis, the foraging rates convert to approximately 18, 20, and 31 s for bees in the control, 0.2 ppm, and 0.8 ppm treatments respectively. Therefore on a typical foraging excursion in which a bee may successfully access 40 complex flowers, bees exposed to no or 0.2 ppm spinosad are estimated to require about 12 to 13 min, whereas bees exposed to 0.8 ppm spinosad would require about 21 min to access the same number of flowers (note that these estimates only involve a bee foraging on one flower type in one patch and do not include other activities such as flying to and from the nest site, and between patches).

3.5 Discussion

Spinosad at a level of 8.0 ppm in pollen was clearly detrimental to bumble bee colony health. The impact was evident first in the proportion of dead adult worker bees, which was greater than the other treatments by week four, two weeks after treatment feeding began. Egg laying by queens did not appear to be directly affected by 8.0 ppm spinosad treatment, but larval development was quickly disrupted to the extent that very few (less than four per colony) pupal cells formed after week three of the experiment. However, bees in the wild are unlikely to be exposed to levels of spinosad in pollen and nectar as high as 8 ppm at current recommended application rates; such a high rate of exposure would only occur if recommended spraying rates or times were not followed.

The only colony health measure suggesting that levels of spinosad within likely concentrations following normal field applications may affect bumble bee colonies was the lower worker weight of bees exposed to 0.8 ppm spinosad during larval development. Our results suggest that levels of spinosad in pollen of 0.8 ppm spinosad will have

minimal immediate ill effects on bumble bee colony health. These results agree with other studies on honey bee and bumble bee colonies that have found minimal to no effects on colony health of spinosad applied at low or medium application rates (Mayes et al. 2003). However, one study found that bumble bee colonies put into greenhouses 0 to 9 days following spinosad application to tomatoes at 120 g AI/ha (comparable to 0.8 ppm spinosad treatment in our experiment) showed some detrimental effects to bumble bee brood and they concluded that there may be a transient effect on bumble bee colonies in greenhouses sprayed with spinosad (Kaneshi 2000). Our procedure of drying the spinosad pollen mixture prior to re-hydration and feeding may have resulted in reduced effects compared to fresh, wet residues that are believed to have greater toxicity to bees (Mayes et al. 2003). Thus, situations in which spinosad residues do not dry prior to bee exposure may cause greater toxicity than our results indicate.

There was no avoidance of treated pollen as indicated by equal pollen consumption among treatments, although bees in our experiment did not have a choice between pollen with or without spinosad. In the wild, where there are multiple pollen sources, bees may avoid pollen containing spinosad. In a study on honey bees foraging in cages on *Phacelia tanacetifolia* Benth., treated with 144 and 540 g AI/ha spinosad, fewer bees were observed foraging on treated crops than on controls (Miles et al. 2002). There was no increase in bee mortality in the treated crops and it was suggested that spinosad residues may be repellent to honey bees.

Although colonies exposed to 0.2 and 0.8 ppm showed only minimal effects on colony health measures, bees that were exposed to 0.8 ppm spinosad during development showed impaired foraging ability on artificial flowers. They took longer to access

complex flowers, resulting in longer handling times and slower foraging rates. Studies on the mode of action of spinosad indicate that exposed insects experience hyper excitation of the nervous system, followed by inhibition of neural firing (Salgado 1998). This process results in initial involuntary muscle tremors followed by paralysis and death. These effects may result from disruption of nicotinic receptors and GABA-gated chloride currents (Watson 2001). The mode of action appears to be similar to insecticides in the chloronicotinoid family, such as imidacloprid, that bind to acetylcholine receptors, but spinosad works through a mechanism that is different from other known insecticides (Mayes et al. 2003). At high doses, imidacloprid causes foraging impairment in both honey bees (Schmuck 1999) and bumble bees (Morandin & Winston 2003; Chapter 2). We noted similar trembling in bees when they were foraging on arrays from the 0.8 ppm spinosad treatment as was observed in bumble bees exposed to 30 ppm imidacloprid foraging on arrays (Morandin & Winston 2003; Chapter 2). Trembling behaviour, most likely caused by excitation of the central nervous system (Salgado 1998), appeared to impair the bees' ability to land and enter the flower tube. In the 0.8 ppm spinosad treatment, we observed that the bees often would land on the lip of an artificial flower, tremble slightly and fall back, and then proceed to enter the flower tube. This behaviour was not observed in bees from the control or 0.2 ppm treatments.

The importance of the decrease in foraging ability that we observed in bumble bees from the 0.8 ppm treatment to colonies in the wild is difficult to assess. Resource availability may play an important role in determining if impaired foraging would be important to colony health and rearing of the reproductive caste. In areas of low resource availability in which colonies are marginally meeting nutritional requirements, any

decline in foraging efficiency of workers may result in lower reproductive output and consequently lower representation in subsequent years. Conversely, if resources are abundant, a decline in foraging efficiency may not have as significant an impact on colony survival or production of reproductive bees. In addition, decreased foraging rates could lead to pollination limitation and lower seed set (Kwon & Saeed 2003).

In summary, we found that spinosad at levels estimated to be twice the likely worst case exposure to bees in the wild resulted in complete colony death within seven weeks after commencement of a four week exposure period. Colonies exposed to more realistic field levels of spinosad in pollen did not show any lethal effects and only minimal immediate colony health effects. However, bees that had developed during their larval stage with 0.8 ppm spinosad treated pollen demonstrated impaired foraging on an artificial complex flower foraging array. Bees need to not only survive exposure to pesticides, but also forage effectively. Our results suggest that testing of novel pesticides should include measurement of sub-lethal foraging effects on adult bees that have come in contact with the pesticide in their adult and larval stage. As we demonstrated in this study, adult bees that have been exposed to a pesticide during larval development may display symptoms of poisoning that are not detected with current tests required by regulatory agencies. Pesticide exposure levels that have previously been thought to be safe for pollinators may prove harmful if larval-exposed adults are screened for sub-lethal foraging effects.

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3.7 Figures

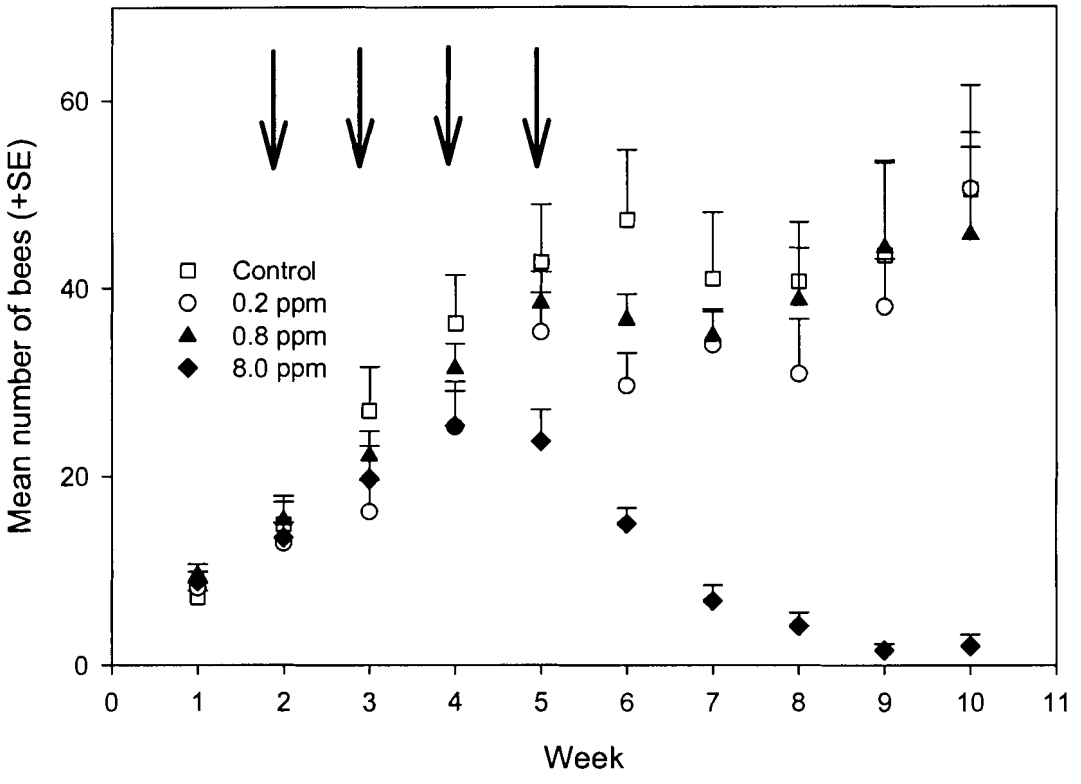

Figure 3.1 Mean number of worker bumble bees +SE in 28 colonies (seven per treatment) in four treatments: control, 0.2 ppm spinosad, 0.8 ppm spinosad, and 8.0 ppm spinosad. Treated pollen was fed to colonies ad libitum during weeks two to five of the experiment, indicated on the graph by arrows.

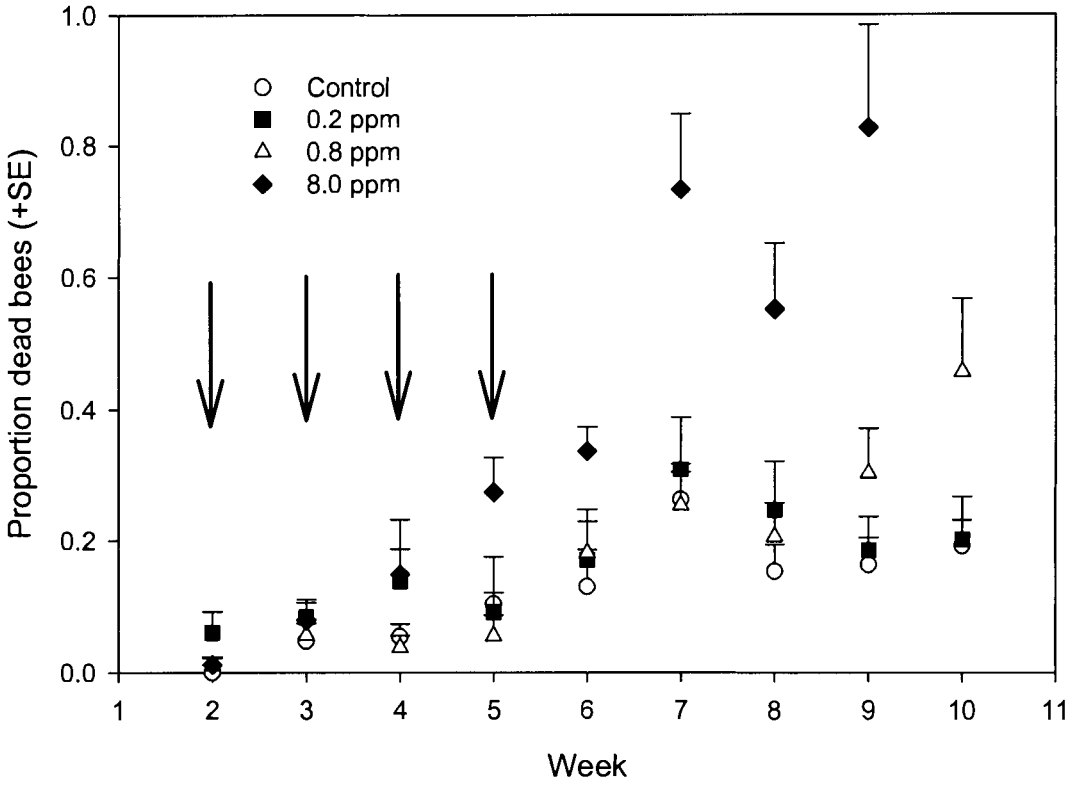

Figure 3.2 **Mean proportion of dead bumble bees +SE found in colonies in four treatments: control, 0.2 ppm spinosad, 0.8 ppm spinosad, and 8.0 ppm spinosad. Arrows indicate weeks where treated pollen was given to colonies.**

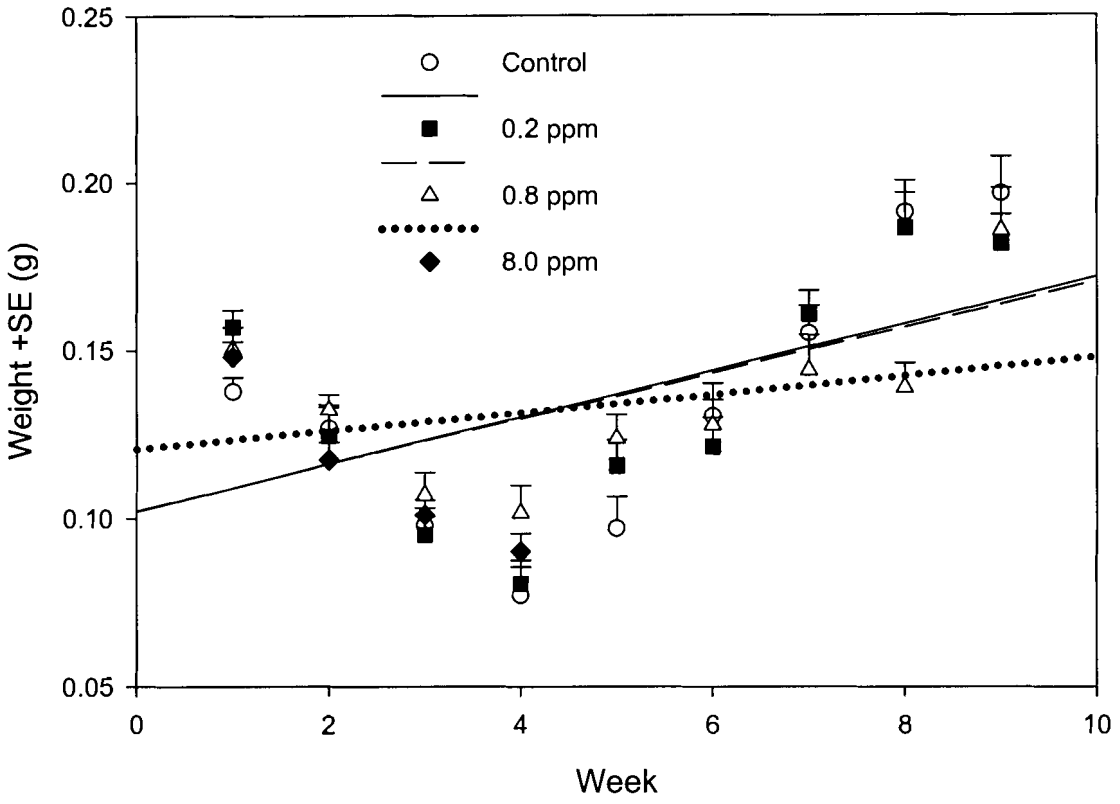

Figure 3.3 **Mean weights of newly emerged worker bees +SE in four treatments: control, 0.2 ppm spinosad, 0.8 ppm spinosad, and 8.0 ppm spinosad. Spinosad treated pollen was fed to bees from weeks two through five of the experiment and bees emerging in weeks five through eight were exposed to spinosad treatment during their entire larval development. Lines were calculated by simple least squares regression from weeks one to ten of the experiment.**

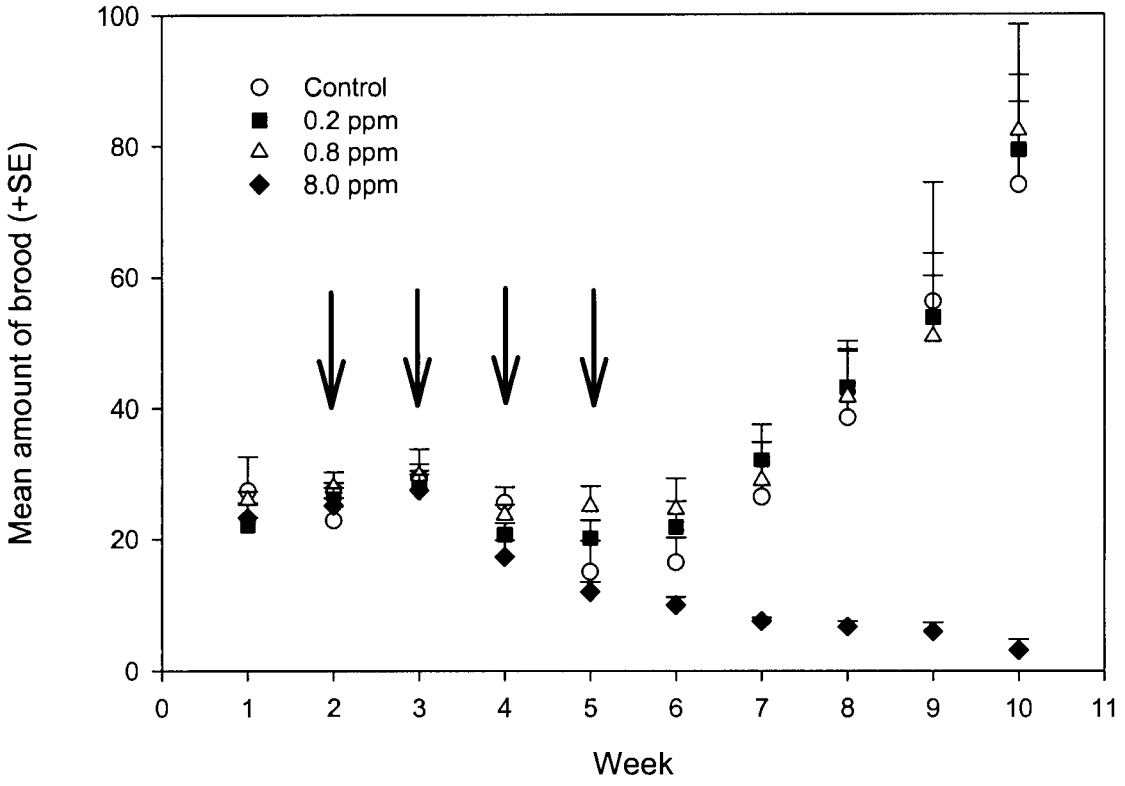

Figure 3.4 **Mean amount of brood +SE in bumble bee colonies in four treatments: control, 0.2 ppm spinosad, 0.8 ppm spinosad, and 8.0 ppm spinosad. The brood number is the estimated sum of egg masses, larval masses, larval cells, and pupae in the colonies. Arrows indicate weeks where treated pollen was given to colonies.**

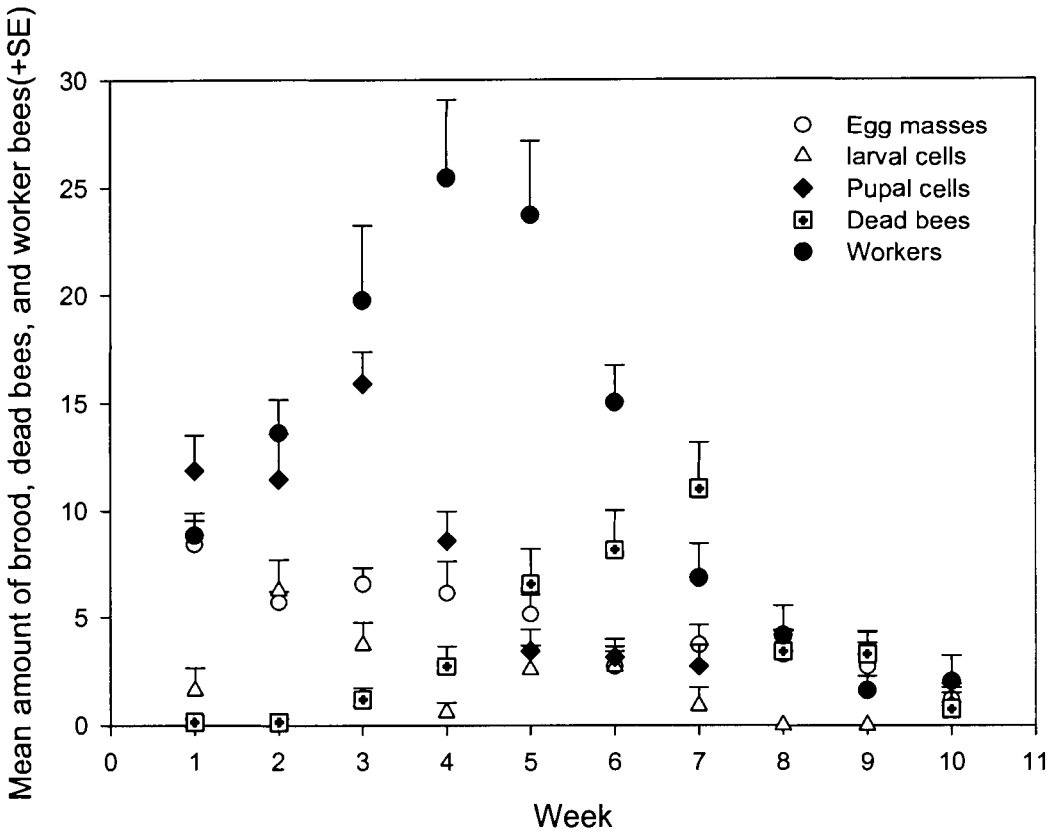

Figure 3.5 The mean +SE number of eggs, larval cells, pupae, worker bees, and dead worker bees each week from seven bumble bee colonies fed pollen with 8.0 ppm spinosad during weeks 2 to 5.

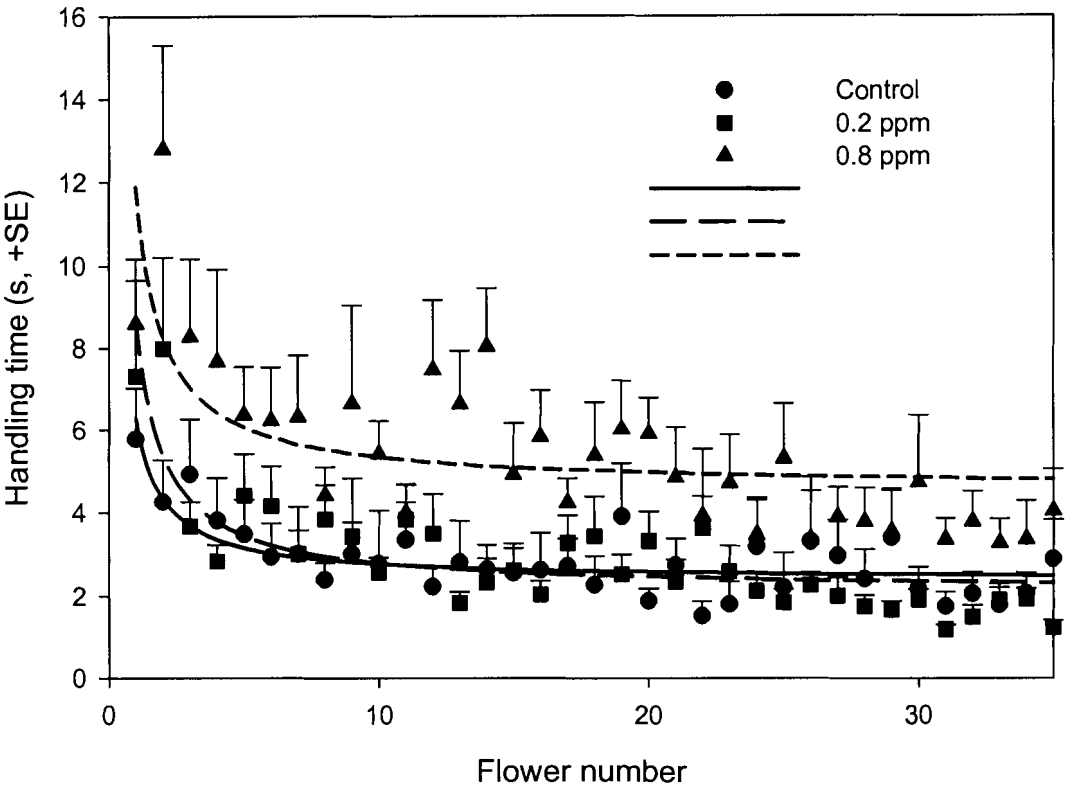

Figure 3.6 **Handling time (s) +SE of bumble bees on arrays of artificial complex flowers in three treatments: control, 0.2 ppm spinosad, and 0.8 ppm spinosad. Treated pollen was fed to bees during weeks 2 to 5 of the experiment and bee handling times are from adult worker bees whose entire larval stage overlapped with the treated pollen feeding period. Handling times were calculated as the total time that bees touched artificial flowers until they successfully accessed the sucrose solution in a flower. Bees were videotaped individually on the foraging arrays for approximately 35 flowers.**

CHAPTER 4
WILD BEE ABUNDANCE AND SEED PRODUCTION IN ORGANIC, CONVENTIONAL, AND GENETICALLY MODIFIED CANOLA

The following chapter has been published in Ecological Applications with Mark L. Winston as co-author.

