

**Wild pollinator contribution to crop yield, a Comparison of landscape characteristics and their effect on seed set of Canola (*Brassica sp.*) in Alberta**

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## **Abstract**

The agricultural production of canola in the Canadian prairies is expanding rapidly, with expected yields of 16 million tonnes per year by the year 2025. It will therefore become increasingly important to better understand the relationship between wild pollinators and semi pollinator-dependent crops like canola (*Brassica napus*). The ecosystems services that wild insects supply could potentially be disrupted by intensive agricultural land use. Biologically diverse landscapes are necessary to uphold insect guided pollination. The loss or dissociation of food or nesting opportunities connected to these landscapes is one of the main causal factors behind a recent decrease in wild pollinators. Most studies which connect canola yields and pollination services involve experimental designs which have input managed honey bee (*Apis mellifera*) into the agricultural system. To expand this body of knowledge, this study investigates whether wild insects play a role in seed setting in Canadian canola under real world agricultural production. We tested whether variance in seed set is attributable to parameters of the surrounding landscape by identifying any yield increases in seed sets of canola as a proxy for yield against contributions by surrounding landscape. Statistical analysis, via ANOVA, was used to check for variance between seed sets from plants found at point measured 20m and 200m into the field across six different canola fields. Findings suggest no variation between seed sets at different distances into the field however significant variance between different growers. Therefore, there may be an effect of different landscape and growing conditions on the final seed sets of large-scale canola cultivation.

**Key Words:** Pollination, Canola, Sustainability, Agriculture, Landscapes

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## 1.0 Introduction

### 1.1 Statement of the Problem

Wild pollinators are frequently labelled as having too sparse an impact on large scale crop systems (Osborne et al. 1999). Their services are seen as inadequate in providing sufficient pollination to food crops in an agriculturally intensive environment. Agriculturally intensive crops are grown at a rate of 4 million metric tonnes per year (Klien et al. 2010). And current yields of oil producing crops in Canada, are considered agriculturally intensive systems. An important oilseed crop within Canadian agriculture, canola (*Brassica napus sp.*) within the production year of 2013 cultivated approximately 16 million tonnes of canola, that is four times the minimal measurement the Millennium Ecosystem Assessment identified as agriculturally intensive crop production (CCC 2013, Reid 2005). The majority of Canadian canola crops are privatized to large corporate entities including Pioneer and Calegen (Kneen 1992) and the privatization of crops can stir conflict between commerce for private gain versus food production for public gain (National Farmers Union 2013). The uprising of large scale intensive agricultural systems including monocultures can cause losses in small scale farming and can diminish important biologically diverse plant selections in the area, as monoculture systems develop and expand (Klien et al. 2010). An important ecosystem service upheld by biologically diverse landscapes is insect guided pollination (Morandin, Winston 2005). The Millennium Ecosystem Assessment proposed the classification of ecosystem services as the link between human welfare and services provided by ecosystems (MEA 2005). Pollination is considered an ecosystem service as the action of pollinating crops allows the plant to develop sexually, and produce fruits and other foods which can be consumed by human (Reid 2005). Pollination by wild insects is viewed as an ecosystem service and is potentially disrupted by agricultural land use (Morandin et al. 2007). These forms of ecosystem services are integral parts of natural resource management for such activities as intensive agriculture expansion, and policy decisions behind these large crop systems (Fisher, Turner 2008).

Reserve areas may be implemented into intensive agricultural landscapes as a precautionary step (Klien et al. 2010). These reserves are thought to aid in maintaining ecosystem services such as pollination (Aizen et al. 2008), large statement which affect agricultural landscapes must be justified. It is very difficult to understand the true impacts agricultural systems have on the biologically diverse habitat and pollinator communities (Morandin, Winston 2005).

Pollinator foraging patterns has been linked to both their dependence on productive pollen gathering and to the distance the bees must travel from their nest (Richards 2001). Foraging bees of this nature prefer creating nesting habitats in undisturbed area such as abandoned burrows, old wood stands and soft top soils in grasslands (Morandin et al. 2007) where activities such as annual ground tilling will not disrupt the foraging bee's home. Unfortunately with current agenda setting being centered on economic gains, precedent for agricultural expansion of profitable crops such as canola, these undisturbed landscapes are on the decline (Kohler et al. 2008). One of the main causal factors behind the current decrease in populations of wild pollinators is the loss of dissociation of important resources to the bees such as food and nesting opportunities (Klien et al. 2010).

## **1.2 Purpose of the Study**

As Canadian agricultural production of canola progresses forward with its plans for expansion into 2025 (CCC 2014) it will become ever-the-more important to recognize the benefits wild pollinators provide for crop systems like canola. The underlying assumption behind the current decrease in populations of wild pollinators will allow for further considerations towards land conservation efforts placed within agricultural policies in order to protect biodiversity of land and insects alike. It is important to recognize impacts of canola on the Canadian landscape and economy, in order to put provide context for this study. The canola industry as a whole (includes farming, processing, distributions) produces about 19.3 billion dollars towards the Canadian economy each year making it Canada's overall best oil seed crop (CCC 2014, Philips 2001). This crop spreads over the prairie provinces of Canada, and of the 16 million tonnes of Canola produced in Canada Alberta produced approximately 35% of the yield (Agriculture Alberta 2013). For this large scale production of a semi pollinator dependant crop it is important to recognize important areas of conservation to wild pollinators which may improve seed sets of canola (Duran 2009, Sabbahi 2009, Morandin et al. 2007). Pollination is found to improve the seed set of some cultivars of *Brassica napus* canola (Manning, Wallis 2005). However other sub varieties of canola are considered autogamous allogamous. Autogamous allogamous implies that the flowers of canola plants are self-fertile and have the ability to produce some seeds and fruits in the absence of animal visitation, but pollination performed by animals can increase the proportion of seed sets, or the quality of the fruit size (Richards 2001).

The attributes of canola's sexual reproduction are important elements of this study. Variance in seed sets of canola as a proxy for pollinator contributions and their associated preferred landscapes surrounding canola cultivated areas within Alberta. Further evidence is required to show whether agricultural landscapes improve pollination services to canola and other semi pollinator dependant crops (Klien et al. 2010). If there is potential for an increase in yield attributable to undisturbed habitat, the potential for larger land conservation efforts in industrial agricultural systems would assumed benefit sustainable agricultural practices into the future.

### **1.3 Research Question**

This study will contribute to knowledge on the wild pollinator's role in canola cultivation through a set of statistical tools working to answer the following research question:

Do wild insects play a role in seed setting in Canadian canola, under real world agricultural production?

This questions will be solved by working through two consecutive questions. First asking, is there any evidence of yield increase due to a plants distance into the field? Subsequent questions move into specific parameters of the study and asks is there is any yield variation can it be correlated to adjacent landscapes? Through solving these questions we will come out with a result either supportive, or unsupportive of the research question.