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4.1 Abstract

The ecological impacts of agriculture are of concern, especially with genetically modified and other intensive modern cropping systems, yet little is known about effects on wild bee populations and subsequent implications for pollination. Pollination deficit (the difference between potential and actual pollination) and bee abundance were measured in organic, conventional, and herbicide resistant genetically modified (GM) canola fields (*Brassica napus* and *B. rapa*) in northern Alberta, Canada in the summer of 2002. Bee abundance data were collected using pan traps and standardized sweep netting, and pollination deficit was assessed by comparing the number of seeds per fruit from open pollinated and supplementally pollinated flowers. There was no pollination deficit in organic fields, a moderate pollination deficit in conventional fields, and the greatest pollination deficit in GM fields. Bee abundance was greatest in organic fields, followed by conventional fields, and lowest in GM fields. Overall, there was a strong, positive relationship between bee abundance at sampling locations and reduced pollination deficits. Seed set in *B. napus* increased with greater bee abundance. Because

B. rapa is an obligate out-crossing species, the lack of pollination deficit in the organic (*B. rapa*) fields likely was due to the high bee abundance rather than a lower dependence of *B. rapa* on pollinators than *B. napus* canola. Our study illustrates the importance of wild bees to agricultural production and suggests that some agroecosystems may better sustain wild bee abundance, resulting in greater seed production. Further research on why some cropping systems such as genetically modified herbicide resistant canola have low wild bee abundance would be useful for management of agroecosystems to promote sustainability of food production.

4.2 Introduction

One of the greatest challenges for ecologists and conservation biologists in recent years has been to understand the impact of established and novel agricultural systems on biodiversity. The onset of genetically modified crops has stimulated considerable research in this area, and in the process revealed large gaps in our knowledge concerning how conventional and alternative agroecosystems interact with the environment around them.

One vital area that has been particularly understudied is the relationship between agriculture and pollinator abundance, in terms of both agricultural impacts on biodiversity and the corresponding effects of diminished bee abundance on crop production (Allen-Wardell et al. 1998). This is important ecologically but also agriculturally, since lower pollinator abundance may lead to reduced yields. While there has been at least some research in conventional systems (e.g., Kremen et al. 2002), no studies have examined how agroecosystems having genetically modified (GM) crops

compare to other cropping systems in their effects on wild bee populations, and how interactions between bees and these new technologies relate to yield and crop production.

Pollination requirements of many crop plants are not well known, and the contribution of native bee communities is unclear (Kearns & Inouye 1997; Kevan & Phillips 2001). Approximately 66% of the world's crop species either benefit from or require animal pollination, primarily provided by bees, and fruit production resulting from animal pollination is essential for about 1/3 of human food in developed countries (O'Toole 1993).

There has been a suspected decline of native pollinators (Torchio 1990; Matheson et al. 1996; e.g., Allen-Wardell et al. 1998). Wild pollinator declines have been associated with low crop yields and even total crop failures (see Kevan 1977; see Allen-Wardell et al. 1998). Further, non-*Apis* bees are of incalculable value for pollination of natural vegetation. Paradoxically, expansion of agriculture both in size and intensity is reducing available foraging and nesting habitats for bees, which may result in increased pollination deficits and lower crop yields (O'Toole 1993; Kremen et al. 2002). Yet, information on the role of wild pollinators in agriculture and the effects of agricultural methods on pollinators is largely speculative, making research critical for understanding this interaction.

Although few studies have examined the relationship between pollinator communities and their environment, Potts et al. (2003) have recently shown a positive relationship between bee diversity and plant diversity (primarily annuals) in a non-agricultural setting. Comparisons of bee populations in natural or uncultivated areas and agricultural areas have found higher bee abundance and/or diversity in natural areas than

in agricultural ecosystems (Mackenzie & Winston 1984; Scott-Dupree & Winston 1987; Banaszak 1996; Calabuig 2001). Williams (1986) found that the number of bumble bees on crops was positively correlated with the crops' proximity to uncultivated land. Calabuig (2001) surveyed solitary bees and bumble bees in semi-natural areas within an agricultural landscape and found that plant species richness and cover in field edges and hedgerows was positively correlated with bee diversity. She suggested that continuity in pollen and nectar availability was beneficial for bumble bee colonies, while a high diversity of plant species could support a large number of oligolectic solitary bee species. In addition, many bees other than bumble bees and oligolectic bees would benefit from floral resources other than local crops in at least three situations: 1) if individual bees live longer than the blooming period of the crop, 2) if the bee's lifespan does not completely overlap with the crop bloom, or 3) if the crop's nectar or pollen does not supply the bee with adequate nutrition. Farming practices that reduce weed diversity in or surrounding crops may result in lower bee abundances and/or diversity (Osborne et al. 1991; Mand et al. 2002; Haughton et al. 2003), possibly lowering seed set.

Studies of pollination deficits of entomophilous (insect pollinated) plant species have been used to infer pollinator declines (see Thomson 2001). In a literature review of pollination supplementation experiments, Burd (1994) found that 62% of 258 wild plant species were pollen limited. Few data are available on pollen limitation of crop species, but a similar literature review found that 59% of 16 cultivars representing 11 species were pollen limited (Mayfield 1998). Comparison of pollination deficits across and within various agricultural cropping systems can provide insight into the abundance and efficacy of associated pollinator populations, and the dependence of a crop on insects for

pollen transfer and seed set. Significant differences in pollination deficit in different cropping systems may indicate that some types of agroecosystems better promote agriculturally beneficial pollinator populations.

Canola (*Brassica* spp.) is the fourth most important crop by acreage seeded and the most important oilseed crop in Canada (Statistics Canada 2003b). Currently, Canada's annual exports of canola seed, oil, and meal alone are valued at over CDN 2 billion dollars (CCC 2005). Approximately 50% of canola crops world-wide are transgenic herbicide-resistant (GM) (James 2004). Organic canola constitutes approximately 0.07% of the canola grown in Canada (Brooks & Barfoot 2004).

Canola flowers secrete large amounts of nectar and are very attractive to many wild bees, including species of *Andrena*, *Halictus*, and *Bombus*. Although the data are conflicting, and differ among canola varieties, there is evidence that insect visits increase canola yield (reviewed in Free 1993; Delaplane & Mayer 2000). There are two species of *Brassica* that have been developed into canola varieties, *B. napus*, or Argentinian canola and *B. rapa*, Polish canola. *Brassica napus* is self-compatible yet studies largely show that insect pollination increases seed production, whereas *B. rapa* is self-incompatible (an obligate outcrosser), and pollinator visits are required for seed production (Mishra et al. 1988; Zuberi & Sarker 1992; Free 1993; Delaplane & Mayer 2000). Zuberi and Sarker (1992) found that without adequate cross pollination, rape seed (*B. campestris* (*rapa*) var. Toria) could not produce high yields and cite multiple examples of similarly inadequate pollen transfer in *B. rapa* under open pollination conditions (Singh 1954; Zuberi & Sarker 1982; Zuberi et al. 1987).

Different cropping methods associated with GM, conventional, and organic canola may affect wild bee abundance in fields. Transgenic herbicide resistant canola fields can be treated with broad-spectrum herbicides after canola emergence, resulting in more effective weed control than in conventional systems. Organic canola growers primarily rely on pre-sowing tillage and fast growing canola varieties for weed management and consequently organic fields tend to have larger amounts and greater diversity of weeds than conventional and GM fields (Morandin et al. in review; Chapter 5). Other differences in cropping methods such as pesticide treatments and field size may also affect wild bee abundance and pollination in different types of canola fields. Chemical pesticide use in conventional and GM crops may cause lower bee numbers in these types of fields than in organic fields which tend not to have pesticide applications, or employ pesticides that are less toxic to bees. In addition, smaller fields, as is characteristic for organic crops (personal observation), may have more bees simply because there is less crop area in relation to uncultivated adjacent area.

We assessed pollination deficits in organic, conventional, and genetically modified canola (*Brassica* spp.) in relation to wild bee abundance as part of a study on the effects of agroecosystems on native bee diversity and abundance. We also examined the relationship between increasing distances into fields and wild bee abundance and seed production. We hypothesized that 1) native bee pollination was required for canola to reach full seed set, 2) different field types would differ in their bee abundance, 3) sampling locations with greater bee abundance, regardless of field type, would have a lower pollination deficit, and 4) bee abundance would diminish with distance into fields.

4.3 Materials and Methods

Data were collected during July – August 2002 near La Crete, Alberta, Canada (~58°N, 116 °E). The region is a patchwork of cultivated fields, boreal forest, and cattle farms and is adjacent to the Peace River. Four replicate fields were selected in each of three field types: organic, conventional, and GM for a total of 12 fields. Within field types, fields were matched for size and crop variety. Organic fields were *B. rapa* Reward (SeCan Association, Ottawa, ON, Canada) canola and certified organic by the Peace River Organic Producers Association (Dawson Creek, BC, Canada). Conventional canola fields were non-genetically modified *B. napus* HyLight Clearfield system 45A71 (Advanta Seeds, Winnipeg, MN, Canada) treated with the herbicide, Odyssey®. GM fields were *B. napus* Roundup Ready DK3235 (Monsanto, St. Louis, MO, USA), treated with the herbicide, Roundup®. In addition to herbicide treatment, conventional and GM fields were all treated once during bloom for pests, with the insecticide, Matador (Syngenta, Guelph, ON, Canada), with the exception of one conventional field and one GM field which were not sprayed with insecticides during bloom. Pesticide application dates were different among fields and we make no attempt in this paper to quantify direct effects of pesticide application on bee populations. No insecticides were used on any of the organic fields in this study. Because organic canola in our study area was *B. rapa*, it would have been more susceptible to pollination deficits under low pollinator conditions than *B. napus* canola, a self compatible species.

Conventional and GM fields were approximately 64 ha (quarter section, 800 x 800 m), and organic fields were smaller ranging from 20 to 50 ha. All fields began blooming within one week of each other (late June) and continued blooming until mid to

late July. Canola fields bloom for two to four weeks depending on location and environmental conditions. Fields were chosen so that treatment replicates were spread throughout the approximately 200,000 ha study location, in order to minimize spatial autocorrelation of treatments and possible confounding effects of environmental similarity. The La Crete, AB area of this study is only recently undergoing intense conversion of forested land to agricultural land and subsequently each study field was in close proximity (no greater than 800 m) to a forested area of at least 16 ha.

Sampling locations in each field were oriented in relation to an uncultivated hedgerow along one side of the field. Hedgerows all had trees and under storey vegetation and were at least 5 m wide. Sampling of bees and pollination limitation were conducted at the same distances from the hedgerow, at 20, 200, and 500 m into fields, with two sample locations at each distance, 200 m apart, for a total of six sample locations per field (Figure 4.1). Because organic fields were mostly smaller than conventional and GM fields, only one of the organic sites had 500 m field sampling locations. At most sites, only one side of a field had a hedgerow and the remaining sides were typically canola, other crops, or roads. Therefore, 500 m collection locations were 300m from field edges but usually 500 m from seminatural areas. However, we included distance from any field side (edge) as a factor in our analyses. To maintain equal sampling effort of bees among locations, fields without 500 m collection sites had one additional collection location at 20 and 200 m.

4.3.1 Pollen Limitation

At each sample location, supplemental pollination experiments were conducted in order to compare seed number in fruit from open (naturally) and supplementally

pollinated flowers (also referred to as control and experimental, respectively). We used methods modified from Zimmerman and Pyke (1988). Six pairs of plants were marked with flagging tape at each sampling location while the field was in full bloom. Within each pair of plants there was one control and one experimental plant and three pollination treatments: three control flowers on control plants (CC), three control flowers on experimental plants (EC), and three experimental flowers on experimental plants (EE), resulting in 18 CC, EC, and EE flowers at each sampling location and a total of 108 flowers per pollination treatment in each field. Three of the four organic fields did not have 500 m collection locations and in these fields the pollination experiment was conducted at four locations resulting in 72 flowers per pollination treatment in each field. Overall, 3,564 flowers were used in the experiment. If resources necessary for seed production are limited, fruits produced from open pollinated flowers on the same plants as supplementally pollinated flowers may have lower seed set (Zimmerman and Pyke 1988). Therefore, we incorporated controls on both experimental plants and on adjacent plants to ensure that differences in seed number between open and supplementally pollinated fruits were due to differences in pollen transfer and not resource availability. A greater number of seeds from fruit in the CC control treatment than in the EC control treatment would indicate that EC controls were suffering lower seed set as a result of shared resources with supplementally pollinated fruit and not from inadequate pollen transfer *per se*.

Stems of flowers were marked with different colours of non-toxic acrylic paint (DecoArt, Stanford, KY). EE flowers were supplementally pollinated with a mixture of pollen, collected with a paintbrush into a Petri dish, from 10 to 15 adjacent flowers, all

from different plants. This pollen mixture was then wiped onto the stigma of EE flowers. Seed pods (siliques) were collected no less than 12 d following supplemental pollination, and the numbers of seeds per silique were counted. Because some siliques could not be found at collection time, actual number of siliques collected was lower than the number of flowers marked.

4.3.2 Bee Collections

In the northern Canadian area of our study, there were few honey bee colonies and honey bees made up less than 2% of all bee captures, so consequently we were able to assess the importance of native bee populations to canola yields in different types of agroecosystems.

Bees were collected during canola bloom at each previously described location, from 02 July to 31 July, using pan traps and standardized sweep netting. Each field was sampled with pan traps once during the bloom for 48 hrs. Pans were left out longer if necessary to compensate for rain, which results in virtually no bee activity, so that all effective collection durations were as similar as possible. When possible, pan trapping was done concurrently at each of one organic, conventional, and GM site. One set of three (blue, white, and yellow), straight-sided, 30 x 50 x 20 cm pan traps was placed on the ground, with the tops of the traps approximately even with the lowest flowers on the racemes at each sampling location. Each pan trap had 1.5 L water, approximately 5 ml glycerol to lower surface tension, and 10 ml of honey. Bees were collected from traps and stored in 70% ethanol for later identification.

Two days of standardized sweep net samples were taken in each field, generally by three different people concurrently in one organic, conventional, and GM field, although it was not always possible to follow this design because of slight differences in the onset of bloom and travel times between fields. Sweep net samples were only conducted on days that were mostly sunny, when the temperature was above 18°C from the beginning to the end of the collection period (approximately 10:00 to 17:00 hrs). The collector followed a standard route between the previously described sampling locations in such a way that two collections were taken each day at each sample location, one at a time between 10:00 to 13:00 hrs, and the second from approximately 14:00 to 17:00 hrs. At each sampling location the collector walked a 30 m transect while making 100, 180° sweeps of the flowering vegetation with a 30 cm diameter sweep net.

4.3.3 Data Analyses

All analyses were done using SAS (SAS 1999). Across all field types least squares means of number of seeds per silique did not differ between CC and EC control flowers ($t_9 = -1.64$, $P = 0.137$) and hence open pollinated data were pooled. We categorized all siliques as either having seeds or having no seeds. The number of siliques in each category was compared between flower treatments within each field type in order to assess if there was a significant difference in the proportion of siliques that set seeds compared to the proportion that did not set seeds between open and supplementally pollinated flower treatments in each field type. Flower treatments were contrasted within field type using the Logistic Procedure (Wald Chi-Square) in SAS, with a binary logic link function for binomial distributions. We also categorized each set of siliques (set = 3 siliques on same plant, either CC, EC, or EE) from 1 to 4, with 1 = all siliques with

between 3 and 10 seeds, 2 = 2 out of 3 siliques with between 3 and 10 seeds, 3 = 1 out of 3 siliques between 3 and 10 seeds, and 4 = no siliques between 3 and 10 seeds. Silique sets were categorized based on 3 to 10 seeds per silique because there was a left hand tail in the histograms of seeds per silique of the open pollinated flowers that differed in the supplementally pollinated histograms in this range. This categorization may also be biologically relevant as it may correspond to siliques that were self-pollinated in self-fertile varieties (see discussion). Categories were compared across field treatments (organic, conventional, and GM) with respect to flower treatment (control versus experimental) using the Logistic Procedure (Wald Chi-Square), with a cumulative logit link function for multinomial distributions. Contrasts were conducted within each field treatment comparing flower treatment.

Silique seed number was compared between flower treatments (open and supplementally pollinated) and among distances (20, 200, and 500 m) using a type 3 sum of squares mixed analysis of variance model (Proc MIXED; Covariance structure = variance components) within field types. In this analysis we included individual plants, collection locations, and fields as random factors, controlling for lack of independence in the data. Plants were of different varieties in conventional and GM treatments and a different species in organic fields, as well as being subject to different cropping practices. Therefore pollination deficit (see below) rather than absolute seed numbers in siliques was used for most analyses to compare differences among field treatment types and in relation to bee abundance.

Pollination deficit was calculated as the difference in mean seed number per fruit between supplementally (N = 3) and open pollinated flowers (N = 6) for each plant pair.

Variation in pollination deficit among field treatments and distances were analysed using a type 3 sum of squares mixed analysis of variance model (Proc MIXED; Covariance structure = variance components), again including all main effects, interactions, and random factors. Where appropriate, orthogonal pairwise comparisons were conducted ("estimate statements" in Proc MIXED). We used separate residual analyses to determine if there was either an effect of distance from the designated hedgerow (always perpendicular to the 20, 200, and 500 m sampling locations) and/or distance from the closest edge on seed deficit. We first derived residuals from the relationship between deficit and distance from the closest edge while controlling for field treatment (Proc GLM). With the derived residuals we tested for a relationship between distance from the designated hedgerow and seed deficit (Proc GLM), expecting a negative trend if there was a relationship. A similar analysis was also performed on residuals from the relationship between seed deficit and distance from the hedgerow while controlling for treatment, and testing for an effect of edge distance (Proc GLM).

Absolute seed deficit values (number of seeds in supplementally pollinated siliques – number of seeds in open pollinated siliques) can be readily comprehended and are direct indicators of the contribution of pollinators to seed output and crop yield. However, because mean supplementally pollinated seed number differed among canola varieties and species, we include analyses to control for this factor. Proportional seed deficit was calculated as the number of seeds in siliques from open pollinated flowers divided by number of seeds in siliques from supplementally pollinated flowers. Some of the values of proportional seed deficit were greater than one and therefore, values were divided by two, the largest proportional seed deficit value, enabling us to normalize the

data with an arcsin square-root transformation. Proportional seed deficit data were analysed using a general linear model (Proc GLM). All reported data in graphs are absolute seed deficit values.

Bee abundances were calculated for each sampling location as the total number of bees collected in both pan traps and sweep nets. The total number of bees collected at each sampling location in each field was compared among treatments using a categorical model with Chi-square distribution statistics (Proc CATMOD) followed by pairwise contrasts of maximum likelihood estimates between organic, conventional, and GM fields.

To elucidate whether pollen limitation was related to differences among sampling locations in bee abundance, we regressed pollination deficit at each sampling location on the corresponding bee abundance across all field treatments.

4.4 Results

In all field types and flower treatments the maximum number of seeds per silique was between 35 and 40. The mean number of seeds per silique ± SE from open and supplementally pollinated flowers was 17.5 ± 0.36 and 18.8 ± 0.49, 16.2 ± 0.38 and 19.6 ± 0.42, and 17.7 ± 0.32 and 23.6 ± 0.31 in organic, conventional, and GM fields respectively. In conventional fields, the percent siliques with no seeds was much higher in control siliques (20.0%) than in experimental siliques (6.3%) (Contrast tests, Wald $X^2_1 = 37.77$, $P < 0.0001$). Similarly, the percentage of siliques with no seeds was much greater in control siliques from GM fields (10.3%) than from experimental siliques (0.5%) (Wald $X^2_1 = 20.26$, $P < 0.0001$). The percent siliques with no seeds in control

and experimental siliques was similar in organic fields, 11.5 and 9.6% respectively (Wald $X^2_1 = 0.96$, $P = 0.327$) (Figure 4.2). Across all field treatment types, there was a lower proportion of siliques with between 3 and 10 seeds from the open versus supplementally pollinated flowers (Wald $X^2_1 = 29.81$, $P < 0.0001$). However, there was a field treatment by flower treatment interaction (Wald $X^2_2 = 9.94$, $P = 0.007$). The difference in the proportion of siliques with 3 to 10 seeds between control and supplementally pollinated siliques was greatest in GM fields (Contrast tests, Wald $X^2_1 = 25.82$, $P < 0.0001$), followed by conventional fields (Wald $X^2_1 = 4.88$, $P = 0.027$), and there was no difference in organic fields (Wald $X^2_1 = 3.10$, $P = 0.078$).

4.4.1 Pollination Deficit

Across all field types, there was a strong effect of flower treatment (open pollinated control and supplementally pollinated) on the number of seeds per silique ($F_{1,9} = 28.73$, $P = 0.0005$). There was also an interaction between flower treatment and field treatment (organic, conventional, and GM) ($F_{2,9} = 4.49$, $P < 0.044$). There was no difference between supplementally pollinated and open pollinated flowers in organic fields ($t_9 = 1.12$, $P = 0.292$) but there was a difference in seed number between the two flower treatments in conventional ($t_9 = 3.31$, $P = 0.0091$) and GM ($t_9 = 5.47$, $P = 0.0004$) canola fields.

There was no relationship between distance from the hedgerow and pollination deficit (Figure 4.3). However, because the 500 m locations were only 300 m from the nearest edge of the field, we conducted analyses of residuals, controlling for either distance from closest edge or distance from the hedgerow, while controlling for

treatment. We found no relationship between seed deficit and distance from hedgerow or edge ($F_{1,394} = 0.24$, $P = 0.623$ and $F_{1,394} = 0.13$, $P = 0.723$, respectively).

There was significant variation in pollination deficit among organic, conventional, and GM fields ($F_{2,9} = 16.02$, $P < 0.0001$) (Figure 4.4). Pairwise comparisons showed a difference in pollination deficit between organic and GM fields ($t_9 = -5.02$, $P < 0.0001$) with a mean deficit per silique ± SE of -1.09 ± 0.63 seeds in organic and -6.07 ± 0.52 seeds in GM fields. The mean pollination deficit ± SE in conventional fields was intermediate between organic and GM at -3.70 ± 0.61 and different from the other field treatments (conventional vs. organic $t_9 = -2.58$, $P = 0.010$; conventional vs. GM $t_9 = -2.99$, $P = 0.003$). The mean percent seed set ● SE in open pollinated plants in each field treatment (number of seeds in siliques from open pollinated flowers divided by number of seeds in siliques from supplementally pollinated flowers for each plant pair) was 99% ± 4%, 84% ± 4%, and 78% ± 2% in organic, conventional, and GM fields respectively. There was no interaction between field treatment and distance from the hedgerow ($F_{4,15} = 0.44$, $P = 0.776$), or effect of distance on proportional deficit ($F_{2,15} = 0.95$, $P = 0.388$). Overall, there was a difference in the percent seed set of open pollinated flowers among field treatments ($F_{2,9} = 9.94$, $P < 0.001$), with the greatest proportional seed set in organic fields (organic vs. conventional $t_9 = 3.28$, $P = 0.001$; organic vs. GM $t_9 = 4.44$, $P < 0.001$). There was no difference between conventional and GM in percent seed set in open pollinated flowers ($t_9 = 1.41$, $P = 0.159$).

4.4.2 Bee abundance

The total number of bees collected in each treatment during bloom was 342, 230, and 101 bees with a proportion of bumble bees to other bees of 1.54, 4.17, and 0.38 in

organic, conventional, and GM fields, respectively. There was no effect of distance from the hedgerow by treatment interaction on bee abundance ($F_{4, 14} = 0.57$, $P = 0.690$) or distance alone on bee abundance ($F_{2, 14} = 1.29$, $P = 0.3073$). Therefore, although only one organic field had 500 m collection locations, data collected from these locations were not excluded from the analyses. Mean numbers of bees ±SE collected within fields were 85.5 ± 7.1, 57.5 ± 7.3, and 25.3 ± 6.5 in organic, conventional, and GM canola respectively, and were different among field types ($\mathbf{X^2} = 118.13$, $df = 2$, $P < 0.0001$). Pairwise comparisons showed that each field treatment was different from the others (organic vs. conventional $\mathbf{X^2} = 21.64$, $df = 1$, $P < 0.0001$; organic vs. GM $\mathbf{X^2} = 116.00$, $df = 1$, $P < 0.0001$; conventional vs. GM $\mathbf{X^2} = 47.53$, $df = 1$, $P < 0.0001$) (Figure 4.4). Species composition and population diversity will be described in a future publication.