### **1.4 Significance of the Study**

Existing studies indicate an increased variance with fruit set at locations isolated from undisturbed landscapes including landscapes which are near-natural (Klien et al. 2010). To date, the majority of studies analysing the significance of canola yields due to pollination services were derived from experimental designs which have input managed honey bee (*Apis sp.*) into the agricultural system. This has resulted in excitement within the research community around the positive correlation in yield of canola with an increase in hives per hectare (Sabbahi 2009, Duran et al. 2009). However this experimental design is neglecting the relationship between wild pollinators and canola yield in real world agricultural production, exclusive from increases in managed hives. From studies by Marandin et al 2007, Metson et al. 2013, Klein et



al 2010, and Kohler 2008, it has been established that landscapes have known effects on their insect communities. It is therefore essential to look at the impacts landscapes have on the pollinations of different crop productions to aid in the long term sustainability of agricultural ecosystems within the Canadian landscapes, in order to protect the biodiversity of insects and natural areas.

### **1.5 Limitations and Delimitations**

Major limitations to be addressed in this study, arise from the long period of storage of the canola pods (which will be used in the analyses) in a deep freeze. The moisture content of the frozen pods has caused some seeds collapse once they have been defrosted. Due to the process of freezing and thawing different shapes of the seed caused the seed counter to experiences difficulty identifying squished or extremely small canola seeds and therefore all final numbers on seed counts should also account for a +/- 2 form the total. This would result in inaccurate estimates of seed sets per pod form each plot. This limitation could be extended to place further problems onto the well fare of the seeds. Some stored pods were packed closer to the bottom of the freezer and could have become squished or ruptured over their 2 years of storage. Squished and ruptured pods may have dislodged seeds which we would be unable to recover for counting and again disrupting the estimate of seeds per pod.

In addition to limitations, there are certain delimitations which I have set for myself to help create the most efficient study possible. First, the data is representative only of the fields and therefore I will not draw conclusions on the canola industry as a whole. Second, Initial pod collection had occurred not simply at 20 m and 200m into the fields but also at 100m. Due to this colossal amount of unprocessed data, the 100m plots were excluded from the fields, to limit the scope to look at the two distances which had the largest and smallest space between their plots and landscapes around the field.

## **2.0 Literature Review**

### **2.1 Large scale agriculture and its impact on landscapes**

The growing human population places a demand on the natural environment to provide us with adequate food and nutrients we need to sustain a healthy diet (Pollen 2006). This growth has left virtually no ecosystem untouched by human influence (Morandin, Winston, Abbott, Franklin 2007). Providing adequate food supply for a growing population is difficult and has led to intensified development in the agricultural industry (Aizen et al. 2008). Agricultural intensification occurs within the cultivating, producing and distributing sectors of the industry. Intensification specifically in the crop cultivating sector is associated with drastic changes in agricultural landscapes (Le Feon et al. 2010). Intensification means a high productivity of pesticides and fertilizers and a decrease in the proportion of natural and semi natural grasslands in agricultural landscapes (Le Feon et al. 2010). Some of the inputs which are commonly increased in the Canadian agricultural landscapes include: increased nitrogen input from fertilizers to arable crops and a number of applications of herbicides insecticides, fungicides and retardants (ibid; Aizen 2008).

Agricultural intensification has led to considerable losses in biological diversity at various spatial scales (Kohler, Verhulst, Van Klink, Kleijn 2008). Converting lands from semi natural habitats such as grassland to croplands favours the channeling of solar and subsidised energy and ultimately rises the agricultural production and economic profitability for some crops (Latterra, Orue, Booman 2012). Unfortunately there are associated biodiversity losses and potential impairment of ecosystems which in return negatively affect agricultural sustainability, and availability of ecosystem services (ibid). Ecosystem services help humans persist, where there is a direct benefit to humans from the natural environment (Garibaldi et al. 2013). Over the last 45 years there has been a net increase in the total area devoted to agriculture and a clear shift in the relative importance of semi natural and natural habitats within agricultural production (Aizen et al. 2010; Le Feon et al. 2010). This shift shadows the importance of biodiversity which positively correlates to provisions of ecosystem services (Kohler, Verhulst, Van Klink, Kleijn 2008). Loss of plant diversity is considered to be the major cause of loss of bee diversity in agricultural habitats (Le Feon et al. 2010). Pollination by wild insects is an essential ecosystem service which is being disrupted by the increase in land use for agricultural practices. These disruption could lead to lower crop yields as a result of too few wild pollinators (Morandin, Winston, Abbott, Franklin 2007).

Not only has the increase in land used for agriculture caused a stir in the reliability of pollinators in the agricultural industry but; global agriculture has become increasingly pollinator dependent over the last five decades (Aizen 2008). A disproportionate increase in the land converted to agricultural production has been centered on pollinator dependant crops and it is unclear how this trade off of natural landscape to pollinator dependant landscapes will degrade the effectiveness of pollination as ecosystem service to the agricultural industry? (ibid; Laterra, Orue, Booman 2012) Altering cropping systems into areas of a majorly pollinator dependent landscape may impose a limit on the rate of yield, and could lead to an impractical need for increasing the services required by the potentially declining pollinator populations (Aizen et al 2008). Therefore it is important to actively seek a deeper understanding of how this change will affect the yields of crops which are used in our daily food system.

In contrast to gloom of limited pollinator services there has also been progress in identifying ways in which to conserve these potential ecosystem services. As stated previously pollinator abundance, visitation rate and consequently pollinator-dependent crop success is favoured by the presence of semi-natural habitats in surrounding landscapes to agricultural practices (Le Feon et al.2010; Morandin Winston, Abbott, Franklin 2007). These habitats could include forest edges or small landscape elements such as rivers, grass strips, weed patches or hedgerows (Philips 1990; Morandin Winston Abbott, Franklin 2007).

To help sustain healthy yields within an agricultural landscape would be to favour an increase in pollinator services, through creation of a healthier ecosystem. To do so the incorporation of landscape mosaics may better conserve ecosystem function than uniform regions of cultivated land (Garibaldi 2013). The idea of pasturelands interspersed amongst tilled areas may provide habitat for wild bees and increase productivity in adjacent crops (Morandin, Winston, Abbott, Franklin 2007). Evidence has shown that pollinator habitat is especially abundant in mosaic landscapes which cover less than 0.5% of the agricultural area and the presence of green linear elements increased pollinator visitation probability by 5-20% (Le Feon et al. 2010). Long term reserve areas have been proposed for agricultural landscapes that would aid in maintenance of ecosystem services such as pollination. The theory that increased natural areas will increase pollinator activity and help to sustain healthy crop yields into the future may hold true for pollinator dependant commodity crops. However there are a multitude of crops grown under intensive agricultural practices which may not be as affected by expansive land conversion and pollinations contributions. In fact in 2005 The European Millennium Assessments found

that there is incomplete evidence on declines in pollinators and the subsequent resulted crop failures (Reid et al. 2005). It is within these crop varieties were further research must be placed to help quantify pollination attributes incorporated in this form of intensive agriculture.

## **2.2 Agricultural Landscapes effect on pollinators**

As for-mentioned there have been steady increases over time in the absolute area of cropping land devoted to cultivation of animal pollinated crops. This increase has had an impact on pollination services as crop land increases and undisturbed pollinator habitat decreases, such as semi natural, grasslands and forests (Morandin, Winston, Abbott, Franklin 2007). Effects on pollinator habitats have consequences pollination demands as there are fewer places for these insects to live and prosper (Aizen et al. 2008). This section will discuss means in which an agricultural landscape can sustain wild pollinator populations through providing resources for food and nesting opportunities.

In a study lead by Morandin and Winston looking into *Wild Bee Abundance and Seed Production in Conventional, Organic and Genetically Modified Canola*, it was found that at study site which has abundant adjacent uncultivated areas contributed to noticeably larger populations of wild bees (2005). Further they found the number of bumble bees on crops was positively correlated with crops proximity to uncultivated land (Morandin Winston 2005). Earlier, having established that wild bee richness and abundance are negatively associated with intensive agriculture due to losses in semi natural habitats (Le Feon et al. 2010), it is also probable that farming practices that reduce weed diversity in or surrounding crops may also result in lower bee abundance (Laterra, Orue, Booman 2012). It is assumed that reducing weed diversity, and other operations which lead to disturbance in proximity to widely grown mass flowering crops has diminished habitat essential for pollinators (Ibid).

However this does not always hold true, fragmentation has also been seen to affect the foraging behaviour of bees. A study performed by Osborne and Williams found bumblebees in particular to be effected by the pollen flow within and between patches of flowering plants (2000). These bees showed a striking site visit consistency of 86-88% of bumble bees re-visiting larger patches of flowering crops compared to smaller patches (Osborne, Williams 2000). Therefore activities brought forth from the agricultural industry such as fragmentation would have an effect on the foraging behaviour

of pollinators (ibid). This study questions the authority of including more undisturbed landscapes into agricultural practices moving forward. However it is important to note that this trend was found for one species of pollinator the bumble bee. Yes, bees can benefit from mass flowering crops including canola, however this can only hold true for limited amounts of time (Le Feon 2010). When a large scale crop is in bloom. Other solitary bees have narrower floral requirements and thus can be directly impacted by the decrease in floral diversity (Kohler Verhults, Van Klink, Kleijn 2008; Le Feon 2010). Where agricultural intensification increased in certain areas it will have a different effect on species with varying life histories (Sydenham, Eldegard, Totland 2014).

Bumble bees not only are more conducive to foraging in larger single species agricultural landscapes but are assumed to have a larger foraging range compared to solitary bees (Le Feon 2010; Osborne Williams 2000). Plants on transects effected bumblebee abundance at experimental plants plots performed by Kohler, Verhults, Van Klink, Kleijn, and resulted in significant positive effects. It was seen that bees declined by about 70-80% in species density and abundance between the edge of the nature reserves and the crop fields (2008). Consequently foraging and nesting resources of solitary bees have to be in closer proximity to their food sources than for bumble bees (Le Feon 2010; Morandin, Winston 2005). If isolation from nesting habitats increase from 0-1km beow-ground nesting bees decreased by 25% and abundance and above ground nesting bees decreased by 47%. (Sydenham, Eldegard, Totland 2014). Therefore undisturbed landscapes would increase the likely hood of solitary bee pollinator services to adjacent agricultural landscape (Morandin, Winston, Abbot, Franklin 2007). Important factors such as spatial patchiness should play a greater role than overall crop abundance because of its on average positive effect on pollinator abundance (Donaldson-Matasci Dornhaus 2012).

Undisturbed landscapes means non-tilled landscapes which help to provide habitat for solitary bees as well as decrease soil erosion caused from wind and water runoff (Philips et al. 1990). In a study performed by Sydenham, Eldegard, Totland it was found that below ground nesting bees were more strongly effected by tilling and increased land used for agriculture (2014). Whereas above ground nesting insects were effected six times more than bees nesting below (Sydenham, Eldegard, Totland 2014) This study highlighted how landscapes can be used as proxies to help quantify pollinator contributions to crops. This concepts can then be applied to surrounding landscapes in agricultural regions

where undisturbed landscapes can act as a proxy for increased wild pollinator densities (Melathopoulos, Cutler, Tyedmers 2015).

### **2.3 Pollinator management in agriculture**

Assumedly pollinators are able to provide benefits to many of the crops in the food system today. An important benefit provided by pollinators is increasing the crops ability to increase fruit set. This service which pollination provides to the human food system has been used to place a monetary value on pollination (Melathopoulos, Cutler, Tyedmers 2015). Being able to identify the benefit from the environment creates deeper compassion for this ecosystem service as to people become more concerned with services which directly affect their wellbeing (Zahvoyska, Bas 2013; Metson, Ziter, Danconse 2013). Unfortunately the value of wild honey bees is seldom reflected in current pollination evaluation or estimates at national or global scales (Melathopoulos, Cutler, Tyedmers 2015). In one of the largest global pollination studies, Garibaldi et al found that in 41 of the major crop systems worldwide, fruit sets increased significantly compared to base line values which excluded pollination, in only 14% of major crop varieties (2013). How can the importance of pollination be stressed if there is no solid understanding that this service has a major effect on the majority of consumer food products. Ecosystem services such as pollination are increasingly studied but still complicated to understand and compare (Metson, Ziter Danconse 2013).

Globally yields of insect pollinated crops are managed for greater pollination through the addition of honey bees and it is unknown how this effects the wild pollinators in these agricultural regions (Garibaldi et al. 2013). It is not clearly understood if the addition of managed honey bees will help overall in increasing pollination services to those crops which pollination is necessary (ibid). There are questions arising out of the substantial evidence which exists for wide spread declines and the negative impacts on pollination services. Currently honey bee populations shifts are poorly documented and even less is known about recent changes in wild pollinator populations and communities (Potts et al. 2010). A study ran by Allen Wardell et al. found that honey bees are given the sole credit for the pollination of 100-150 major crops grown in the USA (1998). In reality native bees, butterflies and moths play an important role in pollination of these crops as well (Allen Wardell et al. 1998). Further evidence put forward by Elle and Button suggested that honey bees may supplement

but cannot replace agricultural pollination by wild insects (2014). What if these assumptions have led some crops necessity for pollination to be falsified? Meaning the crop has been categorized as decreasing in yield due to the loss of bees, where this may not be the case (Potts et al. 2010).