The number of bees collected at each sampling location in each field was used as an index of bee abundance and regressed with pollination deficit at each location. Within each field, bee abundance and pollination deficit data were averaged between replicates, and there was a highly significant decrease in pollination deficit with increasing bee abundance among all field treatment types (inverse exponential decay regression; $y = -8.71e^{(-0.05x)}$, $r^2 = 0.56$, $F_{1, 31} = 40.08$, $P < 0.001$) (Figure 4.5). When *B. napus* varieties (conventional and GM) were analysed, excluding *B. rapa* (organic), again there was a highly significant relationship between bee abundance and pollination deficit ($y = -8.72e^{(-0.05x)}$, $r^2 = 0.48$, $F_{1,22} = 20.57$, $P < 0.001$).

4.5 Discussion

Supplementally pollinated flowers in conventional and GM sites produced siliques with more seeds than adjacent open pollinated flowers, suggesting that 1) yield in

B. napus canola in the Peace River region could benefit from increased bee-mediated pollen transfer, and 2) there were not enough bees and/or other pollinators in the conventional and GM sites to produce full seed set.

Brassica napus is self-fertile, yet insect pollination can increase seed set (summerized in Free 1993) and/or density of siliques (Manning & Boland 2000). However, the degree to which insects increase seed production is variable, possibly due to different cultivars tested, different environmental conditions, and different experimental methods. In this study, GM fields with *B. napus* DK3235, supplemental pollination caused a 33% increase over open pollinated flowers, in seeds per silique, and there was a 21% increase in conventional *B. napus* 45A71. Thus, pollen transfer by wild pollinators was not sufficient for the canola in these fields to reach their full yield potential.

The *B. napus* in our conventional and GM fields were different varieties, and therefore the greater seed deficit in GM fields could have been due to a higher dependence on pollinators for pollen transfer and seed set than the conventional variety examined. However, from our data, it seems unlikely that the conventional canola variety had a lower requirement for pollinators because at collection sites with low bee abundance, pollination deficit values were comparable to pollination deficit values in GM fields with similar pollinator abundances (see Figure 4.5). Our data suggest that the low number of pollinators in the GM fields resulted in the high pollination deficits. Pollinator exclusion experiments would be required to directly test the pollinator requirements of these canola varieties.

In contrast, we found no pollination deficit in organic fields. Since organic canola was *B. rapa*, we were not able to make direct comparisons of absolute seed numbers with GM and conventional canola. However, because *B. rapa* is self incompatible (Ohsawa & Namai 1987; Mishra et al. 1988; Zuberi & Sarker 1992), we predicted that *B. rapa* would be more vulnerable to pollination deficits under inadequate pollinator conditions. Lack of difference in seed number between open pollinated and supplementally pollinated flowers in organic canola was likely a result of sufficient bee numbers to produce full seed set.

The pattern we found in the proportion of siliques with no seeds between open and supplementally pollinated flower treatments showed a much greater effect of supplemental pollination in conventional and GM fields than in organic fields. In the organic fields, the proportion of siliques with no seeds was similar between open and supplementally pollinated siliques (11.5% verses 9.6%) suggesting that the proportion of siliques with no seeds had little to do with lack of pollen transfer. The high proportion of siliques with no seeds from flowers that were supplementally pollinated in organic fields was not anticipated and requires some explanation. In some fields we observed high levels of lygus bug (*Lygus* spp.), a sucking insect which feeds on the sap of reproductive tissue, causing damage to siliques and seeds in canola. No pesticides were used in the organic fields in our experiments and lygus bug damage appeared to be substantial, likely causing the relatively high proportion of siliques with no seeds in both open and supplementally pollinated organic flowers. We made no systematic observations of lygus bug infestation, and although lygus damage is a plausible explanation for the similarly high proportion of flowers that did not set seeds from open and supplementally pollinated

flowers in the organic fields, there are a number of other explanations including a possible lack of nutrients in organic fields resulting in seedless siliques, or higher rates of silique failure could be a characteristic of the *B. rapa* variety that we examined.

The larger difference in the proportion of siliques with no seeds between open and supplementally pollinated flowers in conventional (20.0% vs. 6.3%) and GM (10.3% vs. 0.5%) fields suggests that approximately 69% of the siliques with no seeds in conventional and 95% of siliques with no seeds in GM fields were a direct result of lack of pollen transfer. This is a marked contrast to the organic fields where the proportion of siliques with no seeds had little to do with pollen transfer. The lower response of siliques with no seeds to pollen transfer in conventional fields than in GM fields may have been a result of greater lygus bug damage in conventional fields. However, other explanations include resource limitation, or greater competition with weeds in conventional fields. Conversely, in GM fields, our data indicate that lack of pollen transfer was the primary cause of siliques with low and no seeds.

We found a relatively equal number of seeds per silique from open pollinated flowers in organic, conventional, and GM fields. One interpretation is that there was a similar 'pollinator force' in all field types. However, for a number of reasons, this does not appear likely. We measured greater pollinator abundance in organic, followed by conventional, and lowest in GM fields. Organic canola is self-incompatible, it would therefore require a greater pollinator force to achieve the same number of fertilized ovules as a self-compatible species. In addition, greater lygus bug damage and/or other factors not related to pollinator abundance, as discussed above, caused reduced seed set in organic and conventional fields. Similarly, the lower mean number of seeds per silique

from supplementally pollinated flowers in organic and conventional fields than in GM fields resulted from a larger proportion of siliques with under 11 seeds and was likely not a result of lower potential seed set given ideal conditions (see Figure 4.2). Conventional and GM canola are partially self-fertile and the high proportion of siliques with 3 to 10 seeds in the open pollinated treatment versus the supplementally pollinated treatment may have resulted from flowers that were self pollinated in the absence of insect-mediated pollen transfer.

The 'diminishing' relationship we found between bee abundance and pollination deficit across all fields suggests that seed set increased with bee pollination within canola varieties, up to a threshold. Across field types, pollination deficits approached zero with a bee abundance index above approximately 20 bees per sampling location, suggesting a threshold level for bee abundance sufficient for full pollination in both *B. napus* and *B. rapa* fields. Similar diminishing returns relationships have been found between contact duration of the drone fly, *Eristalis tenax* L. on sweet pepper flowers and fruit quality (Jarlan et al. 1997), and in greenhouse tomatoes pollinated by bumble bees (*Bombus impatiens* Cresson) between anther cone bruising levels (a measure of extent of bumble bee contact) and fruit quality (Morandin et al. 2001). In *B. napus* canola fields we found a striking, positive relationship between bee abundance at sampling sites and pollination deficit, with 48% of the variation in pollination deficit attributable to differences in bee abundance, suggesting that fine scale differences in bee abundance in the fields we tested were associated with measurable differences in pollination levels.

Canola is Canada's most important oil seed crop, and honey bees are sometimes used to supplement pollination and increase plant yields. However current declines in

managed honey bee colonies and increasing demands due to agricultural expansion are focusing attention on the contribution of wild bee populations to crop yields. Our findings support recent concerns over the economic consequences of native bee declines (e.g., Westerkamp & Gottsberger 2000; e.g., Kevan & Phillips 2001). The northern area where our research was conducted is a patchwork of agricultural acreage, logged areas, forest reserves, and regenerating forest. Our research suggests that native pollinator abundance in organic canola fields is adequate for seed set while in conventional and GM fields it is not. MacKenzie and Winston (1984) and Scott-Dupree and Winston (1987) examined pollinator diversity and abundance in berry and orchard crops, and in adjacent uncultivated areas. In both studies they found that wild bee abundance and diversity was greater in the uncultivated areas than on the crops, and similar to our findings in conventional and GM canola, they concluded that pollinators were not abundant enough in the crop areas to provide full pollination.

Our results also suggest that flight distances for wild bees were sufficient for pollination throughout the canola fields we studied, since we found no relationships between bee abundance or seed deficit, and distance into the fields. However, our sites had abundant adjacent uncultivated areas in which wild bees could nest. Research in regions with larger crop acreages and fewer nesting opportunities near fields might reveal different patterns. In addition, bumble and other bees have very different foraging ranges from each other and species composition could change with distance into the field (Calabuig 2001).

Semi-natural habitat is thought to benefit bumble and other bees by providing nesting and continuous, diverse foraging resources in agricultural landscapes (O'Toole

1993; Corbet 1995; Dramstad & Fry 1995). Kremen et al. (2002) found that areas of intense agriculture remote from semi-natural areas have lower pollinator diversity and abundance, insufficient for adequate pollination of watermelon (*Citrullus lanatus*). In their study, organic watermelon farms in close proximity to natural habitat had their pollination requirements met by wild pollinators without supplementation from honey bees, while organic and conventional sites far from natural habitat did not receive adequate pollination.

In the present study, all of our fields had native vegetation nearby (hedgerows and forested). Canola fields were in bloom from two to a maximum of four weeks, making it likely that a single canola field might not have provided enough pollen and nectar resources for bees with life spans longer than bloom, or bees without complete overlap of life and crop bloom timing. Thus, hedgerows, other uncultivated areas, and in-field weeds may provide vital foraging resources pre- and post-bloom. However, one study has found that semi-natural areas are not as important resources for bumble bees as they may be for other bees, possibly because bumble bees' large foraging ranges allow them to access multiple mass flowering crops that flower successively (Westphal et al. 2003). Further work is needed to assess the importance of landscape-level factors and cropping system differences on bee abundance, diversity, and community structure in agroecosystems.

Although insecticide treatments were similar between GM and conventional fields, GM fields were treated with Roundup®, a highly effective herbicide, which resulted in lower weed diversity and abundance within GM fields than in conventional fields (unpublished data), possibly affecting bee abundance. Williams (2002) suggested

that herbicide tolerant crops such as oilseed rape (*B. napus* and *rapa*), because they employ more effective weed control strategies than non-GM rape, will possibly reduce weedy and non-weedy farmland plants causing a reduction in food resources for insects, including bees. The recent Farm Scale Evaluations in Europe (Firbank et al. 2003), are the first large-scale studies comparing GM herbicide tolerant crops to their conventional counterparts. They found that weed diversity, biomass, and bee abundance were lower in GM herbicide tolerant spring oilseed rape (*B. napus*) than in conventional varieties (Haughton et al. 2003; Heard et al. 2003). They proposed that the lower bee numbers in GM herbicide tolerant varieties was an indirect result of herbicide treatments that effectively reduced weeds, and consequently forage for bees. Because organic canola growers in our study relied solely on pre-seeding tillage for weed control, organic fields had the greatest weed diversity and abundance (unpublished data; Chapter 5). In addition, the smaller organic fields may have resulted in greater bee densities simply as a consequence of similar bee source areas (hedgerows and forest) supplying smaller field areas. However, this would not account for differences found between GM and conventional fields which were of similar size. We currently are exploring these and other factors.

Forested regions in our study area are rapidly being cleared and converted to agriculture. The demonstrated limitation in seed set in *B. napus* caused by pollinator scarcity may, over time, become more pronounced as northern agricultural areas become farther removed from natural ecosystems and weed control technologies are further developed. Long-term studies of this and similarly changing regions will be important in determining the importance of wild pollinators to agriculture and food production.

Our study has demonstrated an interesting pattern where wild bee abundance is related to improved crop yields, but a genetically modified crop variety designed to improve yields through weed management might have the undesired consequence of reducing bee abundance in the field. However, it is important to note that other factors may be correlated with field type and be as important to bee abundance. For example, organic farmers tend to locate farther from established farm areas in order to satisfy minimum distance regulations regarding proximity to GM fields, possibly resulting in greater amounts of semi-natural habitat around fields. It is vital to explore these interactions further over a number of years at multiple locations before making broad generalizations concerning particular agroecosystem interactions with pollinator communities. Nevertheless, our research highlights an interaction in which cropping systems may influence bee distribution and abundance within fields, and in turn pollinator deficits may result in decreased yields. Further studies would clearly be of interest for both ecologists and agronomists.

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4.7 Figures

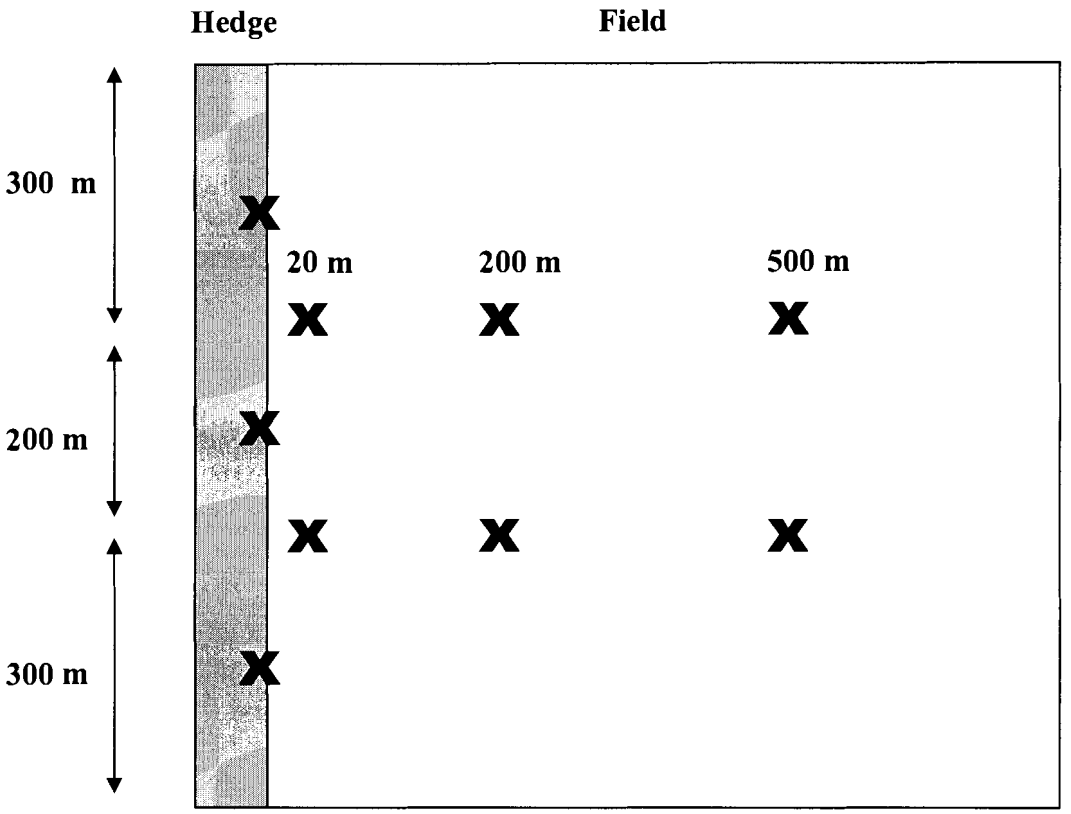

Figure 4.1 **Bee and pollination sample locations in canola fields. The dark region on the left represents a semi-natural hedgerow located at the side of each field from which sample distances were measured.**

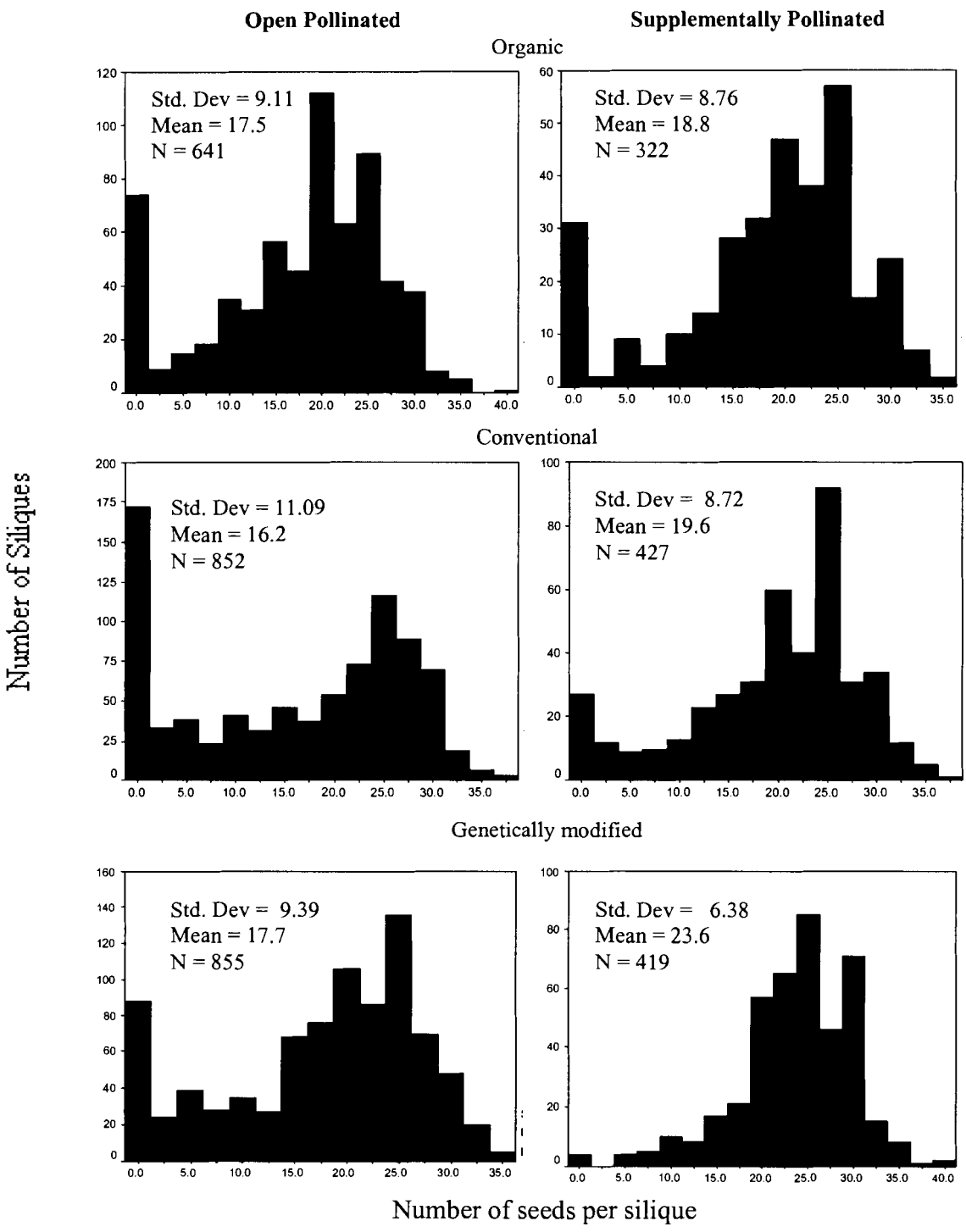

Figure 4.2 Histograms of the number of siliques versus seed number per silique from flowers that were open pollinated (left hand graphs) and from flowers that were supplementally pollinated (right hand graphs) in three types of canola fields, organic (*Brassica rapa*), conventional (*B. napus* 45A71), and genetically modified (*B. napus* DK3235).

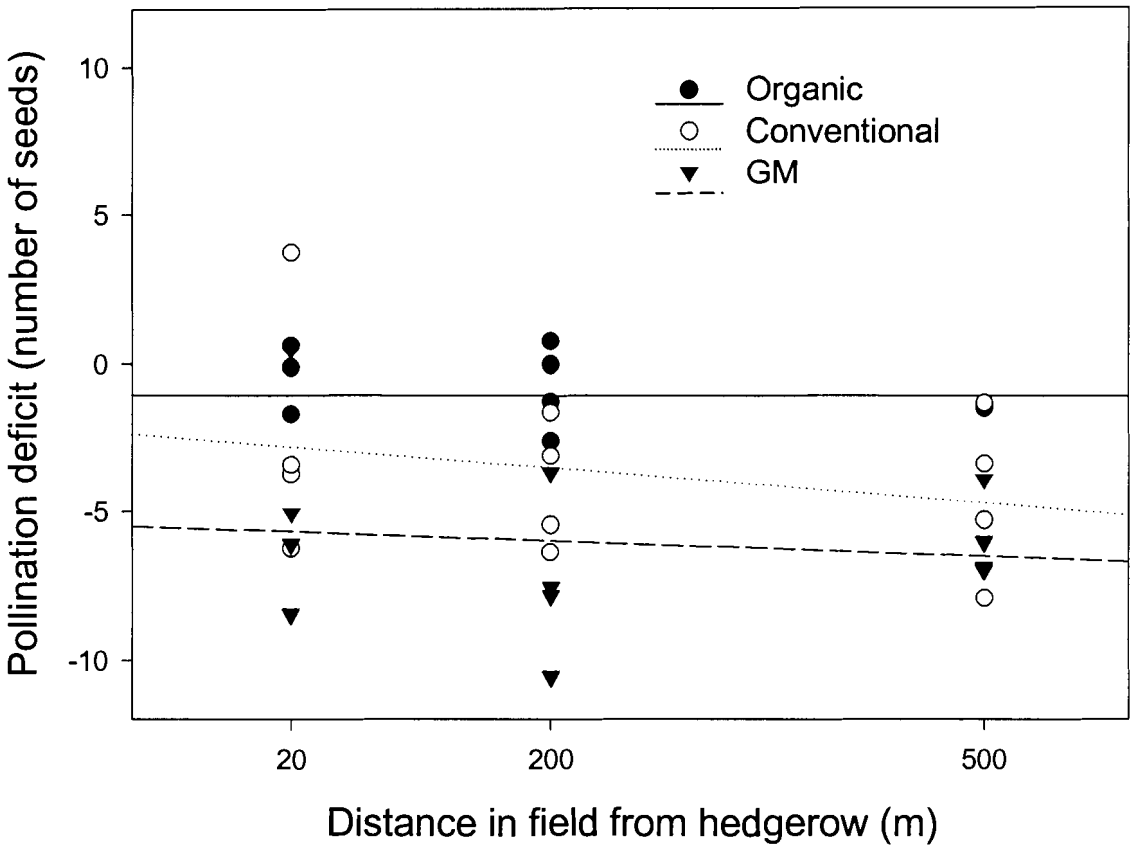

Figure 4.3 **Pollination deficit measured as the difference in the number of seeds from siliques between open and supplementally pollinated canola flowers at different distances into four replicate organic, conventional, and genetically modified fields. $P > 0.05$ for all regressions.**

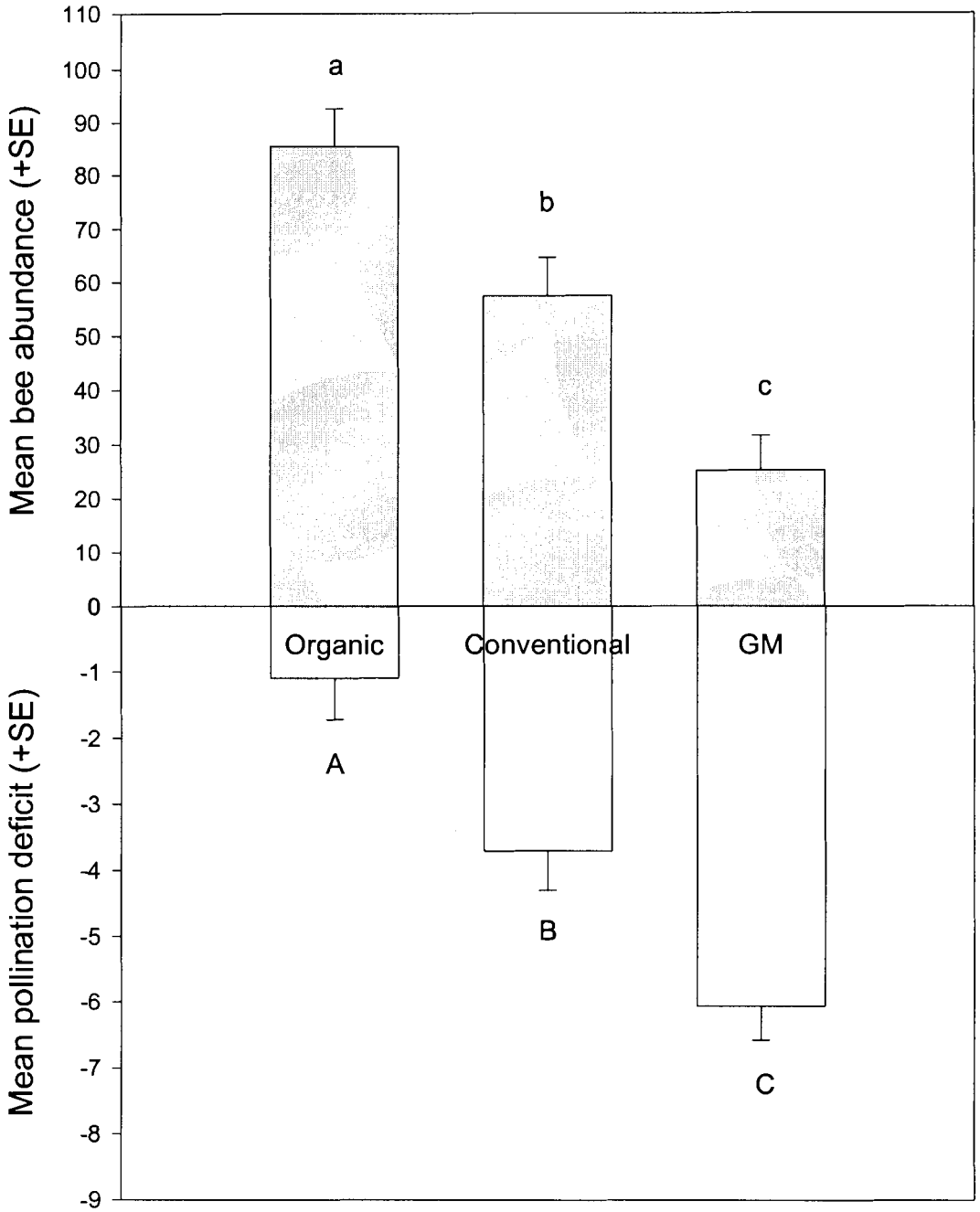

Figure 4.4 **Mean bee abundance and mean pollination deficits for each field type (n = 4). Different small case letters above bars indicate a difference in bee counts at P < 0.05 among field types (CATMOD followed by pairwise contrasts of maximum likelihood estimates: SAS 1999). Different capital letters below bars indicate different levels of pollination deficit at P < 0.05 between field treatments (Proc MIXED followed by comparison of least-squares means: SAS 1999).**

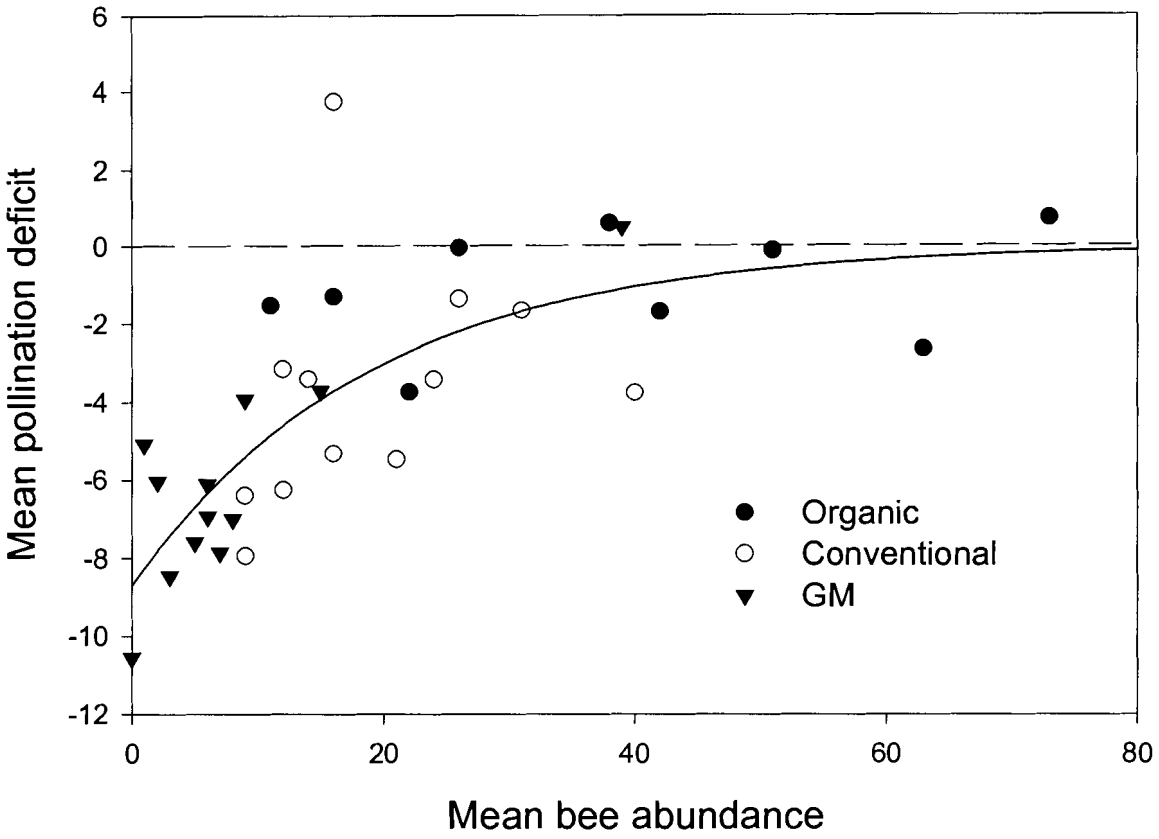

Figure 4.5 **Mean pollination deficit in canola flowers calculated as the difference in the number of seeds per silique in supplementally and open pollinated flowers. Means of pollination deficit and bee abundance are from two replicate distances in each field at 20, 200, and some 500 m distances from a chosen hedgerow. The best fit regression was with an inverse exponential decay function for all field types combined (organic: *Brassica rapa*, and conventional and genetically modified: *B. napus*. $y = -8.71e^{(-0.05x)}$, $r^2 = 0.56$, $F_{1, 31} = 40.08$, $P < 0.001$) and when the *B. napus* fields were analysed alone ($y = -8.72e^{(-0.05x)}$, $r^2 = 0.48$, $F_{1,22} = 20.57$, $P < 0.001$).**

CHAPTER 5
INFLUENCE OF LOCAL AND LARGE SCALE FACTORS ON WILD BEE ABUNDANCE AND DIVERSITY IN CANOLA AGROECOSYSTEMS

In review in Conservation Biology with Mark L. Winston and Terry Griswold as co-authors.

5.1 Abstract

Wild bees are essential pollinators of many crop plants, increasing crop yields, yet little is known about how local habitat and landscape scale factors affect their diversity and abundance. We examined wild bee populations in organic, conventional, and genetically modified (herbicide tolerant) canola (*Brassica* spp.) agroecosystems in relation to local (field-scale) and landscape-level habitat characteristics. Bees were collected in canola fields and adjacent hedges during canola bloom in northern Alberta, Canada in 2002 and 2003. At a local scale, we quantified flowering plant species richness and cover in hedges adjacent to fields and within fields. We classified surrounding land as either cultivated or semi-natural, at increasing scales from 250 m to 1500 m distances from field edges. Bee abundance, species richness, and diversity were found to be greatest in organic fields, lowest in GM fields, intermediate in conventional fields. Of the local scale variables analysed, weed cover and species richness in hedges and fields, weed cover in fields was the most predictive of bee abundance and species richness in fields. On a landscape scale, the amount of uncultivated land 750 m from edges of fields was positively correlated with bee abundance and bee species richness in

fields. Synthesis of local and large scale variables showed that bee abundance in fields was best predicted by the amount of uncultivated land within 750 m from edges and by weed cover in fields. Bumble bees and other wild bees were affected similarly by local- and landscape-level factors. Results from a number of studies support an emerging, and remarkably robust, conclusion that landscape scale differences in the amount of natural or semi-natural land around fields have a large impact on wild bees and crop production. In agriculture with highly effective weed control, maintenance of uncultivated land in surrounding areas could promote greater numbers of wild bees in fields, enhancing crop yield through increased pollination.

5.2 Introduction

Natural ecosystems provide services that are crucial to sustaining the biosphere for human populations (Costanza et al. 1997; Matson et al. 1997). Agricultural expansion into natural areas and intensification of agricultural land-use has resulted in major disruptions to ecosystem function (Matson et al. 1997; Tilman et al. 2002; Swift et al. 2004) and threatens the sustainabilty of high-productivity agroecosystems (Matson et al. 1997). Ecosystem services usually do not benefit growers directly and therefore increased production commonly occurs at the expense of natural ecosystems (Tilman et al. 2002). Agroecosystems generally are poorly monitored, making it difficult to assess economic value of conservation practices (Daily et al. 2000).

Pollination by wild insects is one ecosystem service that provides economic benefits directly to growers and is relatively easy to monitor and quantify (Tilman et al. 2002; Kremen et al. 2004). However, the spatial scale at which pollination services operate is not as clear. Most studies in agricultural systems focus on environmental and

biotic interactions at a local scale, within fields, and neglect surrounding habitat at a landscape scale that may be important for understanding species patterns (Swift et al. 2004). Most pollinators utilize resources at a landscape scale, so that understanding pollinator populations requires assessments of both local and landscape variables (Steffan-Dewenter et al. 2002).

Wild insects are responsible for 20 to 80% of commercial pollination (Southwick & Southwick 1992; Ingram et al. 1996). Approximately 66% of the worlds crops either require or benefit from insect pollination, primarily bees, and about 30% of human food is a direct result of bee pollination (O'Toole 1993). Most bee-pollinated crops depend upon managed honey bee (*Apis mellifera* L.) colonies, and in the United States two million colonies are rented out annually to growers during bloom (Free 1993). Recently, managed honey bee populations experienced serious declines due to newly introduced mite parasites, diseases, and the encroachment of Africanized honey bees (Matheson et al. 1996). With the decline of managed honey bees, and concerns over the stability of crop production, increasing attention has focused on wild pollinators (e.g., Allen-Wardell et al. 1998).

Historically, pollination services provided by wild bees may have gone unnoticed, since bee populations likely were plentiful in agricultural areas, acreages were small, and pollination deficiencies non-existent or unremarkable. Declines in wild bee populations resulting primarily from pesticides and habitat degradation have raised concerns over the future of crop pollination and production (Banaszak 1978; Allen-Wardell et al. 1998). Recent studies in watermelon (Kremen et al. 2002; Kremen et al. 2004), canola (Morandin & Winston 2005; Chapter 4) and coffee (Ricketts 2004; Ricketts et al. 2004)

agroecosystems have shown that crop production is greater in areas that have more abundant and/or diverse wild bee populations.

At the local scale, variation among cropping systems associated with common agricultural practices such as organic farming, farming with genetically modified plants, and conventional farming may cause differences in bee communities (Kremen et al. 2002; Haughton et al. 2003; Morandin & Winston 2005 (Chapter 4)), possibly resulting from differential pesticide use, and differences in weed diversity and cover. With the advent of genetically modified herbicide-tolerant crops, previously unattainable levels of weed control are possible, resulting in extensive monocultures of virtually weed-free plantings.

Larger-scale patterns in land-use that include crop fields and uncultivated land may also affect bee diversity and abundance at a local-scale within fields. Recent studies indicate that greater amounts of natural land around fields increase bee abundance and diversity in fields, and so increase crop yield (Kremen et al. 2004; Ricketts et al. 2004). Determining how local and large-scale factors influence wild bee populations, and at what scale landscape characteristics are most important to bee populations, is essential for agricultural land-management and sustainability of food production.

Canola (*Brassica* spp.) is the most important oilseed crop in Canada and is increasingly grown in the United States and other parts of the world. Seed set in many varieties of canola benefit from bee-mediated pollen transfer (Delaplane & Mayer 2000). There is therefore a direct economic benefit of wild bee populations in improving seed set and thereby increasing oil yield extracted from canola seeds. Previously (Morandin & Winston 2005; Chapter 4), we found that bee abundance was lower in herbicide-tolerant

genetically modified (GM) canola fields than in conventional fields, and greatest in organic fields, and that there was a greater pollination deficit in areas that had lower wild bee abundance in a location where growers rely on resident wild pollinator populations for seed production. In that study, we demonstrated a relationship between bee abundance and seed set, and proposed hypotheses to explain variation among fields in bee abundance.

In this study, we examine the importance of local and landscape variables on wild bee species richness, diversity, and abundance in canola agroecosystems. We also assess the scale at which land-use patterns most influence bee populations in fields. We hypothesized that greater flowering weed cover and/or species richness would be associated with greater bee abundance and more diverse populations. We also hypothesized that fields with more semi-natural, uncultivated land around them would have greater bee abundance and diversity. Because bumble bees tend to have larger foraging ranges than other wild bees (e.g., Westphal et al. 2003) we hypothesized that bumble bee populations would be influenced by land-use patterns at a larger spatial scale than other wild bees.

5.3 Methods

5.3.1 Study Site

Our study took place near La Crete, Northern Alberta, Canada (~58°N, 116 °E) during the summers of 2002 and 2003. The area is made up of aspen parkland, boreal forest, and a mosaic of cleared agricultural land, aspen woodland, grassland, shrubland, and wetlands. The primary crops are canola, wheat, and soybean. In 2002, four

genetically modified, four conventional, and four organic fields were examined, and in 2003, five fields were examined from each field type. In one of the organic fields in 2003, only a very low proportion of seeds germinated due to extremely dry conditions, and this field was excluded from the analyses. Fields were of the same crop variety within field types. Organic fields contained *B. rapa* Reward (SeCan Association, Ottawa, ON, Canada) canola and were certified organic by the Peace River Organic Producers Association (Dawson Creek, BC, Canada). Conventional canola fields contained non-genetically modified *B. napus* HyLite Clearfield system 45A71 (Advanta Seeds, Winnipeg, MN, Canada) in 2002 and *B. napus* HyLite Clearfield 289 (Advanta Seeds, Winnipeg, MN, Canada) in 2003, treated with the herbicide Odyssey and two fields in 2003, with Lontrel and Select. GM fields contained *B. napus* Roundup Ready DK3235 (Monsanto, St. Louis, MO, USA), treated with the herbicide Roundup. No herbicides were used on any of the organic fields in this study. Conventional and GM fields were treated once during or close to bloom for pests, with the insecticide, Matador (Syngenta, Guelph, ON, Canada), with the exception of one conventional field and one GM field in 2002, and two GM fields and two conventional fields in 2003 which were not sprayed with insecticides during or close to bloom (within 10 d). Pesticide application dates were different among fields and we make no attempt to quantify direct effects of pesticide application on bee populations. No insecticides were used on any of the organic fields in this study.

All fields were approximately 64 ha (quarter section, 800 x 800 m) except three organic fields in 2002 that were smaller, ranging from 20 to 50 ha. Plants in all fields in both years began blooming within one week of each other, in late June, and remained in

bloom until mid to late July. As much as possible, fields were chosen so that treatment replicates were spread throughout the approximately 200,000 ha study location.

5.3.2 Collection Locations

Sampling locations in each field were oriented in relation to an uncultivated edge along one side of the field. Uncultivated edges consisted of trees and under-storey vegetation and were at least 5 m wide. While most uncultivated strips contained trees, we refer to them as hedges to distinguish them from other field edges that tended to border other cultivated fields. Sampling of bees and vegetation was conducted at the same distances from the hedge, at 20, 200, and 500 m into fields, with two sample locations at each distance, 200 m apart, for a total of six sample locations per field. Because organic fields were mostly smaller than conventional and GM fields in 2002, only one organic site had 500 m field sampling locations. To maintain equal sampling effort among locations, organic fields in 2002 (lacking 500 m collection sites) had one additional collection location at 20 and 200 m. In all analyses of variance, distance was included in models. In each field, three sampling locations were located along the hedge, at 200 m from either lateral edge, and each 200 m apart. The three hedge collection locations were approximately 1 m from the edge of the canola crop.

5.3.3 Bee Collections

Bees were collected during canola bloom at each previously described location, from 02 July to 31 July in both years, using pan traps and standardized sweep netting. Three pan traps (one blue, yellow, and white) were placed at each collection location during mid bloom for 48 h (see Morandin and Winston 2005; Chapter 4). If there was

rain during this time, pans were left out an extra amount of time in order that sampling times were as standard as possible among fields. Sweep net samples (see Morandin and Winston 2005; Chapter 4) were done once in each field, during mid bloom for an entire day. Collectors began at one hedge collection location and completed two circuits of the field, one in the morning and one in the afternoon. Bees were pinned or preserved in 70% ethanol for later identification.

5.3.4 Vegetation Surveys

Vegetation surveys were conducted at each collection location in the hedge and in the field. Hedge surveys were conducted immediately prior to the canola bloom and field surveys were conducted during canola bloom. At each collection location in the hedge, all herbaceous and shrubby flowering plants were identified to species along a 50 m transect. Transects began approximately 1 m back from the edge of the canola crop and continued on an angle of approximately 10^{o} away from the canola, into the hedge. A survey of plant species and cover was made in five, 1 m x 1 m quadrats randomly placed along each transect in the hedge, approximately 10 m apart. In fields, three 1 m x 1 m quadrats were assessed at each location. Flowering plants within a vertical plane of the quadrat were identified and cover was scored based using the following scale: 0 = not present, 1 = <5%, 2 = 5 – 10%, 3 = 10 – 25%, 4 = 25 – 50%, 5 = 50 – 90%, and 6 = >90% of quadrat area. In order to standardize estimates among surveyors, we used percentage cover diagrams when assessing vegetation cover.

5.3.5 GIS Analysis of Surrounding Land

The land surrounding each field was assessed using ArcGIS (ESRI 2002). We used a 5 m resolution satellite image of the study region from 2001 (Mackenzie Municipal District office). We created a vector layer of fields on the raster image and created six successive, concentric buffer zones of 250 m around fields, so that the outer edge of the largest region was 1500 m from the outer edge of the field (Figure 5.1). We chose to analyse land at these spatial scales because a number of studies suggest that wild pollinators are most affected by habitat on spatial scales up to 1000 m radius (Osborne et al. 1999; Steffan-Dewenter et al. 2001; Steffan-Dewenter et al. 2002). While some studies indicate that bumble bees may be affected by land-use patterns at spatial scales from two up to several kilometers (Walther-Hellwig & Frankl 2000; Westphal et al. 2003), mark-recapture data indicates that maximum bumble bee foraging distances from nests are up to 1750 m for some species while others tend to forage closer to nests (Walther-Hellwig & Frankl 2000). The amount of land in each buffer was quantified as either cultivated or uncultivated, based on the reflectance of the raster image. Through examination of the raster layer and ground surveys we reclassified the satellite image by categorizing uncultivated land as pixels with a value of 0 to 150 and cultivated land as pixels with a value of 151 to 255. Cultivated land rarely contained pixels with a reflectance value below 151. However, uncultivated land that was grassy (without shrubs or larger vegetation) had pixels greater than 150 and therefore the estimation of uncultivated land is an underestimate. We did not consider this significant because most uncultivated areas had minimal area of continuous grass type vegetation. Total amount of uncultivated land was estimated for each successively increasing size buffer.

5.3.6 Data Analyses

The total number of flowering plant species in each field, other than canola, and in each hedgerow was calculated. A total flowering plant cover score was calculated for each hedgerow and field (field flowering plants from here on will be referred to as 'weeds') by adding all cover values.

We first present analyses with only field type (organic, conventional, GM) and year as predictor variables on bee abundance, species richness, and diversity. We then present models in which we have incorporated multiple quantitative and class predictor variables in order to assess the importance of all measured factors. We estimated bee abundance and bee species richness using a jackknife procedure (Jack 1, 100 iterations; EstimateS) (Colwell 2005), and Shannon's diversity index (H). For each field, estimates were made separately for field and hedge bees. We used mixed model analyses of variance (Proc MIXED; SAS 1999) with type 3 estimation, and bee abundance, bee species richness, and Shannon's diversity index as response variables, field type as the fixed effect, and year and year by field treatment as random effects. We analysed plant data in a similar manner in relation to field type.

In order to assess which buffer size was the best predictor of bee abundance in fields, and maintain independence among different buffer sizes, we used a forward selection regression model based on maximum R^2 improvement (Proc REG; SAS 1999). The uncultivated land value from the buffer size that was the best predictor of bee abundance, species richness, and diversity in fields (i.e., the first scale selected in the model) was used in subsequent models. We performed a multiple regression, with backwards elimination (Proc REG; SAS 1999) of plant species richness and cover in the

hedgerow, and weed richness and cover in the field in order to assess which were the best local scale predictors of bee abundance, species richness, and diversity in fields. We used a backwards selection model in this second case because we wanted to identify all predictors that significantly contributed to variation with respect to all other variables measured. Variables that added little predictive ability to the model were not included in subsequent analyses of variance.

We then present results from synthesis analyses. A mixed model analysis of variance (Proc MIXED; SAS 1999) was used to compare mean bee abundance, species richness, and diversity among field types. The main effects were field type (class variable), amount of uncultivated land, and the best local scale predictors (quantitative variables; see above). Interactions between field type and quantitative variables were included in the model as main effects. Year and field type by year were included as random factors. If interaction terms were significant, we only report main effects in which the slopes of lines are all either positive or negative (i.e., the trend is in the same direction for all field types). In all mixed model analyses, if interaction terms had a $p > 0.200$, we removed the interaction term from the model and report results from the reduced model. In cases where the variance increased with the mean, we analysed $\log_{10}$ transformed data; however, reported means and graphs are from non-transformed data.

Bumble bees live in colonies and are more socially advanced than most other bees (solitary to primitively social), have larger bodies than all other bees in our study area, and are thought to forage longer distances than other bees (e.g., Steffan-Dewenter et al. 2002). Uncultivated land may affect bumble and other bees differently and we therefore

also conducted analyses of local and large scale factors with bumble and other wild bees as two separate groups.

5.4 Results

The total number of field and hedge bees collected was 1125 at 12 sites in 2002 and 1184 at 14 sites in 2003. We collected 76 wild bee species in 18 genera. The six most abundant genera (followed by actual number of individuals collected) were *Bombus* (1290), *Megachile* (209), *Hylaeus* (196), *Anthophora* (173), *Osmia* (85), *Lasioglossum* (83) and *Halictus* (69) (see Table 5.1 for complete list of species).

5.4.1 Field type and wild bees

There were no interactions between year and field type, between years, or among field types and bee abundance, species richness, or diversity in any analyses. Hedges adjacent to organic fields had greater bee abundance than hedges adjacent to GM fields but there was no difference in bee species richness or diversity (Figure 5.2). In fields, bee abundance, species richness, and diversity were greatest in organic fields, followed by conventional fields, and lowest in GM fields in both years (Figure 5.2).

With all field types pooled, there was a positive relationship between numbers of bumble bees and numbers of other bees collected in fields ($F_{1,24} = 7.54$, $p = 0.011$, $r^2 = 0.24$). In organic fields, there was a strong relationship between bumble and other bees ($r^2 = 0.45$), there was a weaker relationship in GM fields ($r^2 = 0.12$), and no relationship in conventional fields. There was a positive, linear relationship between numbers of bees collected in hedges and numbers of bees collected in fields ($F_{1,24} = 25.72$, $p < 0.001$, $r^2 =$

0.52). There also was a positive linear relationship between species richness in hedges and adjacent fields ($F_{1,24} = 4.57$, $p = 0.043$).

5.4.2 Field type and flowering plants

The number of species of flowering plants in hedges adjacent to fields ± SE was 26.5 ± 1.43, 26.6 ± 1.62, and 20.7 ± 1.54 in organic, conventional, and GM fields respectively. There were significantly more flowering plant species and cover in hedges by organic and conventional fields than GM fields ($F_{2,22} = 6.93$, $p = 0.005$ and $F_{2,22} = 3.87$, $p = 0.036$ respectively).

The number of species of weeds and weed cover in fields were both significantly different among field types, with the greatest number of species and cover in organic fields and lowest in GM fields ($F_{2,22} = 17.91$, $p < 0.001$ and $F_{2,22} = 10.99$, $p < 0.001$; Figure 5.3).

5.4.3 Flowering plants and wild bees

Weed cover was the most important predictor variable of both the abundance of wild bees in fields ($F_{1,24} = 34.07$, $p < 0.001$, $R^2 = 0.59$) and bee species richness ($F_{1,24} = 11.04$, $p = 0.003$, $R^2 = 0.32$). Because the relationship between weed cover and bee species richness appeared to be nonlinear, we analysed the relationship using nonlinear regression (Proc NLIN; SAS 1999)($F_{1,24} = 15.79$, $p < 0.001$, $r^2 = 0.40$; Figure 5.4). For Shannon's diversity index, weed species richness was the only local scale predictor remaining in the final model ($F_{1,24} = 4.09$, $p = 0.054$, $R^2 = 0.15$).

Weed cover in fields was the best local-scale quantitative predictor variable of both the numbers of bumble bees collected in fields ($F_{1,24} = 26.22$, $p < 0.001$, $R^2 = 0.52$) and the numbers of other bees collected in fields ($F_{1,24} = 12.04$, $p = 0.002$, $R^2 = 0.33$).