Fright tactics such as those for-mentioned with the loss of foods due to the loss of pollinators are lacking supporting evidence. The interactions between managed and wild pollinators should be further understood before managed bees can be placed increasingly into already disruptive agricultural intensification. It is therefore important moving forward to incorporate the observed proportions of both wild and managed bees as there are at least 17000 species of wild bees worldwide and they contribute substantially to the pollination of crops (Melathopoulos, Cutler, Tyedmers 2015; Winfree, Gros, Kremen 2011). Having accurate estimates of this value could improve land use planning by quantifying the costs and benefits of conserving habitat for pollinators in agricultural systems (Winfree, Gros, Kremen 2011). This type of information collection would require educational programs to help better understand which species serve as wild pollinators for which cultivated plants (Allen Wardell et al. 1998). This work would be additionally remarkable as it incorporates parameters which account for how much pollination can take place before yields reach a maximum, and therefore be able to quantify if further pollinator contributions are necessary in the production of some crop varieties (Melathopoulos, Cutler, Tyedmers 2015).

#### **2.4 Pollinator contributions to Canada's canola industry**

The Canadian Canola Industry is contributing to the reduction of undisturbed landscapes in efforts to increase production. Between the springs of 2011 to 2012 the total acreage of canola harvested within Canada jumped from 18 753.8 to 21 743.8 (000) acres (Stats Can 2014). That is an accumulated 0.768 tonnes of canola per acre (ibid). This large sum of food product harvested from this annually tilled landscape contributes largely to the Canadian economy. Between the years of 2009 to 2012 the industry indirectly accounted for 249 000 jobs within the farming, transportation and processing sectors of Canola Production (CCGA 2014; Billinger 2013). The crop itself brought in approximately 19.3 billion dollars for the Canadian economy (Billinger 2013). These statistics have ranked canola as Canada's most important oil seed crop, not only does it contribute positively to the wealth of the nation but it can be found in a plethora of

food and food products as well as biodiesels (AAFC 2014). However important this crop may be for the Canadian economy a sustainable food system must also include the effects this crop has on society and the environment. The remainder of this chapter will focus on how intensive agricultural practices play into the production of canola and the significance of looking at pollination contributions to this food industry.

#### **2.4.1 Canola as intensive agriculture and the role of pollinators**

Canola plants (*Brassica sp.*) are Canadian invented species of oilseed rape (CCGA 2014). Canola is most commonly grown in similar agricultural areas as wheat, barley and soybean, all of these crops are grown at a large scale, meaning over 14 million tonnes per year (AAFC 2014) and dominate the Canadian prairie landscape (Morandin, Winston, Abbott Franklin 2007). These crops are harvested each year and their fields are often tilled in both the fall and spring the action of turning the ground through tilling practices eradicated potential nesting habitat for many solitary bee species [See section 2.2] (Klien et al. 2010; Morandin, Winston, Abbott, Franklin 2007) .

Currently the canola industry within Albertan agriculture specifically, cultivates 37.5 canola plants per acre on an average of 6 455 000 acres (Stats Can 2014). This huge area of land taken up by mass flowering canola plants causes there to be a copious amount of pollen and nectar available for limited time periods during the spring blooming season (Bowie et al. 2010; Huang et al. 2004). This huge resource being available for such a short period of time combined with its disruptive maintenance of tilling may prove to be indecent for the sustainability of solitary pollinators.

Much of the research which looks into bees relationship with canola (*Brassica rapa* and *Brassica napus*) has proven the crop is self-compatible (Morandin, Winston 2005; Manning, Wallis 2005). This corresponds to Pott et al. findings that the many of the world's highest volume crops benefit simply from wind pollination and the input of insect and other pollinators can be seen as redundant (2010). It is also important to note that canola plants benefit from other resource inputs, not simply pollination contributions. Other inputs include the use of zinc, lead, nitrogen and sulfur based fertilizers which have a positive effect on the seed yield, number of pods per plant, and height of plant (Yasari, Patwardhan 2006). On multiple occasions it has been found that insect pollination increases pollen deposition in canola crops leading to increased fruit set per plant and seed production and decrease in the variance of fruit sets (Morandin Winston 2005; Garibaldi et al. 2010; Klein et al. 2010; Richards 2001). A 2004 study from Huang et al. notices that, "there



are many indications that pollination from a variety of species can ensure optimal crop development...even in crops that are only partly self-compatible.” (2004).

These findings still pose difficulty in identifying ways to distinctly quantify pollinator’s significance in canola’s autogamous nature (Manning, Wallis 2005). There are two major components to full pollination within a crop field: one at the flower scale, where a flower must receive a certain amount of pollen deposited in order to set a marketable fruit. Two, at the field scale fruit set will asymptote as the percentage of flowers that are fully pollinated approaches a saturation point (Winfree, Gros, Kremen 2011). Using adjacent landscapes such as grassy field margins can help to identify quantities of pollinators in surrounding areas, and there must be an understanding of how benefits these pollinators contribute to final yields of crops such as canola.

#### **2.4.2 Moving Forward with Canola**

Improper reporting of pollinator contributions to canola could impose a threat to the industry as improper allocation of pollinator resources can prove to be ineffective (Potts et al. 2010). Considering the increases in pollinator dependant crops which are dominating agricultural landscapes and leading to the decrease in available pollinator resource, it is difficult to tell how this will affect the future yields of commodity crops in our food system, or wild pollinator’s health. With these knowledge gaps the Canadian government has put forward a coalition of 30 organizations from across food agriculture and conservation agencies (CCC 2014). These groups are working towards a bee healthy road map where they will take action to help promote a healthy population of honey bees, native and managed pollinators and create thriving ecosystems (ibid). Identification of habitat that could promote wild bee populations and crop yield in intensive agricultural areas are important for maintaining sustainable production in bee pollinated crops and should be taken into account when looking for benefits to pollinators in intensive agricultural schemes (Moradin, Winston, Abbott, Franklin 2007).

Other factors which must be taken into consideration for increasing knowledge on pollination and yields in commodity crops could include pesticide treatments or field sizes, as both of these factors may also affect wild bee abundance and pollination in different types of canola crops (Morandin, Winston 2005). Further research should begin to

look at the impact of interactive effects as most studies look at the impact of specific drivers in isolation and therefore evidence of interactive effects in agricultural systems are sparse (Potts et al. 2010). There is ample information indicating how landscapes affect communities of insects in agro-ecosystems. However evaluation of landscape impacts on crop pollination is lacking. Through using the accumulated knowledge on different natural and semi natural habitats and their relationships to pollinators it is possible to correlate bee abundance with yield increases or decreases within adjacent canola crops to help decrease this knowledge gap.

### 3.0 Methods

This research project began in the summer of 2012 in three regions of Alberta Canada. A total of 6 fields were to be collected from where two fields were chosen from

**Table 1: Outline of Field Locations within northern, Central and Southern Alberta**

<b>Region</b>	<b>Field Name</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Closest Town</b>
<b>North</b>	N1	55.305545	-119.211379	Grand Prairie
	N2	55.428589	-119.223538	
<b>Central</b>	C1	53.476665	-112.199675	Grand Cache
	C2	53.654127	-111.916098	
<b>South</b>	S1	51.012688	-112.782109	Gleichen
	S2	50.926681	-112.759246	

each northern, central and southern Alberta (refer to Table 1). The use of these fields was granted to us from their individual land owners. Rationale behind the use of two fields from each region stemmed from their observed flower density's (Melathopoulos unpublished), as well as time restraints placed on this project, and the unavailable time to process and analyses additional fields. One field per region was classified as having high pollinator potential due to its high density of flowers produced (detail in Appendix A) the other field would be classified as a lower intensity due to lower production of blooms per plant. Flower densities were observed by randomly selecting 40 plants per plot during the spring bloom between July 6<sup>th</sup> and July 27<sup>th</sup> 2012. Within the 6 fields a total of 72 plots were thrown, or 12 plots per field. Each plot was marked using flagging tape at 20m and 200m perpendicular from the western edge of field, as well as 20 m and 200 m again perpendicular from the eastern side of the field (Modified from Morandin, Winston 2005). It was of significant importance to collect plants from the East and West edges of the field in order to attribute any potential pollen transfer to prevailing winds moving west to east across Canada. An unbalanced design will be used to analyse final results within each field as there was an unequal representation of plants collected between the eastern and western halves of each field.

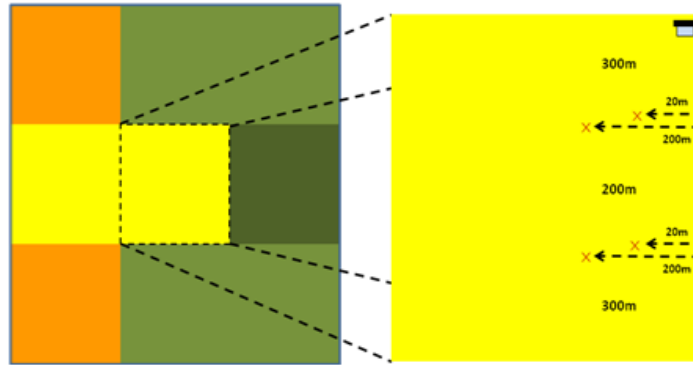


Figure 1: Visual Representation of how measurements will be taken from each of the 6 Albertan Canola fields (Melathopoulos Unpublished 2012)

These symmetrical plots were measured using a measuring wheel, during the pre-bloom season in Alberta, the pre bloom season occurs before peak summer heat in early June (AOF 2014, Mordain & Winston 2005). It was ideal to measure and plot pre-bloom as after the plants were fully developed accurate measurements would be difficult to achieve, as the plants would be difficult to navigate through without disrupting their development.

The fields were designated into three different areas of the province in order to differentiate between the landscapes adjacent to each field and common within each region. This categorization of adjacent land use will be used to divide the 6 fields used into sub bin of disturbed and undisturbed landscapes. The scope of adjacent field measurements will be modeled after Morandin, Winston, Abbott, Franklin study from 2007, where they found that the bee abundance in a field was most affected by habitat surrounding the field within a distance of 750m from field edge (Morandin et al. 2007). In addition, the mosaic of agricultural fields found in the most intense agricultural landscapes of southern Alberta are on average 800m by 800m therefore we will be taking into account approximately 75% of adjacent agricultural landscapes using this prescribed measurement (ibid).

Northern Alberta was classified as an area with fewer settlements, and less agricultural development. It was assumed that northern Alberta on average had the largest percentage of undisturbed land adjacent to N1 and N2. Central Alberta was classified as having a mixed landscape of both disturbed landscapes, including canola (*Brassica sp.*), wheat, corn and oats as well as undisturbed grasslands and pasture lands. Southern fields in Alberta were assumed to have the highest percentages of adjacent agricultural development, as this region has a high human populations as well as higher percentages of arable land and therefore the most disturbed landscape (Gov. Alberta 2012).

### **3.1 Plant Selection**

At each 20 m and 200m measurement in the fields 3 plots were flagged, and 3 plants were taken from each plot. Plants were randomly selected from swathed canola by walking and using a step measurement then selecting the plant closest to the observers leading foot. This process was repeated three times per plot or 36 times per field. The canola plants were selected pre harvest and laid to dry for one day before packaging into labeled bags. When the plants were sorted the main stem and branching stems of the plant were put in separate bags. It was important to separate the main pods from branching pods as the branching stems begin pod development secondary to the main stem. The branching pods are more heavily influenced by frost stress, moisture stress and heat stresses which may affect their seed sets (AOF 2014). Through this collection method the main stem acts as a control for seed yields per plant, having been less effected by environmental stresses later in the planting season. Plants were collected after swathing in order to reduce negative impacts of human interactions, such as trampling, on the growing canola crop.

### **3.2 Pod and Seed Sorting**

Seeds collected from the six fields were stored in a deep freeze over the course of two years (2012-2014). Once collected each field was categorized into individually labeled bags, which referenced, which field (N1, N2, C1, C2, S1, S2), which plant and at what distance pods were collected from. In order to separate the seeds from the pods, plants were laid out onto paper towed aluminum trays over the course of a day to dry out completely. The main stem pods and branching stem pods were laid on separate trays and labelled accordingly. Once the pods were dried, they were counted by hand their final numbers were entered into an excel spreadsheet. Once counted pods and seed sets were separated by hand. Any pods which were experiencing mold were cleaned using paper toweling. Again terminal seeds were kept separate from branching seeds and repackaged into individual envelopes to be stored until they will be counted. Envelopes were labeled with the identical information present on previous plant holding bags. Both pods and seed sets will be counted in order to solve for an average seed set per pod, on each plant collected. The correlation between seeds per pod is important in understanding the efforts pollinators have contributed to each 20m or 200m region in the field. Studies have

found that insect pollination increases seed production in *Brassica napus* canola, as well as pollinator visits helped to increase the number of pods produced per plant (Morandin & Winston 2005, Sabbahi et al 2009, Duran 2009). Whereas simply measuring the weight of seeds produced would be seen as inadequate. Duran et al. found a negative relationship between yield and grain weight of canola (2009). The larger the contribution of insect pollinators to canola plants, the larger quantity of seeds per pod, and the lighter the weight of each seed (Sabbahi et al. 2009, Manning & Wallis 2005).

### **3.3 Data Analyses**

To determine an average seed set per plant we will use the Elmor C1 seed counter set to speed 7, and adjusted to seed size 3 to fit the average canola seed.

#### **Question 1: Is there any evidence of a yield increase in Canola crop dependant on its locations within the field?**

A statistical analysis will be done using ANOVA to look for any significant difference in the seed set per plant produced at 20m comparatively to 200m. The variables under analysis will be number of seeds per pod and measured distance into the field 20 m or 200m. This comparison will be made for each grower's field. There will be graphical depictions illustrating the average seed sets from plants within, North, Central and South agricultural regions. The potential to use pseudo-replication for each distance plot within the field may be utilised if variation between plant samples have no significant difference and seed sets can be assumed to have similar yields dependent on which field they were cultivated.