5.4.4 Uncultivated land

We analysed field bee numbers, richness, and diversity in relation to the amount of uncultivated land within buffer zones of increasing size. The purpose was two-fold; to find at what scale variation in amount of uncultivated land most affected bee abundance and diversity, and then to use uncultivated land values at this scale as a predictor in local and landscape factor synthesis analyses of variance. The amount of uncultivated land within 750 m of field edges was the size of buffer that was the best predictor of field bee abundance and field bee species richness (Figure 5.5). Addition of spatial information greater than 750 m did not increase the predictability of the model, and therefore we used the 750 m uncultivated land value in later analyses. There was not a significant correlation between amount of uncultivated land and diversity of wild bees at any scale. 750 m uncultivated land values also were the best predictors of both abundance of bumble bees and other bees when analysed separately. There was a strong, positive correlation between the amount of uncultivated land and bumble bees ($F_{1,24} = 28.79$, $p < 0.001$, $r^2 = 0.55$), and a weaker, but still significant, positive correlation between the amount of uncultivated land and other wild bees ($F_{1,24} = 4.99$, $p = 0.035$, $r^2 = 0.17$).

There was a difference in the amount of uncultivated land, within 750 m of field edges, among field types ($F_{2,23} = 5.11$, $p = 0.014$), with a mean amount ± SE of 176.3 ± 25.3, 108.8± 25.5, and 78.4 ± 12.7 ha around organic, conventional, and GM fields respectively. Pairwise comparisons of least squares means showed that organic fields

had more uncultivated land around them than conventional (t_{23} = 2.17, p = 0.040) and GM (t_{23} = 3.14, p = 0.005) fields.

5.4.5 Local and Landscape Scale Factor Synthesis

Within Hedges

For bee abundance, species richness, and Shannon's diversity, the only significant relationship was between the amount of uncultivated land within 750 m of field edges and bee abundance ($F_{1,20.7}$ = 4.66, p = 0.043).

Within Fields

There was an interaction between year and field type ($F_{2,16}$ = 8.99, p =0.002), an interaction between weed cover and field type ($F_{2,16.5}$ = 7.58, p = 0.004; Figure 5.6), an effect of weed cover ($F_{1,16.1}$ = 14.87, p = 0.001), and an effect of uncultivated land within 750 m of field edges ($F_{1,15.6}$ =6.62, p = 0.021) on bee abundance (Figure 5.7). The effect of field type on the number of bees collected was marginally non-significant ($F_{2,6.44}$ = 3.93, p = 0.077). When weed cover was removed from the analysis (which is related to field type; see Figure 5.3), there was an effect of field type ($F_{2,21}$ = 3.75, p = 0.040).

Using the same model and field species richness as the response variable, there was an interaction between weed cover and field type ($F_{2,18.5}$ = 4.67, p = 0.023), an effect of weed cover ($F_{1,18.7}$ = 7.89, p = 0.011), and an effect of field type ($F_{2,18.8}$ = 4.33, p = 0.029). For Shannon's diversity index as the response variable, the only significant effect was field weed species richness ($F_{1,14.8}$ = 5.08, p = 0.040).

With the number of bumble bees collected in fields as the response variable we found that there were interactions between uncultivated land and field type ($F_{2,14.1}$ = 4.38,

$p = 0.033$) and weed cover and field type ($F_{2,14.5} = 5.04$, $p = 0.022$). There was an effect of uncultivated land ($F_{1,14.4} = 5.05$, $p = 0.041$) and of field cover ($F_{1,14.2} = 9.04$, $p = 0.009$). When we modelled the number of other wild bees collected in relation to field type, year, weed cover, and uncultivated land, there was an effect of uncultivated land within 750 m of field edges ($F_{1,17} = 5.78$, $p = 0.028$) and an effect of field type ($F_{2,17} = 11.10$, $p = 0.001$).

5.5 Discussion

Both local scale differences in fields and landscape scale differences up to 750 m from field edges were important to wild bee populations. The future of sustainable agriculture depends on identification of landscape scales and factors important to the functioning of ecosystem services such as pollination (Kareiva & Wennergren 1995; Westphal et al. 2003) and therefore, it is crucial that we better understand these complex systems. In our study, bee abundance was greatest in organic fields, intermediate in conventional fields, and lowest in GM fields, similar to what we found in a subset of our data from 2002 (Morandin & Winston 2005; Chapter 4), but when we controlled for weed cover and amount of uncultivated land in a 750 m distance from edges, there was no longer a difference between field types, suggesting that these quantitative variables were driving variation in bee abundance. Similarly, bee species richness and diversity in fields increased with greater amounts of weeds (weed cover and richness, respectively) but was not related to surrounding uncultivated land when local and landscape variables were accounted for.

Not unexpectedly, differences in weed cover were strongly associated with field type. There were no herbicides applied to organic canola fields in our study and growers

used mainly pre-sowing tillage to control weeds, although some manually pull weeds as well. The conventional canola in our study was bred for herbicide resistance (as is most conventional canola in Canada), and broad-leaf herbicides can be applied after plant emergence, resulting in weed control that is more effective than in organic fields. The genetically modified, herbicide tolerant canola in our study was modified for resistance to Roundup, a broad spectrum herbicide that provides extremely effective weed control.

A number of studies comparing bee abundance and diversity in semi-natural and agricultural areas have shown that bee abundance and/or diversity is greater in semi-natural areas than crop fields (Mackenzie & Winston 1984; Banaszak 1996; Calabuig 2001), and it has been suggested that high diversity of flowering plant species can better support larger, more diverse bee populations (e.g., Calabuig 2001; Potts et al. 2003). A number of researchers have proposed that farming practices reducing weed abundance and diversity within and adjacent to agricultural areas may lower bee abundance and diversity, but few studies have directly assessed this relationship. As part of a large-scale study in Scotland (Firbank et al. 2003), comparisons were made of weeds in herbicide-tolerant GM and conventional canola. Weed diversity and biomass, and bee abundance, were lower in GM fields than in conventional fields (Haughton et al. 2003; Heard et al. 2003; Bohan 2005)

Conversely, Kremen et al. (2004) found that there was no effect of crop type (local scale factors) on bee abundance in conventional and organic watermelon fields. Rather, they found that the amount of natural land (upland habitat) at a scale of 1200 to 4800 m was the only factor associated with observed bee abundance. We found large differences in weed cover between GM fields and other field types and perhaps the lack

of extreme variation in field weed cover in Kremen et al. (2004) partially led to the conflicting results between studies. Agriculture with genetically modified herbicide tolerant crops allows fields to be virtually free of weeds and our data indicate that this influences bee populations in a substantial way.

Within organic, conventional, and GM fields, 30, 23, and 40%, respectively, of variation in bee abundance was explained by field weed cover (see Figure 5.6). Bee species richness within field types also was correlated with field weed cover. Fields with greater weed cover may be attractive to more bees and a wider range of bee species from the ambient population, resulting in larger numbers than fields with less weed cover.

Hedges adjacent to GM fields had the lowest flowering plant species richness and cover and also had the lowest bee abundance and sampled species richness. Herbicides applied to fields may have drifted to adjacent areas, causing the lower weed species richness and cover. If the lower number of sampled species of bees and bee abundance around GM fields was caused by lower weed species richness and cover around GM fields, careful application of herbicides in order to minimize drift into semi-natural adjacent areas may help increase numbers of bee species and abundance adjacent to fields. If edges of fields are acting as source areas for bees pollinating crops, larger edge populations ultimately may improve crop pollination. There was a significant positive relationship between bee numbers in hedges and in fields, and research for developing methods to enhance edge bee populations may prove valuable.

Bee abundance in both hedges and fields increased with more uncultivated land surrounding fields. Because bumble bees are thought to have larger foraging ranges than other bees, it was hypothesized that bumble bees would be influenced by patterns of land-

use that occur at larger landscape-level spatial scales than other wild bees (Cresswell et al. 2000; Walther-Hellwig & Frankl 2000; Steffan-Dewenter et al. 2001; Steffan-Dewenter et al. 2002; Westphal et al. 2003). Unexpectedly, we found that both bumble bee and other wild bee abundance was most influenced by amount of uncultivated land at a 750 m landscape scale and patterns of land-use at scales greater than 750 m did not add to the predictive ability of our models. Conversely, a study by Steffan-Dewenter et al. (2001) found that solitary bees were influenced by habitat patterns on a small spatial scale (250 m) while bumble bees were increasingly associated with patterns in habitat structure up to a scale of 3000 m.

How bee communities respond to habitat variation, and the importance of land-use at different landscape scales, likely differ in relation to the bee community, climate, and habitat characteristics. In areas where mass flowering crops successively bloom throughout the summer, bumble bees may have large enough foraging ranges resulting in minimal requirements for uncultivated land (Westphal et al. 2003). In our study area, canola was the main flowering crop and plants only bloomed in July. Bumble bees in our study environment would be dependent on food sources other than crops for part of their life cycle, which may explain why we found a strong relationship between bumble bee abundance in fields and the amount of uncultivated land. In addition, semi-natural land provides nest sites for wild bees (Osborne et al. 1991; Svensson et al. 2000), which is likely part of the reason bee abundance increased with greater amounts of uncultivated land. Although variation among studies is evident in the exact scale at which landscape factors affect bee communities, there is remarkable robustness in the emerging conclusion that landscape scale differences in the amount of natural or semi-natural land

around fields have a large impact on wild bees and crop production in agroecosystems.

Organic fields in our study had more uncultivated land around them than conventional and GM fields, possibly because in order to be certified organic, there cannot be any GM canola varieties within a one mile radius (1600 m) of organic fields. Therefore, organic fields tended to be farther from the centre of the study area, and closer to larger areas of uncultivated land than conventional and GM fields. We found no relationship between bee richness, evenness, and diversity in fields and the amount of uncultivated land.

In a related experiment in the same study area, we showed that pollination and seed set were lower in areas where bee abundance was lower (Morandin & Winston 2005). GM herbicide tolerant canola is designed to minimize weed abundance in fields for the purpose of improving crop yield. Effective weed management in canola can significantly improve yield (Harker 2001) and growers are unlikely to adopt poorer weed control methods in order to improve bee abundance and seed set. If lower weed cover is the causative factor for the lower bee abundance found in GM fields compared to conventionally grown canola (Haughton et al. 2003; Heard et al. 2003; Morandin & Winston 2005 (Chapter 4)), then growers of GM canola would benefit by promoting wild bee populations through other methods. For example, bee abundance increases in fields, within and across different cropping methods, with greater amounts of uncultivated land within 750 m of field edges. Management of land in semi-natural states in close proximity to agricultural areas may benefit canola production, particularly in cropping systems where effective in-field weed control is possible.

Our data show that both local (field level), and larger landscape scale factors influence bee communities. The link between bee abundance in canola and seed production make this an economically important relationship that if exploited could benefit both grower and land conservation interests. Utilization of information on how ecosystem services that afford direct economic benefit to growers are affected by local and large scale land differences can aid in agricultural planning at the landscape level that unite technological advances with sustainability. Agricultural expansion and intensification is occurring rapidly and only with an understanding of ecosystem function at local and large scales can we design agroecosystems that maintain crucial ecosystem services.

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5.7 Tables

Table 5.1 **Wild bees collected in the area of La Crete, AB, Canada in the summers of 2002 and 2003 in and in hedges around canola fields using pan trapping and sweep net sampling. Samples that could not be named to species were separated based on morphology and are listed as sp #. Samples were identified with help from the USDA Bee Systematics Laboratory in Logan, Utah, USA.**

Species	Organic (n = 8)	Conventional (n = 9)	GM (n = 9)
Andrena sp 1	2	5	2
Andrena miranda	3	5	3
Andrena nivalis	5	2	4
Andrena thaspii	6	14	10
Anthophora bomboides	0	2	1
Anthophora terminalis	115	25	30
Bombus sp 1	2	0	4
Bombus alboanalis	61	34	7
Bombus borealis	13	16	17
Bombus californicus	0	0	2
Bombus flavifrons	39	22	11
Bombus frigidus	44	69	13
Bombus melanopygus	109	108	21
Bombus mixtus	4	1	2
Bombus nevadensis	20	22	8
Bombus rufocinctus	99	140	55
Bombus suckleyi	6	1	5
Bombus ternarius	34	21	4
Bombus terricola	109	83	16
Bombus vagans	33	25	10
Coelioxys funeraria	14	2	2
Coelioxys moesta	23	1	3
Coelioxys porterae	4	1	0
Coelioxys sodalis	1	0	1
Colletes hyalinus	1	1	0
Colletes impunctatus	0	2	1
Everra froter	2	0	0
Halictus confusus	39	8	12
Halictus rubicundus	7	0	3
Heriades variolose	1	0	0
Heterosarus parvus	4	10	15
Heterosarus sp 1	2	2	1
Hoplitis albifrons	6	0	1
Hoplitis producta	2	1	3
Hoplitis spoliata	4	4	0
Hylaeus affinis	2	0	0
Hylaeus annulatus	93	26	32
Hylaeus basalis	4	0	0

Species	Organic (N = 8)	Conventional (N = 9)	GM (N = 9)
Hylaeus messillae	3	0	4
Hylaeus modestus	23	0	3
Hylaeus sp 1	1	1	0
Hylaeus verticalis	3	1	0
Lasioglossum athabascense	5	0	3
Lasioglossum (Dialictus) sp 1	1	1	1
Lasioglossum (Dialictus) sp 2	7	6	11
Lasioglossum (Dialictus) sp 3	2	2	3
Lasioglossum (Dialictus) sp 4	1	0	2
Lasioglossum (Dialictus) sp 5	0	1	0
Lasioglossum (Dialictus) sp 6	1	0	0
Lasioglossum (Dialictus) sp 7	0	0	1
Lasioglossum (Evylaeus) sp 1	2	0	2
Lasioglossum (Evylaeus) sp 2	11	4	14
Lasioglossum (Evylaeus) sp 3	0	1	1
Megachile frigida	9	1	0
Megachile gemula	9	1	1
Megachile giliae	4	2	1
Megachile inermis	66	12	12
Megachile melanophaea	3	3	7
Megachile nivalis	2	0	0
Megachile perihirta	3	0	1
Megachile pugnata	4	0	0
Megachile relativa	51	10	7
Melissodes rustica	0	1	12
Nomada sp 1	0	1	0
Nomada sp 2	0	2	2
Osmia bucephala	16	6	12
Osmia nigriveatris	2	2	1
Osmia proxima	2	7	3
Osmia simillima	3	4	7
Osmia sp 1	3	3	1
Osmia tersula	1	9	2
Osmia tristella	1	0	0
Sphecodes sp 1	1	0	0
Sphecodes sp 2	1	0	4
Sphecodes sp 3	1	0	0
Stelis foederalis	2	0	1
SUM	**1157**	**734**	**418**

5.8 Figures

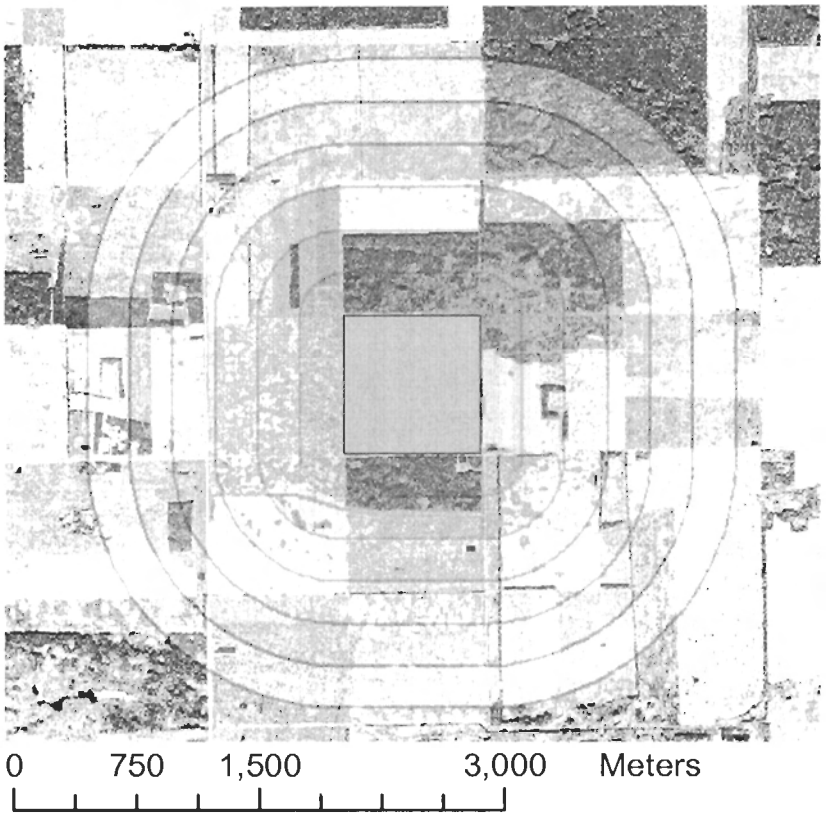
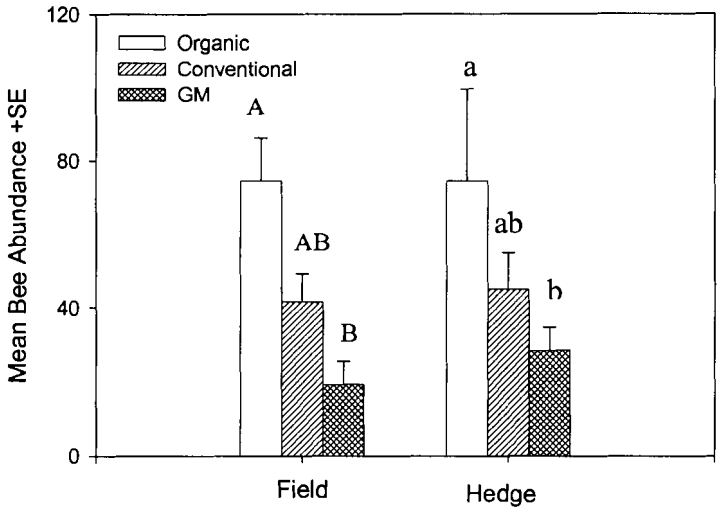
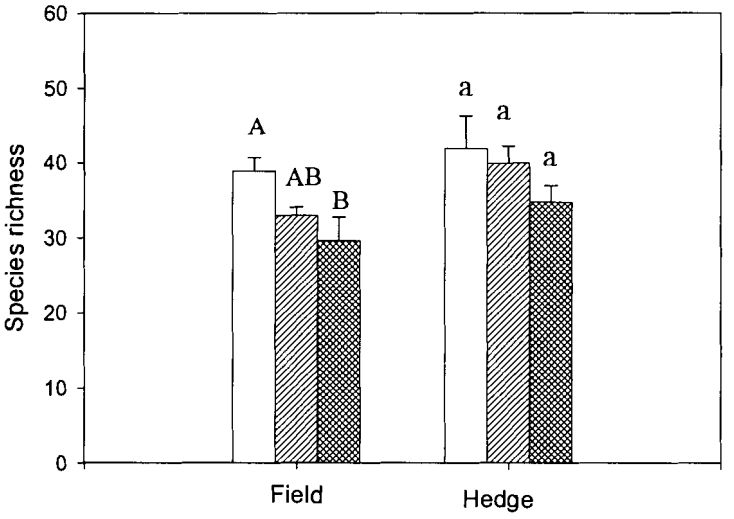


Figure 5.1 **An ArcView GIS satellite raster image (5 m resolution) with a vector overlay of a canola field. Six buffer zones were created around each field at increasing sizes up to 1500 m from the field edge, at 250 m increments. Land was scored in each buffer as either cultivated or uncultivated based on reflectance of the satellite image.**

a


Organic
Conventional
GM
Mean Bee Abundance +SE
120
80
40
0
A
AB
B
a
ab
b
Field
Hedge

b


Species richness
60
50
40
30
20
10
0
A
AB
B
a
a
a
Field
Hedge

c

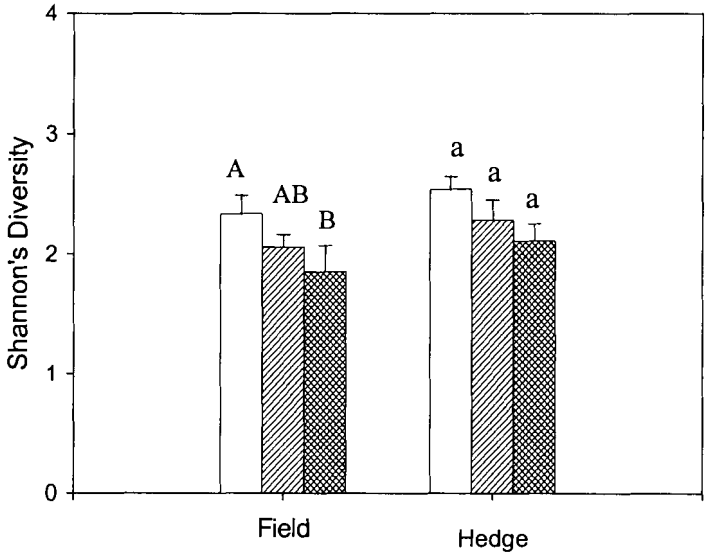

Shannon's Diversity
4
3
2
1
0
A
AB
B
a
a
a
Field
Hedge

Figure 5.2 **Field and hedge bee response variables in relation to type of canola field (organic, conventional, and GM). Bars are means for each field type +SE. Within hedge or field collections, bars with the same letters indicate that there was no difference between least-squares means ($P > 0.05$).**

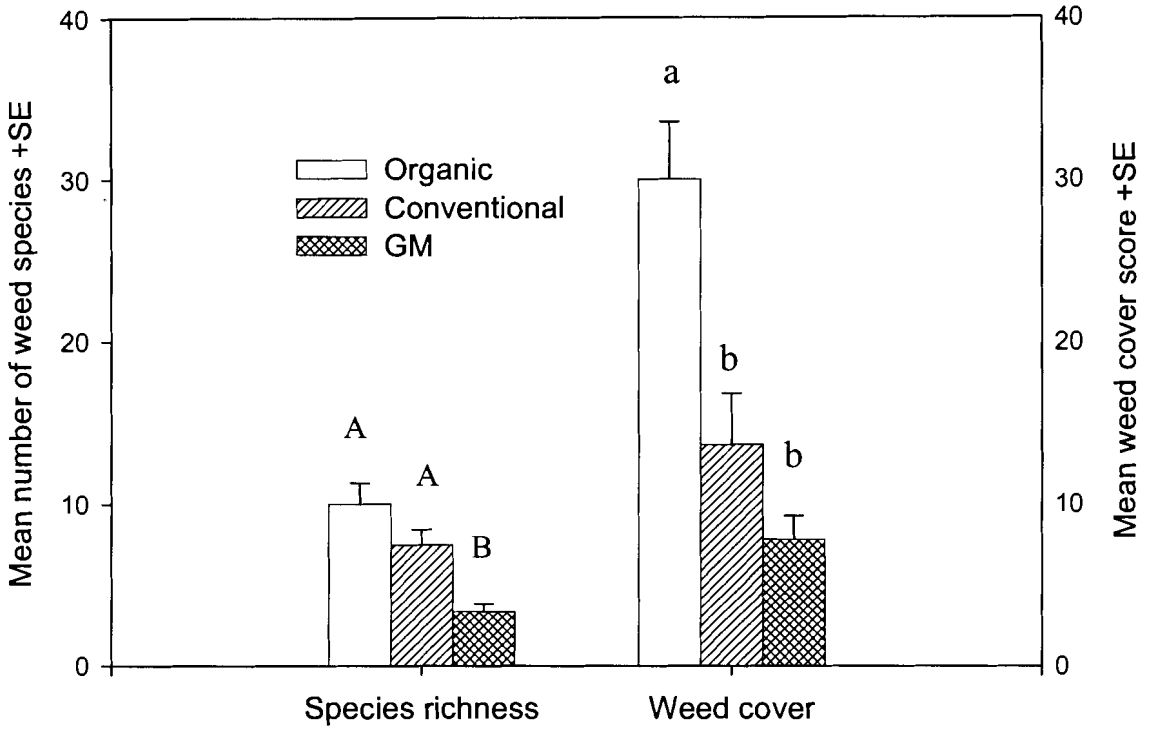


Figure 5.3 **Mean weed species richness and weed cover +SE in organic, conventional, and GM canola fields. Bars with the same letters indicate that there was no difference between least-squares means (P > 0.05).**