#### **Question 2: If there is variance within the seed set can this relationship correlated to characteristic in the adjacent landscapes.**

To compare seed sets in relation to adjacent landscaped, digitalized maps of the 6 canola fields will be used as a tool to describe land cover within a 750m radius from the edge of each field. The adjacent landscapes will be characterized into bins of disturbed or undisturbed. Undisturbed landscaped pertain to a natural or semi natural landscape that does not undergo any tilling (Morandin et al. 2007). Landscapes within the maps which will qualify as undisturbed include: woodlands, headlands, marginal grasslands, grasslands, wetlands and semi natural pastureland (Richards 2001,

Morandin 2005). These landscapes are important as they have been found to be more inclined to house a higher species richness of pollination species including wild bee populations (Klein et al. 2010, Morandin et al. 2007, Calabuig as referenced in Morandin and Winston 2005). Disturbed landscapes will be any agricultural field which undergoes annual tilling, including: canola, corn, wheat or grains (Melathopoulos unpublished). Once the fields have been separated into bins, statistical analysis using ANOVA will measure the significant difference in average seed per pod produced corresponding to the each grower's fields.

## 4.0 Results

### 4.1 Distance Analysis

A sum of 160 plants were collected from six field's located in, southern, central and northern regions of Alberta. The first test of normality measured all plants as independent variables within six field (Figure 2). Seed sets per pod were used as a proxy for levels of pollination within the field and transformed through a square root function to meet assumptions of normality for ANOVA. All statistical tests were run through R. The independent variable, plants, was

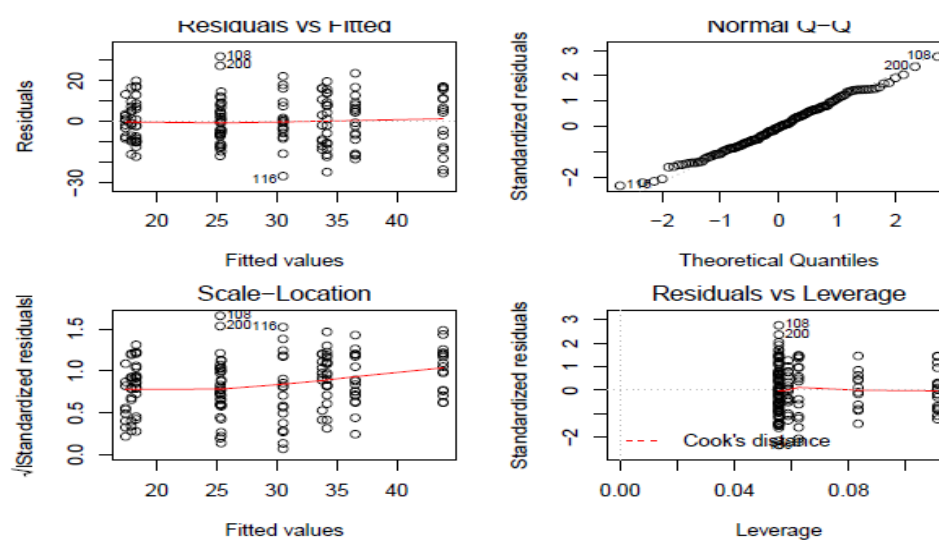


Figure 2: Model Diagnostic graphs illustrating assumptions met of an ANOVA, Assumptions of normality met through transforming data using square root functions. Correlation of distance into the field (20 m and 200m) and the specific grower of Canola (*Brassica sp.*) [N1, N2, C1, C2, S1, S2]. All plants considered independent of one another within growers fields, total N = 160 plants,  $F_{1,4} = 2.94$   $P = 0.022 > P_{\alpha}$ . No significant differences found in transformed data relating distance to yield for each growing location. Data Collected Alberta Canada 2012.

compared to the particular grower (N1, N2, C1, C2, S1, S2) and the distance they were collected from within each field. These variables were correlated to answer the first hypothesis, *is there an increase in yield of canola dependant on its location within the field?*



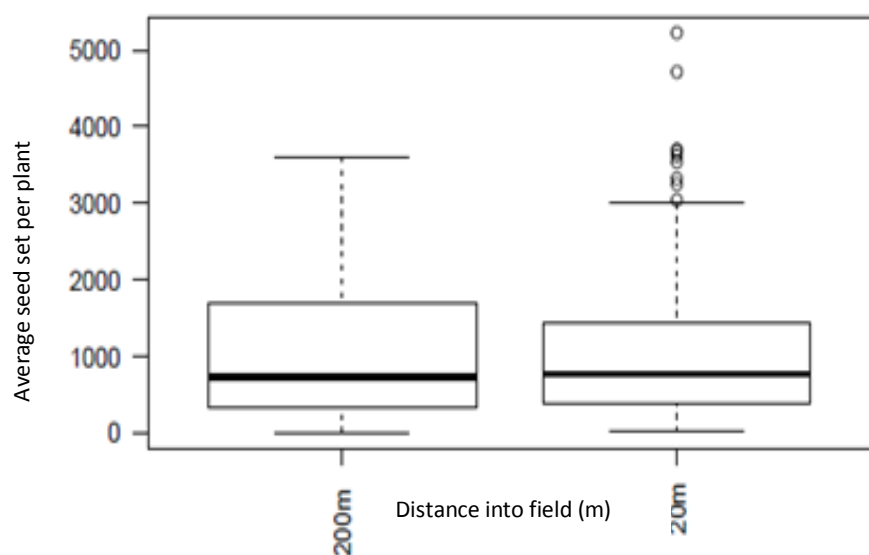


Figure 3: Boxplot of standardized residual after transformation. Average number of seeds per canola (*Brassica sp.*) pod found at all growing locations of 200m versus 20m with standard error. Plants at each location considered independent of one another. N=160, No significant difference at  $P = 0.05$  Data collected in Alberta Canada 2012.