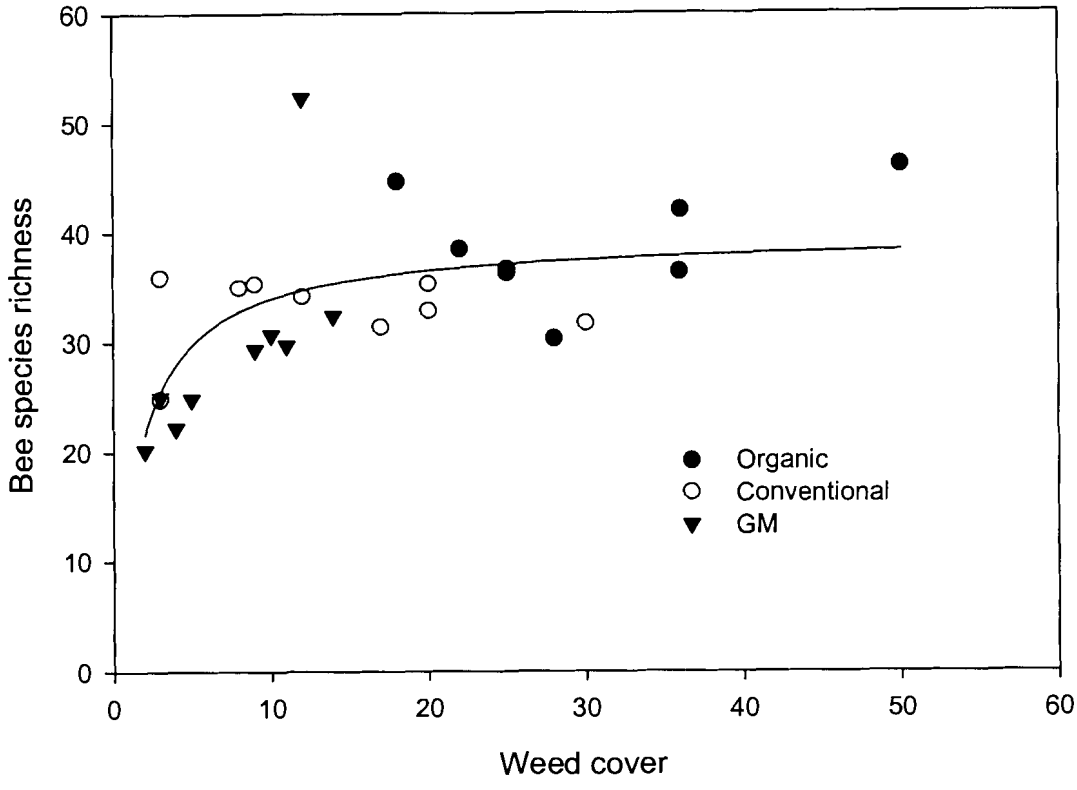


Figure 5.4 **Estimated bee species richness in each field (n = 26 fields) regressed on weed cover index. Weed cover index was calculated for each field by summing cover scores from three quadrats each at six collection locations. The plotted line is the equation: Bee species richness = 20.61(Weed cover index)/(5.86 + Weed cover index) ($F_{1,24} = 15.7$, $P < 0.001$, $r^2 = 0.40$).**

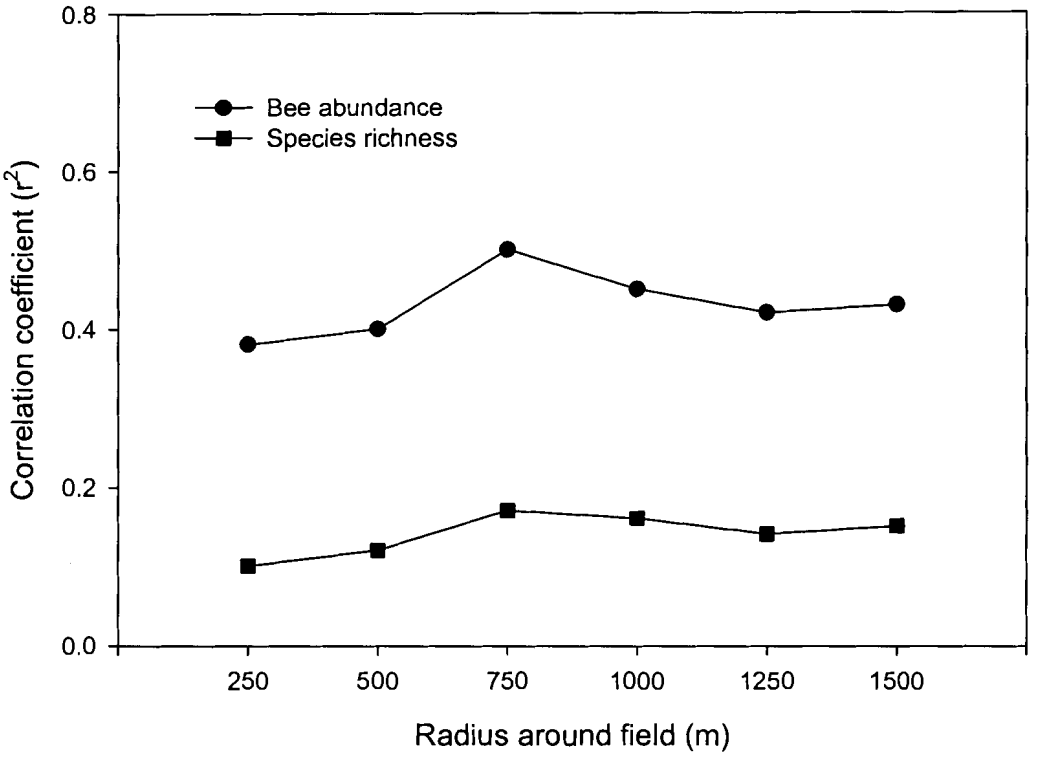


Figure 5.5 Correlation coefficients of bee abundance and bee species richness in relation to the amount of uncultivated land at six scales around fields.

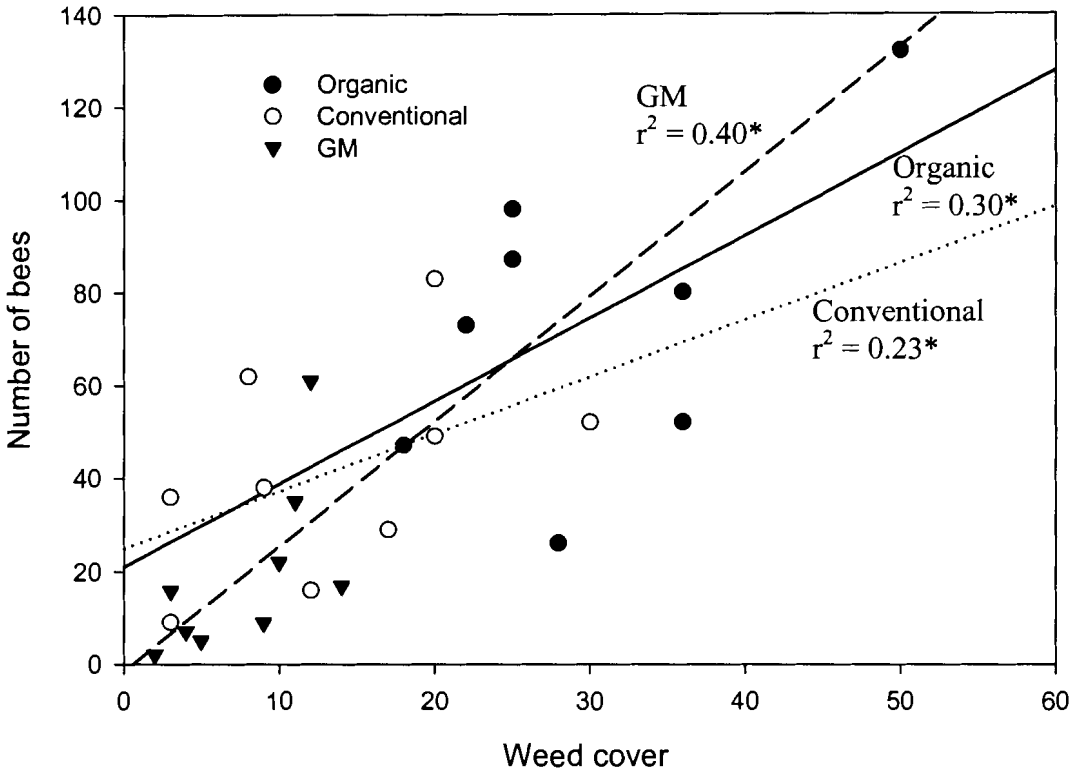


Figure 5.6 **Numbers of bees collected in fields in relation to weed cover index calculated for each field by summing cover scores from three 1 x 1 m quadrats each at six collection locations. Organic, conventional, and GM fields are plotted separately because there was a significant interaction between field type and weed cover on bee abundance.**

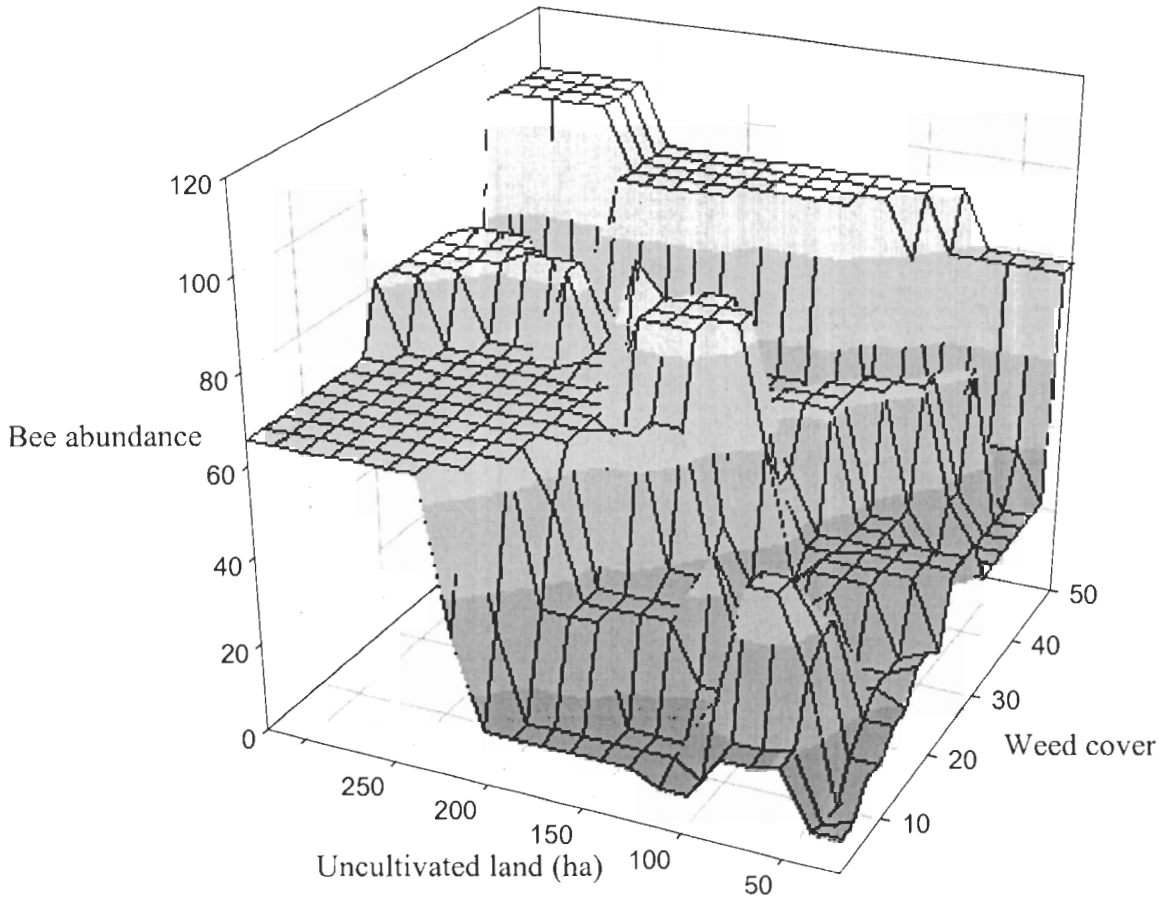


Figure 5.7 A surface plot of the number of bees collected in fields in relation to the amount of uncultivated land within 750 m of field edges and weed cover in fields. The surface was created using linear interpolation.

CHAPTER 6
POLLINATORS PROVIDE ECONOMIC INCENTIVE TO PRESERVE NATURAL LAND IN AGROECOSYSTEMS

The following chapter is provisionally accepted (20 Sept 2005) as a Short Communication in Agriculture, Ecosystems & Environment with Mark L. Winston as co-author.

6.1 Abstract

Natural habitats are considered inherently indispensable to the global economy by conservationists, but few natural ecosystems afford direct and quantifiable economic benefits. Quantification of natural land value can provide compelling evidence favouring preservation over development. Wild bees are important pollinators of many crop plants, and natural patches in agroecosystems enhance pollinator services and crop yield. Bee abundance was greater in fields with more uncultivated land within 750 m of field edges and seed set was greater in fields with higher bee abundance. A cost-benefit model that estimates profit in canola agroecosystems with different proportions of uncultivated land is presented. Yield and profit could be maximized with 30% of land uncultivated within 750 m of field edges. Sustainable agricultural production depends on the integration of development with preservation of natural ecosystem services.

6.2 Introduction

Conversion of land for agriculture is one of the major causes of diminishing natural ecosystems and biodiversity globally (Banaszak 1978; Vitousek et al. 1997; Allen-Wardell et al. 1998; Lambin et al. 2001; Schmucki et al. 2002). Natural patches

within and surrounding cropland often are viewed negatively by producers as a source of weedy plants and other pest species. But conservationists view natural patches differently, as providing biodiversity refuges and habitat corridors (Schmucki et al. 2002; Tscharntke et al. 2002). Pollinating insects such as wild bees, benefit from natural areas in agroecosystems (Free 1993; Roubik 1995), yet despite the economic contribution of wild bees to crop production (Kremen et al. 2002; Kremen et al. 2004; Ricketts et al. 2004), they rarely are considered in agricultural landscape planning. Economic valuation of ecosystem services can be used as incentive for natural land preservation, benefiting biodiversity, agricultural production, and ecosystem processes. We test the hypothesis that natural land preserves in agroecosystems can give significant economic benefit to growers by promoting wild bee populations that enhance seed production and yield.

6.3 Methods

Canola (*Brassica* spp.) provides an excellent model system to assess the benefits of natural land preserves in agricultural landscapes. Canola is the most important oilseed crop in Canada and is of increasing importance globally. Seed set in canola is improved by insect-mediated pollen transfer (Free 1993), resulting in a direct increase in crop yield. In July of 2002 and 2003, wild bee populations, surrounding habitat, and canola seed production near La Crete, Alberta, Canada (~58°N, 116 °E) were examined. The area was aspen parkland and was being cleared rapidly for agriculture. In 2002 four non-transgenic, conventional (cv. 45A71; Advanta Seeds, Winnipeg, MN, Canada), four genetically modified herbicide tolerant (GMHT) no-till (cv. DK3235; Monsanto, St. Louis, MO, USA), and four GMHT regularly tilled (cv. DK3235) canola fields (*Brassica napus*) were assessed. In 2003 five conventional (cv. CL289; Advanta Seeds, Winnipeg,

MN, Canada) and five GMHT (cv. DK3235) canola fields were assessed. All fields were 800 x 800 m (quarter section, 158 ac, 64 ha) and collection locations were measured from a chosen hedge row. In each field there were two collection locations at 20, 200, and 500 m from the hedge, 200 m apart and 300 m from the lateral edge of the field. Pollination effectiveness of bee populations was assessed by comparing seed set from open pollinated and supplementally pollinated flowers (seed deficit) (Morandin & Winston 2005; Chapter 4). Six pairs of plants were selected at each collection location with three control flowers on each plant and three experimental flowers on one plant per pair, for a total of 324 flowers per field (216 control flowers and 108 experimental flowers). Control flowers were marked but not manipulated in any other way and experimental flowers were marked and manually pollinated with pollen from adjacent plants. Bee abundance was assessed using pan trapping and sweep netting (Morandin & Winston 2005; Chapter 4). Each field was sampled with yellow, blue, and white pan traps once during the bloom for 48 hrs. One day of standardized sweep net samples were conducted in each field, on days that were mostly sunny, when the temperature was above 18°C from the beginning to the end of the collection period (approximately 10:00 to 17:00 hrs) (see Morandin & Winston 2005; Chapter 4 for more detailed description of methods).

6.4 Results and Discussion

There was a strong, diminishing returns relationship between bee abundance estimates in fields and seed set (Figure 6.1). Fields with moderate to high bee abundance had close to maximum yields, with seed set deficits that approached zero. Canola varieties in our study had a mean (± SE) potential seed set of 25.0 ± 0.2 seeds/pod with full pollination (n = 2350 from 22 fields), but mean (± SE) actual set was 18.1 ± 0.2

seeds/pod (n = 4708 pods from 22 fields). Even more substantial losses of seeds due directly to lack of pollen transfer were evident in a number of fields. For example, one GMHT field in 2002 had a mean number of seeds/pod of 10.2 ± 0.7 from open pollinated flowers (n = 216), and 23.3 ± 0.6 from supplementally pollinated flowers (n = 108), a loss of greater than 50% of seeds due directly to poor pollination. This field also had one of the lowest bee abundance estimates (bee abundance index: 6). We found a similar relationship when we analyzed a subset of our 2002 data (Morandin & Winston 2005; Chapter 4).

Bee abundance previously was found to increase with increases in both weed cover in fields and uncultivated land around fields (Morandin et al. in review; Chapter 5). Uncultivated land amounts from a 250 to 1500 m scale around fields at increments of 250 m were analyzed in relation to bee abundance and it was determined that a scale of 750 m was most predictive of in-field bee abundance (Morandin et al. in review; Chapter 5). Weeds in canola fields are known to reduce crop yield (Harker 2001), and extremely effective weed control is possible with GMHT canola, because broad spectrum herbicides can be applied after crop emergence. While growers are not likely to adopt poorer weed control strategies in order to increase wild bee abundance in fields, if land set asides provide greater economic benefit than cultivation, preservation may be favoured. Analysis of land within 750 m of field edges in conventional and GMHT canola fields in this study indicated that seed production and crop yield can be increased by greater amounts of uncultivated habitat (Figure 6.2).

The importance of natural land to crop yield is not unique to canola crops. Similar relationships have been found in watermelon (Kremen et al. 2004) and coffee

(Ricketts et al. 2004) agricultural landscapes where pollen deposition and crop yield were positively related to the amount of uncultivated land in proximity to fields, indicating a pervasive association between crop production and pollination services provided by bees from natural areas.

Given these relationships between bee abundance, seed deficit from inadequate pollination, and uncultivated land, the potential economic benefit of uncultivated area in a typical canola agroecosystem was calculated. Mean seed set in open-pollinated canola fields was 18.1 ± 0.2 seeds/pod. Mean amount of uncultivated land within 750 m of field edges was 37.9 ± 6.2 ha or approximately 9% (n = 13 fields). The other 91% was used primarily for crops. In 2002 and 2003, the GMHT and conventional varieties in our study yielded on average 1120, 1568, 1344, and 1568 kg/ha respectively (AFRD 2005) (using conversion factor 1bu/ac = 56 kg/ha). Taking an average yield of 1400 kg/ha, a typical quarter section (64 ha) of canola would yield 89 600 kg. Prices for canola seed have fluctuated between $0.25-$0.45 kg (all dollar values in Canadian currency) for the last 5 years (ACPC 2005). Using a typical but conservative price estimate for the 2002 and 2003 seasons of $0.31/kg, gross revenue was $27 776 per quarter section. Approximately $20 000 of this was input costs, resulting in a profit of $7776 per section (AFRD 2003, 2005).

A typical agricultural landscape with canola is shown in Figure 6.3a. All five fields have approximately 38 ha of uncultivated land within 750 m of field edges, and a profit, at 1400 kg/ha and a market value of $0.31/kg, of $7776. The profit from the five canola fields in this landscape is $38 880. However, if the centre of the five sections had not been ploughed for cultivation or was allowed to revert to a semi-natural state (Figure

6.3b), there would be 104 ha of uncultivated land within 750 m from all four cultivated field edges, and the bee abundance index (± 95% CI) would increase from a mean (± 95% CI) of 26.8 ● 6.0 to 67.6 ● 16.0, with corresponding pollination deficit changes from -6.3 to -2.8, an increase of 3.5 seeds/pod. Yield would increase from 1400 to 1680 kg/ha and gross revenue per field would equal $33 331. Because wild pollinators provide a 'free' pollination service, input costs per field would remain the same ($20 000 per quarter section) and profit would be $13 331 per quarter section, a 71% increase in profit per field. Net value of canola in this second landscape scenario (four fields) is $53 324, a 37% increase in landscape profit over five fields without a central uncultivated area. Harvesting and transport costs may slightly increase with greater yields but we do not include this in our analyses.

In order to assess optimum uncultivated land area, a generalized model of profit from canola in a 576 ha area along a continuum, from all land being canola fields to the area made up entirely of uncultivated land is presented (Figure 6.4). Agroecosystems were examined in this size area because it is a likely scale at which bees utilize their environment (Osborne et al. 1999; Steffan-Dewenter et al. 2001; Steffan-Dewenter 2002; Morandin et al. in review). The model assumes that the area is a closed system and there is no outside uncultivated land from which pollinators can come into the landscape, and that addition of uncultivated land contributes pollinator services equally in all fields. Uncultivated land added predominantly to one side of the modeled landscape would have less of an impact on overall seed set if canola within bees' foraging range was already maximally pollinated. Rather than modelling unrealistic full seed set, the model was

constrained, not allowing seed set greater than what was achieved with full pollination in this study (i.e., seed deficit values must be no greater than zero).

Landscape profit rises sharply with an increase from zero to approximately 20% uncultivated land. Rate of profit increase decreases from 20 to 30% uncultivated land, indicating that the most benefit of uncultivated land is seen when increasing from low to moderate amounts. Above 31%, seed set no longer increases and declining amounts of cultivated land result in a sharp linear decline in profit. Thus, maximum landscape profit is achieved with just over 30% of the landscape uncultivated.

Canola is grown in rotation with other crops and while we do not attempt to extend the calculations beyond one year, it is reasonable to expect that landscape profit would fluctuate yearly for a given proportion of uncultivated land. Profit would benefit more from uncultivated land in years in which crops predominantly were composed of species that benefit from wild bee pollination services as opposed to years in which the majority of the landscape was composed of crops that did not require bee pollination.

In our study area, uncultivated land was a mix of open aspen parkland, aspen forests, wetlands, and shrub lands. Agroecosystems with densely closed forest in uncultivated areas would not be as good habitat for wild bees as open habitat. We did not distinguish between types of uncultivated land in our study, but more refined data on habitat types would be valuable for future studies concerning agroecosystem landscape planning.

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6.6 Figures

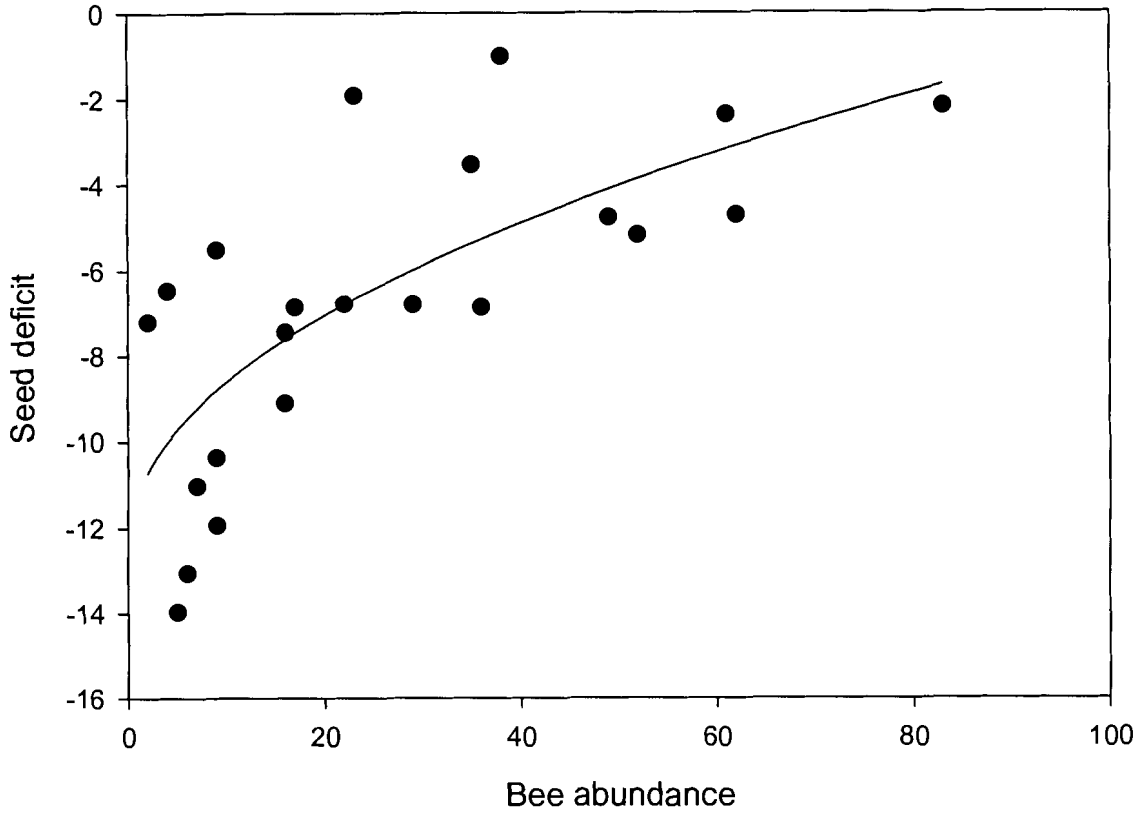

Figure 6.1 **Seed deficit in relation to bee abundance. Seed deficit is the difference in the number of seeds in canola pods from flowers that were supplementally pollinated and the number of seeds per pod from flowers that were pollinated by ambient insect populations. There was a diminishing returns relationship ($f(x) = -12.54 + 1.29x^{0.48}$, $r^2 = 0.50$) between bee abundance and seed deficit.**

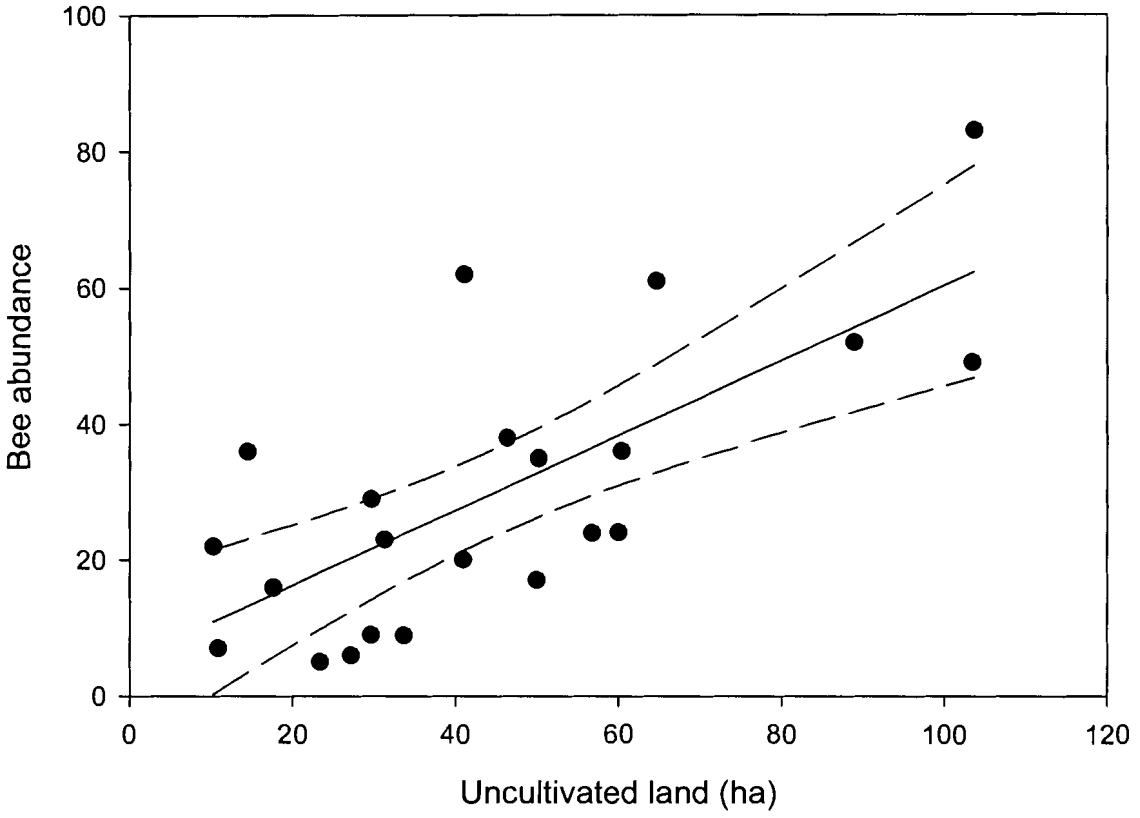

Figure 6.2 **Wild bee abundance, assessed using pan trapping and sweep netting, in conventional and genetically modified herbicide tolerant canola fields in relation to the amount of uncultivated land within 750 m of field edges. $f(x) = 3.42 + 0.617x$, $r^2 = 0.62$, $F_{1,20} = 33.03$, $P < 0.0001$.**

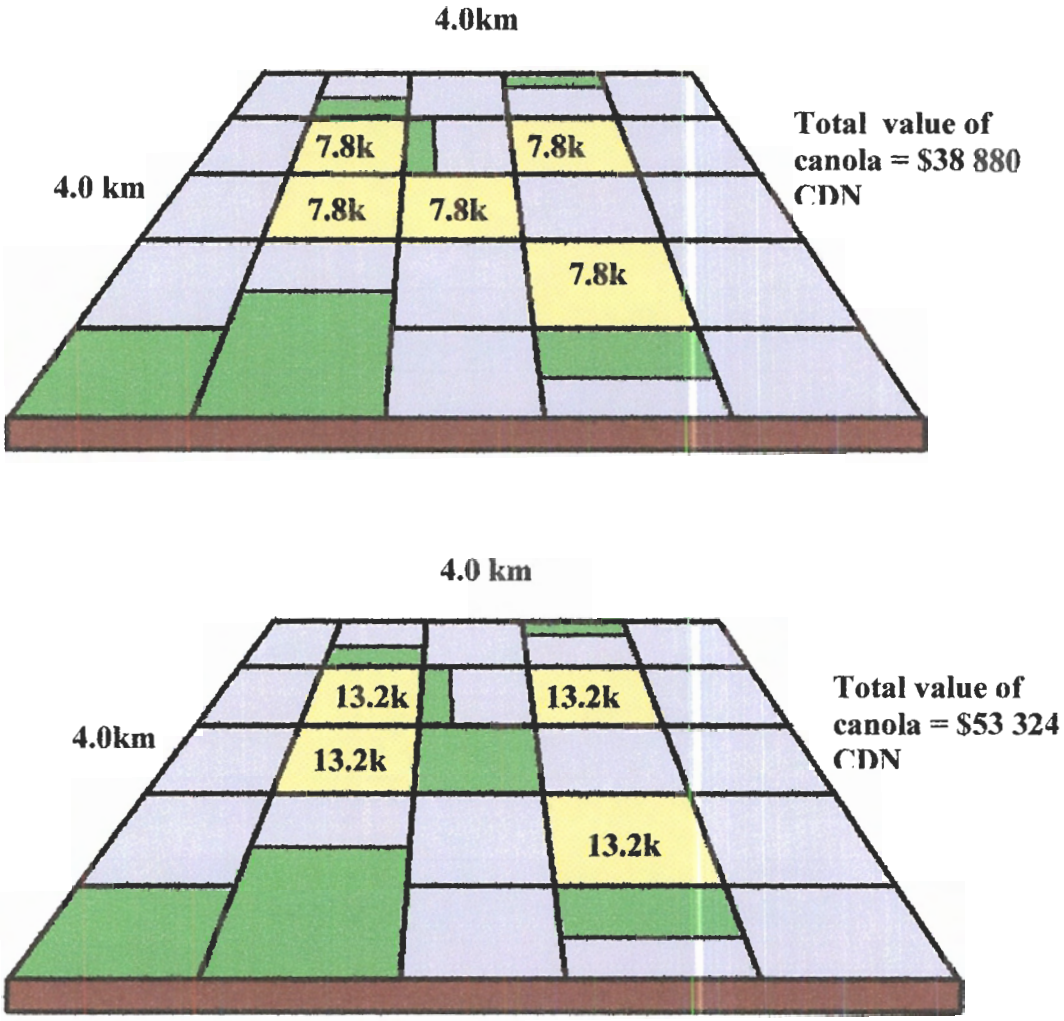

Figure 6.3 Typical agroecosystem landscapes with canola in northern Alberta. The large square is 4 x 4 km, main squares are 800 x 800 m (quarter section), yellow squares are canola, blue are other crops, and green areas are uncultivated land. a. Landscape with five canola fields. Each field in this landscape would make a profit of $7776 CDN and a landscape profit of $38 880. b. Landscape with four canola fields and a central uncultivated section. Profit for each field is $13 180, and landscape profit is $53 324, an increase of 37% over landscape a.

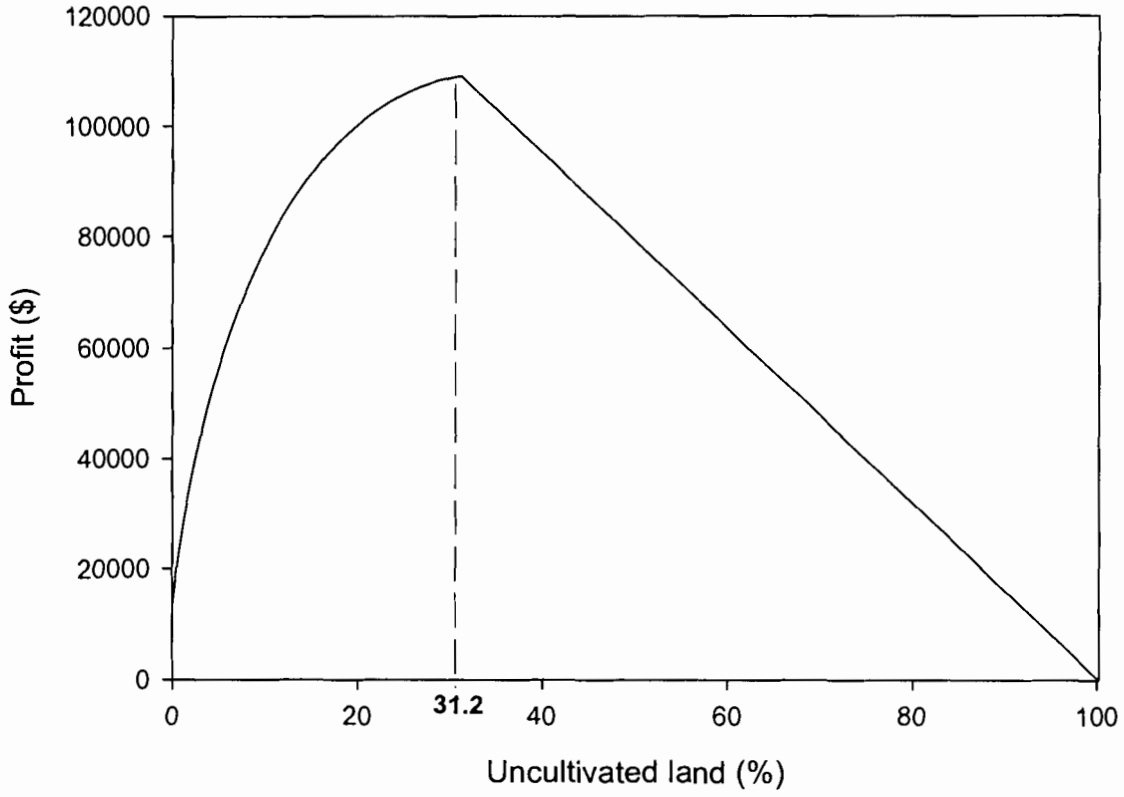

Figure 6.4 **Model of canola profit on a landscape scale defined by the equation: f(x) = ((((3.42+0.617x)$^{0.48}$1.29-12.54)1580+17604)/64)(576-x). Seed deficit was not allowed to increase above zero (creating the linear decline after 31.2% uncultivated land). Land is either canola or uncultivated.**

CHAPTER 7
CAN PASTURELAND INCREASE WILD BEE ABUNDANCE IN AGRICULTURALLY INTENSE AREAS?

The following chapter is in review in Basic and Applied Ecology with Mark L. Winston, Virginia A. Abbott, and Michelle T. Franklin as co-authors.

7.1 Abstract

Agricultural intensification and expansion are major present and future causes of global ecosystem disruption. Natural and semi-natural reserve areas in agroecosystems are thought to be important for preservation of essential ecosystem services such as pollination, but data about land use patterns and pollinator abundance are lacking. We assessed wild bee populations in canola fields in an agriculturally intense area where virtually all land was either tilled agricultural fields or semi-natural grazed pasturelands, with the expectation that mosaics of land use types may better support ecosystem services than homogenous crop areas. Fields were chosen in two categories, five with little or no pastureland (<6%) and five with at least 15% pastureland within an 800 m distance of field edges. Fields in the high pasture category had more bumble bees than low pasture fields and 94% of the variation in bumble bee abundance in fields was explained by variation in the amount of pastureland nearby. There was a trend towards greater abundance of other bees in the high pasture category and significantly more other bees in the centre of high pasture fields than low pasture fields. Lower bee abundance in fields with little pastureland around them could result in reduced pollination and seed set unless supplemented with managed pollinators such as honey bees. In areas with intense

agriculture we show that mosaics of land use types can be better for wild bee populations and potentially for crop production than landscapes that are homogenous tilled crop areas. Design and management of agroecosystems that integrate land use and ecosystem function is a practical approach for promoting sustainable agricultural.

7.2 Introduction

Humans have transformed over half of the earth's surface and no ecosystems are untouched by human influence (Vitousek et al. 1997). Modern agricultural landscapes are highly disrupted, raising the concern that intensive agriculture is not sustainable (Matson et al. 1997). If current trends of agricultural intensification and land conversion continue, it is expected that disruption of the earth's ecosystems caused by agriculture will be the major cause of non-climatic global change (Tilman et al. 2001). Pollination by wild insects is an essential ecosystem service potentially disrupted by agricultural land use. However without insect pollination, crop production would be dramatically lowered in approximately 66% of crop species (Roubik 1995), severely impacting global food supply. Areas of intensive agriculture, with little natural land, are suffering lower crop yields as a result of too few wild pollinators (Kremen et al. 2004; Ricketts 2004; Morandin & Winston 2005). Managed honey bees have become the most important managed pollinators world-wide, but extensive losses of colonies have occurred in the last 20 years and conservation of wild bee populations is of increasing concern (Watanabe 1994; Matheson et al. 1996; Allen-Wardell et al. 1998).

Ideally, more natural land areas can be established or maintained within agricultural regions to provide essential and/or beneficial ecosystem services such as flood control, soil creation and regeneration, water purification, atmospheric carbon

dioxide storage (Tilman et al. 2002 and references there-in), and crop pollination (Allen-Wardell et al. 1998). Annually tilled crop lands are highly disturbed and landscapes that incorporate mosaics of managed land use types may better conserve ecosystem function than uniform regions of cultivated land (Sanderson et al. 2002). Organisms such as bees, in homogenously tilled agricultural landscapes, that utilize the environment on a large scale could benefit from land mosaics, with semi-natural areas or areas of less intensely managed agriculture (Banaszak 1992). Thus, pasturelands interspersed amongst tilled areas may provide habitat for wild bees and increase productivity in adjacent crops.

Wild bees require both nesting and foraging habitats (e.g., Kearns & Inouye 1997). Crops may provide abundant floral resources while in bloom, but crop plants often bloom in synchronous periods usually lasting only a few weeks. On a larger landscape level, successive crop bloom could provide more continuous forage than single fields (Westphal et al. 2003). Fields must bloom successively, within foraging range, throughout a bee's life for crop landscapes to provide adequate food supply to wild bees. While this situation may benefit bumble bees and other bees with large foraging ranges (Westphal et al. 2003), most other bee species are thought to have foraging ranges that are less than one kilometre (Steffan-Dewenter et al. 2002), and some as little as a few hundred meters.

Wild bees also require nesting sites, often old rodent burrows, hollows in twigs or grass, brush piles, or holes in soft sand and dirt (Kearns & Inouye 1997). Potential nesting habitat can be disrupted in wheat, canola, barley, and soybean fields, which dominate the Canadian prairies (Statistics Canada 2005) because these annual crops are harvested each year and fields are tilled, often in the fall and spring.

In agricultural landscapes dominated by tilled crop fields, field margins likely are the only land that provides nesting habitat for wild bees. But pasturelands are untilled, semi-natural grasslands that might provide suitable nesting habitat and forage when nearby crops are not in bloom. Identification of habitat that could promote wild bee populations and consequently crop yield in intensive agricultural areas is important for maintaining sustainable production from bee-pollinated crops.

Canola (*Brassica napus*) vies with wheat as Canada's most valuable crop. Annual exports of canola seeds, oil, and meal produced from seeds are valued at over $3 billion (CDN) and the canola industry contributes over $6 billion annually to the Canadian economy (CCC 2005), and is of increasing importance in the United States. Canola is an annual crop and fields are tilled before planting, although some genetically modified herbicide-tolerant canola fields are directly seeded (all fields were tilled in our study). Because canola seed production is increased by insect pollination (e.g., Free 1993), examination of landscape factors that promote wild bee populations in canola agroecosystems is important economically as well as for conservation of biodiversity.

We examined wild bee populations in canola fields in southern Alberta, Canada, in relation to surrounding crop and pasture land. We hypothesized that canola fields with greater proportions of surrounding pastureland within 800 m of field edges would have greater bee abundance than canola fields surrounded by tilled cropped land.

7.3 Methods

Data were collected in southern Alberta, Canada, near the city of Lethbridge. The area is a mix of semi-natural grassland pasture and barley, wheat, soybean, and canola

fields. We chose ten canola (*B. napus* DK3235) fields ranging in size from 24 to 64 ha. Five fields had a minimum of 15% (approximately 64 ha) pastureland in an 800 m distance from field edges and five had less than 6% pastureland in an 800 m distance from field edges. In the 'high' pasture category, pastureland ranged from 72 to 256 ha. In the 'low' pasture category, pastureland ranged between 0 and 24 ha although four of the five fields had less than 2 ha of pastureland within an 800 m of field edges.

Six bee collection sites were located in fields, two each at 20 m, 100 m, and 300 m from a chosen edge. Collection sites at the same distance from the edge were 200 m from perpendicular edges and 200 m from each other.

7.3.1 Bee Collections

Bees were collected using pan trapping and sweep net sampling at each collection location. A set of three pan traps (one blue, yellow, and white; approximately 30 x 50 x 20 cm) were placed at each collection location twice during bloom for 48 h each time. Each pan trap had 1.5 L water, approximately 5 ml glycerol to lower surface tension, and 10 ml of honey (Morandin and Winston 2005; Chapter 4). One day of sweep net samples and visual observations were conducted in each field, from approximately 10 am until approximately 4 pm. Collectors started at one collection location on the edge of the field and followed a route so that each collection location was sampled in the morning and afternoon. Sweep net samples were done along a 30 m transect at each location. The collector, using a 30 cm diameter sweep net, walked the transect until they had completed 50 sweeps directed towards bees that could be seen on canola flowers, or simply into the canola flowers if no bees were observed. Each sweep net sample lasted approximately 60 s.

Visual observations were conducted in a 1 m x 1 m quadrat randomly placed at each collection site. The quadrat was observed for 5 minutes and only bees landing within the quadrat were recorded. Due to the difficulty of identifying bees to genus visually, observations were split into three categories: Bumble bees, honey bees and all other bees. Visual and sweep net samples were only conducted on days when the temperature was above 18°C and mostly sunny.

7.3.2 Surrounding Land

We quantified land types within an 800 m distance from field edges by ground surveys for a total of approximately 512 ha (8 sections each 800 x 800 m) of surrounding area surveyed. We chose to examine land at this scale because in a previous study examining habitat from a 250 to 1500 m scale around canola fields, we found that bee abundance in fields was most affected by habitat within a 750 m distance of field edges (Morandin et al. in review; Chapter 5). Agricultural land in Alberta generally is divided into 800 x 800 m sections (quarter of a mile^2 sections), making examination of the eight quarter sections around each field a practical and relevant area to investigate. We categorized land as crop, pastureland, natural grassland, and swampland. We found that virtually all land was either tilled cropland or pastureland and therefore these were the only land categories included in analyses. The pasturelands in our study were composed of grasses, small shrubs, and wildflowers.

In some cases pastureland was only located on one side of fields while for other fields pastureland was more dispersed around fields, so we were unable to orient collection locations in relation to pastureland. Instead, collections reflect overall bee

abundance in fields with respect to a chosen side that could be easily accessed with minimal disturbance to fields.

7.3.3 Data analysis

We identified bees to genus and compared genus richness estimated using a jackknife procedure (Jack1; EstimateS 2005) between low and high pasture fields using ANOVA. Proportional representation of the top three genera of wild bees was compared between field treatments using Chi-square analysis.

We analysed bumble bee (*Bombus* spp.) and "other" wild bee abundance data separately. Bumble bees are larger than most other bees and have different nesting requirements. Perhaps more importantly, bumble bees are thought to have foraging ranges of one to several kilometres whereas other bees are believed to only forage within a few hundred meters of their nesting sites, potentially resulting in very different responses of these to groups of bees to landscape characteristics (Walther-Hellwig & Frankl 2000; Westphal et al. 2003).

Bee abundance was calculated from bees collected in pan traps and sweep nets and was compared among treatments using a mixed model ANOVA with a nested design. We included field treatment (high and low pasture) and distance into the field as main effects, and field nested within treatment and collection side nested within distance, field, sample method, and field treatment as random effects. We included all interaction terms and computed pairwise comparisons of least squares means of main effects and interactions. Visual observation data were analysed separately, using a mixed model ANOVA with the same design as described above. Because data from visual

observations resulted in far less data than pan trap and sweep net samples, the majority of analyses have been conducted on collection data rather than visual data. Only 29 of the 656 bees were collected by sweep net sampling and we therefore included pan and sweep net samples together rather than doing separate analyses. Unless otherwise stated, all reported results are from collected bees. Data were poisson distributed and we therefore square root transformed raw numbers from each trap location. All reported means are from the non-transformed data. Because there was a range of amounts of pastureland in high and low pasture field treatments, we also include regression analyses of bumble and other bee abundance in relation to pasture area.

7.4 Results

7.4.1 Genera composition

A total of 656 bees from 20 genera were collected in the 10 fields (Table 7.1). The most common genera were *Lasioglossum* (42.5%), *Bombus* (26.1%), and *Andrena* (14.3%). In the low pasture fields a total of 145 bees were collected from 15 genera with the most common being *Lasioglossum* (37.9%), *Andrena* (24.1%), and *Bombus* (16.5%). In the high pasture fields a total of 511 bees were collected from 17 genera with the most common being *Lasioglossum* (43.4%), *Bombus* (28.8%), and *Andrena* (11.5%). Estimated genus richness ± SE was 9.1 ± 1.14 and 13.9 ± 2.63 in low and high pasture fields respectively. There was a trend towards greater genera richness in high pasture fields but there was no significant difference between pasture categories ($F_{1,8} = 2.73$, $P = 0.137$).

There was a difference in the proportional representation of the top three genera of wild bees between high and low pasture fields ($\chi^2_3 = 19.73$, $P < 0.001$). The data that most contributed to the significant Chi-square value were the relatively smaller representation from the genus *Bombus* and relatively larger representation from the genus *Andrena* in the low pasture fields.

7.4.2 Bee abundance and pastureland

Bumble bees

There was no interaction between bumble bee abundance at different distances into fields and pasture category ($F_{2,46} = 0.97$ $P = 0.386$) and marginally no effect of distance ($F_{2,46} = 2.82$, $P = 0.070$). There were more bumble bees in the high pasture category than the low pasture category ($F_{1,8} = 13.15$, $P = 0.007$; Figure 7.1). Bumble bee abundance was significantly greater in the high pasture category at all distances (20 m, $t_{46} = -2.83$, $P = 0.007$; 100 m, $t_{46} = -2.36$, $P = 0.022$; 300 m, $t_{46} = -3.72$, $P < 0.001$; Table 7.2). While there was a trend towards greater bumble bee abundance with distance into fields in the high pasture category there was no significant difference in bumble bee abundance among distances in either pasture category (Category*Distance sliced by category; low pasture $F_{2,46} = 1.36$, $P = 0.267$, high pasture $F_{2,46} = 2.43$, $P = 0.099$). There was no significant difference in bumble bee abundance between field types from visual assessments ($F_{1,7} = 1.20$, $P = 0.308$), however there only was a marginally non significant difference between least squares means ($t_7 = -2.10$, $P = 0.074$) with greater bumble bee abundance in high pasture fields than low pasture fields (0.21 ± 0.08 SE bees/5min observation vs. 0.01 ± 0.07 SE bees/5min observation respectively).