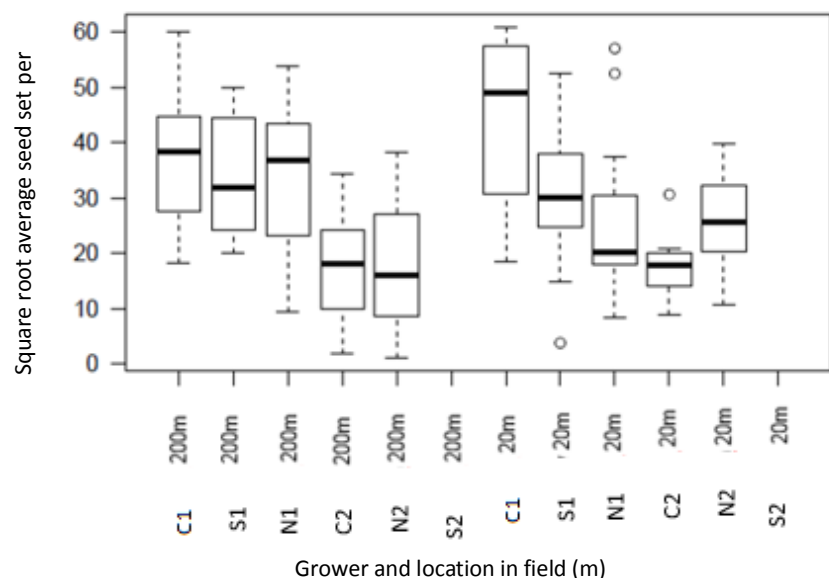


Figure 4: Square root of average seed per canola (*Brassica sp.*) pod found at each location within growers fields. Distance paired with each field. Standard error for each seed set. Plants were not pooled and considered independent of one another within each fields. N=160, N = 160 plants,  $F_{1,4} = 2.94$   $P = 0.022 > P_{\alpha 0.05}$  No significant difference between distances into field. Data collected in Alberta Canada 2012.

As a result there is no significant difference between the canola yields grown at 20m versus canola grown at 200m when all plants at those designated distances were pooled (Figure 3). Variance in seed sets per pod at 20m contained a higher amount of outliers then seed sets at 200m this could be a result of edge effects pertaining to the different locations of each grower.

Separating the pooled distances into their designated grower's field upheld the same outcome from Figure 2. The distance canola is grown into the field does not play a significant role in terms of final yields in relation to seed set per plant. There is no significant difference found between 20m and 200m distances at each growing location  $F_{1,4} = 2.94$   $P = 0.022 > P_{\alpha 0.05}$ . Figure 3 shows no notable variance between yield and distance, however it does show a potential for significant variance in yield between the different growers themselves. In particular the two central growers C1 and C2 at 20 m distances vary greatly in their final seed sets, as do N1 and N2 at 200m distances.

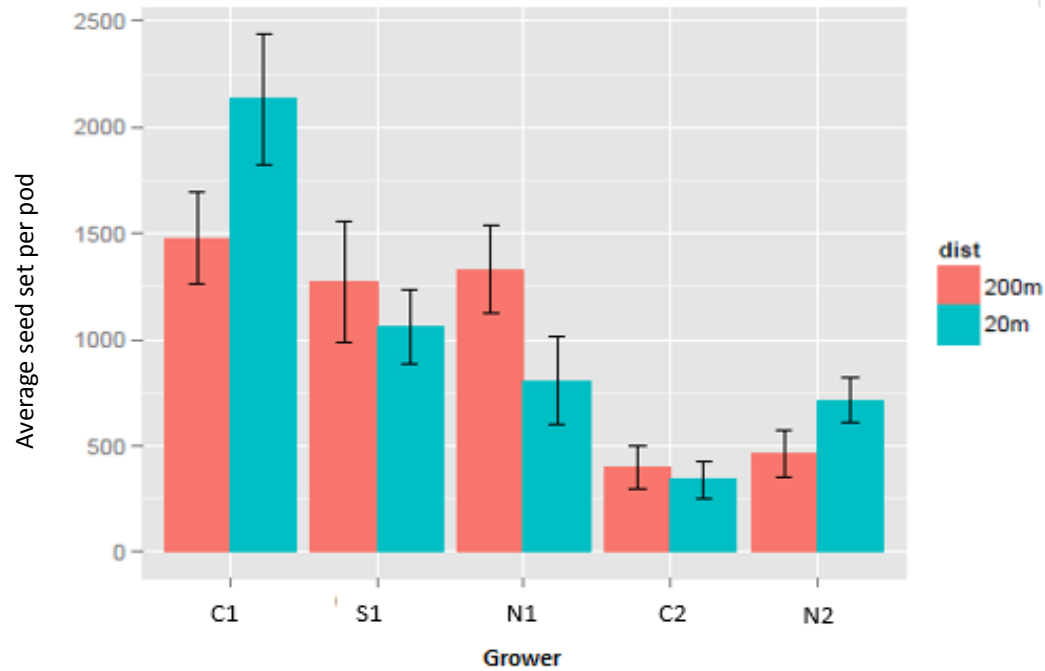


Figure 5: Difference in seed set per canola pod (*Brassica sp.*) in relation to distance 20 m versus 200 m for each grower location with standard error. Plant to plant variation assumed in this model. S2 not included as data was limited to on 20 m distance and no comparison could be show at the 200m distance. N=153, No significant difference at P=0.05, distance into the field is not a factor affecting final yield of canola. Variation between the different grower's final yields. Data collected in Alberta Canada 2012.

Paired comparisons between the final seed set per pod at each distance for each growing location show uneven distribution between 20m and 200m field plots. Both show a slight skew to the left as a result of the square root function applied to increase normality of the original data. Further S2 could not be included in this figure as it only holds data for seed sets at 20m and could not be compared to the 200m yields. In some fields seed sets is higher at 20m then at 200m for example C1 and N2, however at others seed set appears higher in the 200m plots. This pattern does not hold true to the hypothesis and seed sets of canola does not vary depending on its location within the field. Further seed set being a proxy for contributions from pollinators speculates that pollination throughout the canola field does not diminish yields due to the plants location into the field.

## 4.2 Conservative Model

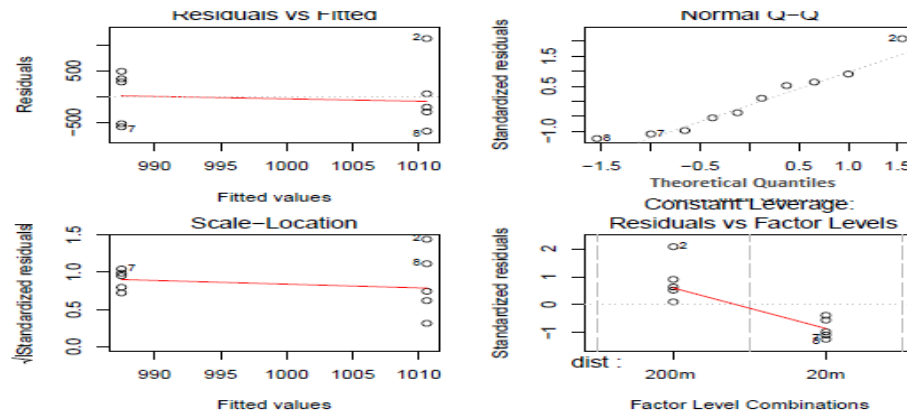


Figure 6: Conservative model diagnostic graphs illustrating assumptions met of an ANOVA, assumptions of normality met through pooling plants per field. Average seed set per canola (*Brassica sp.*) from each field location reduced to single variable to correct for variation within each field. Looking for correlations of distance into the field (20 m and 200m).  $N = 9$  plants,  $F_{1,8} = 0$   $P = 0.95 > P_{\alpha}$  No significant differences found in pooled data relating distance to yield. Data Collected Alberta Canada 2012.