Other bees

There was no interaction between field treatment and distance into fields in other bee abundance ($F_{2,46} = 2.39$, $P = 0.103$) and no effect of distance on other bee abundance ($F_{2,46} = 0.65$, $P = 0.526$). There were significantly more other bees in the high pasture category than low pasture category ($F_{1,8} = 5.82$, $P = 0.042$; Figure 7.1). Other bee abundance was significantly greater in the high pasture category at 100 m ($t_{46} = -2.29$, $P = 0.027$) and at 300 m ($t_{46} = -2.93$, $P = 0.005$) but no significant difference between field categories in abundance of other bees at 20 m ($t_{46} = -1.12$, $P = 0.232$; Table 7.2). There was a trend towards more other bees with distance into fields in the high pasture category but there was no significant effect of distance in either pasture category (Category*Distance sliced by category; low pasture $F_{2,46} = 0.63$, $P = 0.538$, high pasture $F_{2,46} = 2.41$, $P = 0.101$). There was no significant difference in other bee abundance estimated from visual observations ($F_{1,7} = 2.68$, $P = 0.146$) but differences of least squares means indicated a trend towards more other bees in the high pasture category than the low pasture category (1.04 ± 0.32 SE bees/5min observation vs. 0.33 ± 0.29 SE bees/5min observation respectively; $t_7 = 1.87$, $P = 0.103$).

There was a significant relationship between area of pastureland and bumble bee abundance in fields ($F_{1,8} = 159.54$, $P < 0.001$, $r^2 = 0.95$) but not between area of pastureland and other bee abundance ($F_{1,8} = 1.22$, $P = 0.302$, $r^2 = 0.13$; Figure 7.2). Other bee abundance at 300 m (centre) into fields was significantly correlated with the amount of pastureland around fields when one outlier field was removed from the analysis (this field had three times more other bees in the centre than any other field and an

intermediate amount of pastureland) ($F_{1,7}$ =10.64, P = 0.014, r^2 = 0.60; Figure 7.3; with outlier, $F_{1,8}$ = 1.49, P = 0.257, r^2 = 0.16).

7.5 Discussion

Canola fields with semi-natural pastureland within 800 m of field edges had more bumble bees than fields that were almost completely surrounded by tilled crop land. Although bumble bee species differ in their nest site preferences (Richards 1978; Svensson et al. 2000), uncultivated areas are much more commonly used for nesting sites than cultivated areas (Fussell & Corbet 1992; Svensson et al. 2000). Svensson et al. (2000) conducted the most extensive study of bumble bee nest selection in agricultural landscapes, observing 147 nest-seeking queens over a variety of land-use types including pastures, cultivated fields, and uncultivated fields. While they found some nest seeking queen bumble bees in crops that were not tilled annually, they found no nest seeking queens in annually tilled fields. Bumble bee queens in their study were found searching for nests in areas with withered grass and tussocks typical of uncultivated areas. Underground nesting bumble bees likely depend on the presence of abandoned rodent burrows (Harder 1986), that also would not be frequent in tilled agricultural fields.

In the 10 fields in our study location, 94% of the variation in bumble bee abundance in fields was explained by differences in the amount of pastureland within 800 m of field edges. Virtually all land was either annually tilled crop or semi-natural pastureland, and therefore our data suggest that only pasture areas provided suitable nesting area for bumble bees in our study region.

Semi-natural land is thought to be important for bumble bees in agricultural landscapes (Corbet 1995; Dramstad & Fry 1995; Kells & Goulson 2003) and our data support this hypothesis. However, there is some disagreement over the importance of uncultivated land to bumble bee abundance in agroecosystems. Wesphal et al. (2003) found a positive relationship between bumble bees and mass flowering crops on a landscape scale and no relationship between bumble bee abundance and semi-natural land. They hypothesized that because bumble bees have foraging ranges up to a few kilometres and are more general foragers than other bees, they benefit from mass flowering crops on a landscape scale and are less dependent on semi-natural land (Westphal et al. 2003 and references there-in). However, there were grasslands, forests, and settlement areas within their landscapes and these were not included in their analysis of semi-natural land and bumble bee abundance, and they may have provided suitable habitat for bumble bees. The difference between our study and Westphal et al. (2003) highlights the complexity of pollinator population dynamics at a landscape scale.

Semi-natural areas are thought to be important for nesting and foraging habitat for non-*Bombus* wild bees (Corbet 1995) and we also found a trend towards more non-bumble, wild bees in fields from the high pastureland treatment. The difference between low and high pasture fields in the number of other bees was more pronounced at greater distances into fields, and in the centres of fields, high pasture fields had significantly more other bees than low pasture fields. Similarly, Steffan-Dewenter and Tscharntke (1999) found that wild bee abundance decreased with distance from grasslands and concluded that maintaining the connectivity of natural habitats is essential to crop and endangered wild plants. Our collection locations were not necessarily oriented with

respect to pastureland around fields, and high variation in other bee abundance at edge collections was evident. High pasture fields may have shown a trend towards more bumble and other bees with distance into fields because in centres of fields there could have been a cumulative effect of source habitat from more than one side contributing to bee abundance.

Overall other bee abundance was not correlated with the amount of semi-natural land around fields. Most bees have smaller foraging ranges than bumble bees (e.g., Westphal et al. 2003) and therefore differences in nesting habitat (e.g., pastureland, edge habitat suitability) in relation to the edge of fields where collections were oriented could have caused the large variation observed at field edges. Interestingly, abundance of other bees in the centre of fields was highly correlated with the amount of surrounding pastureland ($r^2 = 0.60$ with one outlier field removed from the analysis). This may be because bee abundance in centres of fields is a reflection of overall surrounding habitat and less influenced by small-scale variation in habitat near collection locations. Greater bumble and other bee abundance in centres of fields with more pastureland around them suggest that these fields are receiving more homogenous pollination than ones with little or no pastureland around them and few wild bees towards the centre.

Ground nesting bees such as *Lasioglossum* and ground or cavity nesting bumble bee species (Michener et al. 1994) would not be able to nest in tilled canola fields and this may explain why we found fewer bees from these genera in fields with little or no pastureland around them. There was not a large decrease in the number of *Andrena* collected in low pasture fields than high pasture fields, possibly because their preferred nesting habitat is in banks (Michener et al. 1994). In our study area the untilled field

margins adjacent to roads were usually steep banks a few meters wide and may have been good nesting habitat for *Andrena*.

Greater abundance of wild bees has been found to increase production in canola (Morandin & Winston 2005; Chapter 4), watermelon (Kremen et al. 2002), and coffee (Ricketts 2004) crops. With declines in managed honey bee colonies over the last 20 years resulting from pests, diseases, and breeding with Africanized honey bees, and suspected declines of wild bees there are concerns that crop pollination and production are in jeopardy (Watanabe 1994; Allen-Wardell et al. 1998). Long-term reserve areas have been proposed for agricultural landscapes that would aid in maintenance of ecosystem services such as pollination by wild insects (Kremen et al. 2004). Although minimal disturbance by humans and human industry in the form of natural reserve areas is likely the best way to preserve natural ecosystem function, our results show that diversity in land use also can significantly benefit ecosystem services. Understanding how ecosystems function on a landscape scale will aid in development of agroecosystems that are both profitable and sustainable.

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7.7 Tables

Table 7.1 Total number of bees, divided by genera, collected in five canola fields with little or no pastureland and five canola fields with at least 15% pastureland within 800 m of field edges. Bees were collected using pan traps and directed sweep netting, with the same collection effort in each field.

Genus	Low Pasture Fields (n = 5)	High Pasture Fields (n = 5)
Agapostemon	2	0
Andrena	35	59
Anthidium	1	1
Bombus	24	147
Colletes	5	17
Diadasia	5	5
Dufourea	3	8
Epeolus	1	2
Halictus	0	4
Hoplitus	1	0
Hylaeus	0	1
Lasioglossum	57	222
Megachile	5	3
Melissodes	0	4
Nomada	1	1
Osmia	0	3
Panurginus	3	16
Perdita	1	1
Psithryus	1	0
Sphecodes	0	17
Total	145	511

Table 7.2 **Mean number of wild bees collected at sampling locations at different distances into canola fields using pan trapping and sweep net sampling. Five fields had little or no pastureland around them and five had at least 15% pastureland within 800 m of field edges. Mean abundances are means from each sampling distance averaged across fields. Rows with different letters within bumble bees and other bees indicate significant differences between abundance at each distance ($p < 0.05$).**

	Bumble bees ± SE		Other bees ± SE	
Distance (m)	Low Pasture	High Pasture	Low Pasture	High Pasture
20	0.2 ± 0.1a	3.4 ± 0.9b	4.6 ± 1.5a	9.1 ± 2.4a
100	1.1 ± 0.4a	4.5 ± 1.0b	3.3 ± 1.6a	12.7 ± 3.7b
300	0.7 ± 0.3a	7.8 ± 2.2b	2.8 ± 0.9a	17.0 ± 5.0b

7.8 Figures

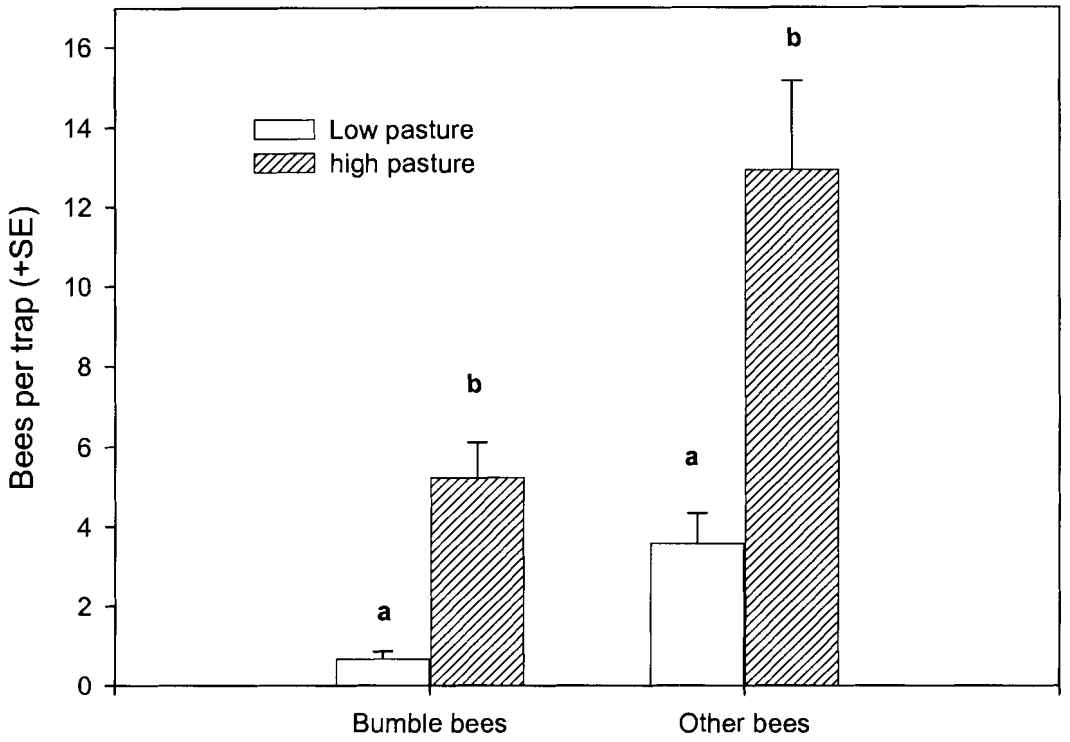

Figure 7.1 **Mean number of bumble (*Bombus* spp.) and other wild bees collected in 10 canola fields. Five fields had little or no pastureland within 800 m of field edges and five had at least 15% pastureland within 800 m of field edges. Different letters over bars from the two field categories indicates a significant difference in bee abundance at $p < 0.05$.**

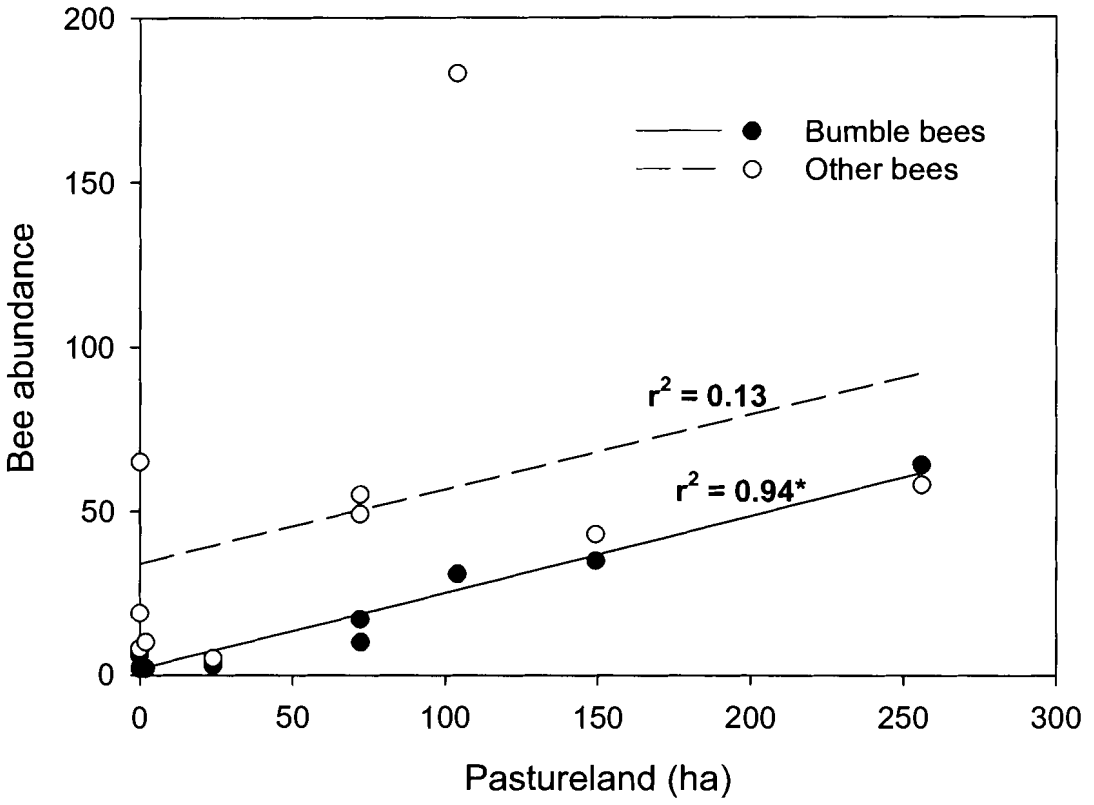

Figure 7.2 The relationship between number of bumble (*Bombus* spp.) and other wild bees, and amount of pastureland within 800 m of 10 canola fields. Virtually all land was either tilled crop land or pastureland.

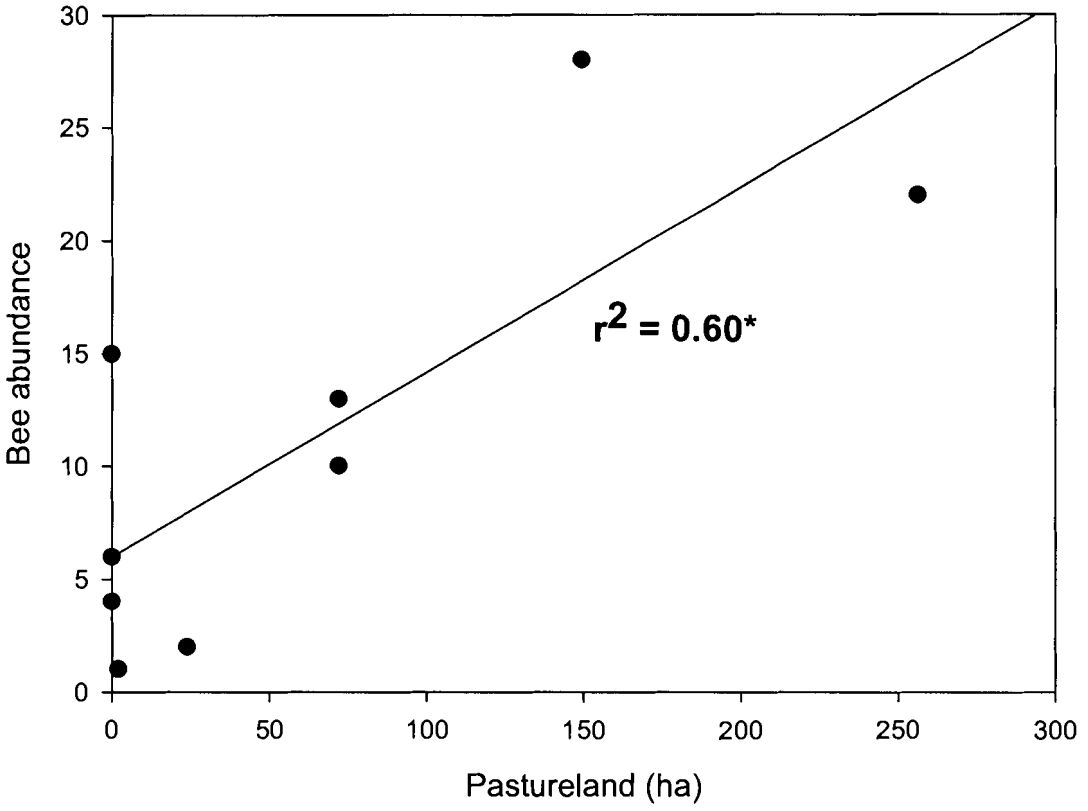

Figure 7.3 The relationship between abundance of wild bees (excluding bumble bees) in the centres (300 m from edge) of 10 canola fields and amount of pastureland within 800 m of field edges.

CHAPTER 8
CONCLUSIONS

8.1 Overview

Modern agriculture can negatively impact wild bee populations, resulting in lower yields in bee-pollinated crops. Identification of practices that harm bees, and methods to improve bee health and populations are essential for development of agricultural practices and agroecosystems that not only are sustainable, but profitable. Bt GM crops currently are the most common insect resistant transgenic plants, and our research suggests that this method of pest control is harmless to bees (Chapter 2). Another GM pesticidal protein, chitinase, also did not harm bumble bees. If these two GM pesticides are not toxic to other wild bees, the use of those GM pest control technologies might conserve pollinator biodiversity and abundance, and improve crop yield, compared to pest control based in synthetic chemicals. Still, each new GM pesticidal protein should be tested on a variety of wild bees, for lethal, sub-lethal, and foraging effects before wide-spread use in the environment.

I did find a negative effect of the nicotinoid insecticide imidacloprid on bumble bee foraging ability when bees were fed elevated doses (Chapter 2). However, exposure at realistic levels of imidacloprid encountered in the field did not harm bees. Our finding of negative impact at higher doses emphasizes the importance of following use recommendations in order to prevent negative ecosystem impacts.

While spinosad can be harmful to honey bees, current use recommendations only require that honey bees not be present when spraying occurs and make no mention of wild bees. We found that elevated doses of spinosad rapidly kill bumble bee colonies and levels found in the field can impair foraging ability (Chapter 3). Timing of applications and doses need to be managed in order that this pesticide is used effectively while causing minimal harm to wild bees. Our research suggest that pesticides developed from naturally generated compounds, even if thought to be reduced risk to the environment, can cause substantial impacts to bees and warrant full impact assessment prior to commercial use.

Our research also highlights hidden effects that pesticides may have on bees. In addition to few bees other than honey bees being tested, sub-lethal effects, long-term exposure, and exposure during larval development often are overlooked. I developed a method of testing for foraging effects of pesticides on wild bees that proved to be effective at revealing sub-lethal impacts that would normally go undetected. I suggest that this or similar methods be incorporated into routine testing as part of the regulation of new pesticides.

Caution need be applied when interpreting data from toxicity studies that show 'no effect'. A finding of no significant effect of pesticides could result from low power to test for differences between treatment and control means, falsely indicating that pesticides are safe, when in fact experiments with greater power could find differences. For this reason, it is important when reporting and interpreting results of studies that carry large implications when no effect is found, that power be reported, as I have done in these studies. Alternatively, magnitude of differences that could be found significant

given variation and sample size in a particular experiment based on an acceptable risk of type II error may better demonstrate the ability of an experiment to predict pesticide impact.

Little is known regarding the importance of wild bees to the majority of crops, and there is little incentive to develop technologies and management that take into account wild bee health. I show (Chapter 4) that wild bees significantly increase seed set in canola, and technologies that reduce weed cover in fields may be resulting in fewer wild bees and lower seed set. Multiple factors influence bee abundance (Chapter 5), and bumble bees and other bees were most affected by habitat within 750 m of edges of fields. Identification of the scale that is important to wild bee populations is mandatory for development of agroecosystem landscapes that promote bee populations. Analysis of landscape and local factors allowed me to identify weed cover in fields and area of surrounding uncultivated land as the most important factors associated with bee abundance in fields. Bee species richness and diversity also were related to surrounding habitat and field weeds. Interestingly, when I controlled for variation in these local and landscape factors in analyses, there was no difference in bee abundance among field types (organic, conventional, and GM) suggesting that the factors we quantified were important for bee communities.

Weed control technologies are beneficial to crop production, and currently over 80% of canola grown in Canada is GM herbicide tolerant. But my data suggest that maintenance of semi-natural surrounding land within 750 m of fields can increase wild bee abundance even in fields with extremely effective weed control. Because bees are so important for pollination of canola, there is a trade-off between conversion of land to

agriculture and maintenance of semi-natural land that serves as source area for bees. Based on my data I developed a model that showed canola agroecosystems with 20-30% of land left in a semi-natural state would maximize profits (Chapter 6). While I model a simple system with canola, any crop that benefits from wild bee pollination similarly would benefit from landscapes that maintain semi-natural land.

Mosaics of land uses may be better for pollinator populations than homogenously tilled landscapes in agriculturally intense areas where there is little or no natural land. Wild bees were more abundant in fields that had grazed pastureland around them (at least 15% of area within 800m of field edges) than fields that only had tilled crop acreage around them (Chapter 7).

8.2 Synthesis

Agricultural technologies are constantly changing and advancing, and new technologies often are adopted before environmental impacts are reasonably well understood. While world hunger is more related to food distribution and unequal access than insufficient food production *per se* (Matson et al. 1997) with the world's population expected to grow from 6.5 billion at present to over 9 billion by 2050 (U.S. Census 2005) agricultural expansion and intensification is inevitable (Tilman et al. 2001). Neglecting environmental impacts of agriculture threatens to disrupt ecosystem services, reducing or eliminating any yield benefit from intensification and expansion, and cause irreversible environmental impact (Tilman et al. 2002). Wild bees have been suspected (Corbet et al. 1991; Allen-Wardell et al. 1998) and more recently shown (Kremen et al. 2004; Ricketts et al. 2004; Morandin & Winston 2005 (Chapter 4)) to be an economically important component of agricultural production. I have shown that some new agricultural products

and practices negatively impact wild bee populations and crop production while others are not harmful.

In North America, we have increasingly relied on honey bees as our primary crop pollinators over the last hundred years and pollination by honey bees is currently valued at $1 billion per year in Canada and $15 billion (CDN) per year in the United States alone (Watanabe 1994; Nasr 2005). In the past small farms likely had all of their pollination requirements met by wild pollinators. As farming intensified during the green revolution of the 1960's, pollination by managed honey bees increased, and declines of wild pollinators may have resulted from increased contact with pesticides and decreased forage and nesting habitat. But substantial declines over the last 15 years in managed honey bee colonies (Watanabe 1994; Matheson et al. 1996) followed by unprecedented colony death estimated at 50% of North American colonies in one year (2004/2005), has resulted in the price of colonies more than doubling and pollination needs not being met (Nasr 2005). These events make conservation of wild bees crucial to crop production and global food supply. Only with knowledge of how modern agricultural practices affect wild bee populations can regulatory policy be modified and agroecosystems designed and managed in ways that promote pollinators populations and biodiversity, and ensure sustainable food production.

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