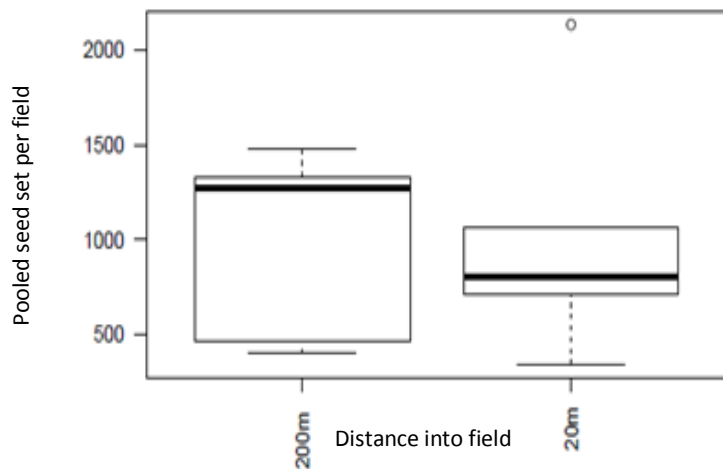


Figure 7: Boxplot of standardized residuals, average seed set per canola plant (*Brassica sp.*) standard error term at 200m distance more variable than at 20m distance. Model assumptions for normality not likely met due to variability seen in figure 3 between each field.  $N=9$  no significant difference between distances. Data collected from Alberta Canada 2012.

Figure 6 uses pseudo-replication placing plants found within a plot from each field into a single variable. Reducing  $N$  from  $N=160$  to  $N=9$  replicates. Here S2 was left out due to insufficient data. These pooled samples assume each plant per 20m or 200m plot would yield similar seed sets. Again there is no significant difference between seed sets and distance into each field  $F_{1,8} = 0$   $P = 0.95 > P_{0.05}$ . Figure 6 shows no such pattern, and an increased variability between the standard error terms at 200m compared to 20m.

With the data in figures 6 and 7 depicting the same results as those found in figures 2 through 5 there is justification in the ability to pool plants within each field location with no resulting false inflation of replication. Meaning it can be assumed that plants found at certain distances within each field can be predicted to yield similar seed sets per plant.

### 4.3 Undisturbed vs. Disturbed landscapes

Figures 4 and 5 show potential variance in seed sets per grower. Between all six fields two were recognized as undisturbed landscapes C2 and N1 (S2 was also recognized as undisturbed however there was insufficient data to prove any correlation between landscape and seed set for this particular field). Leaving the remaining fields to be categorized as having disturbed surrounding landscapes S1, C1, N2 (Appendix B).

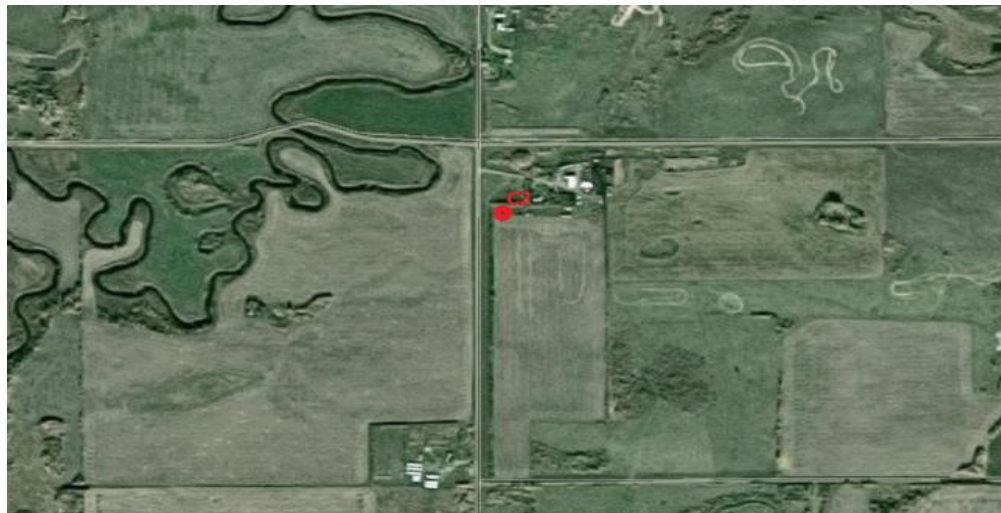


Figure 8: Visualization of field C2 in Central Alberta agricultural landscape. Surrounding landscape features include pasturelands, aspen forest, wetland, alfalfa and corn. Image taken from: <https://www.google.ca/maps/place/Bens+Lake,+Two+Hills+County+No.+21,+AB+T0B/@53.6544067,-111.919202,1950m/data=!3m1!1e3!4m2!3m1!1s0x53a0bcbb373c80c5:0x31955b00e6a8fcd6>

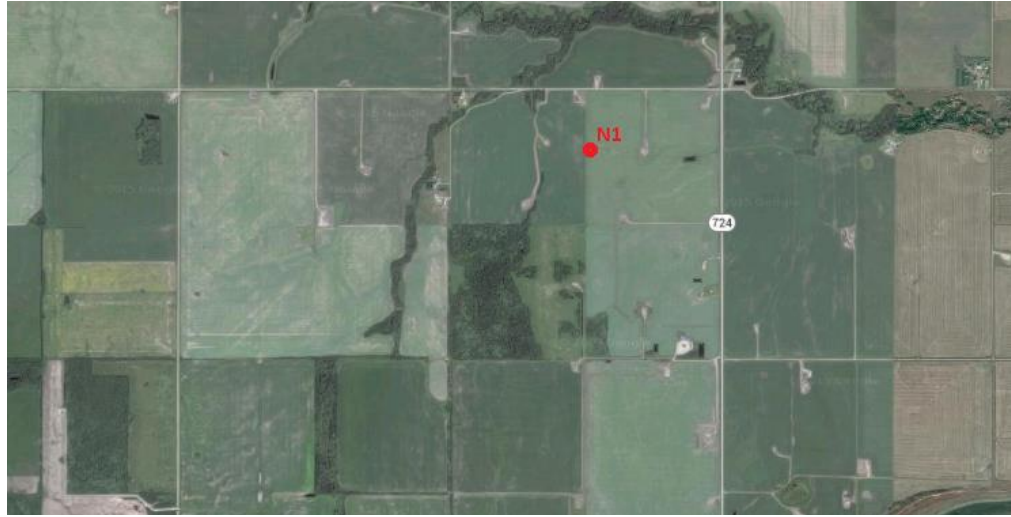


Figure 9: Visualization of field N1 in northern Albertan agricultural landscape. Surrounding landscape features include: grazed pastureland, willow trees, hay fields, and wheat. Image taken from: <https://www.google.ca/maps/@55.270067,-119.1901309,15211m/data=!3m1!1e3>

The statistical analysis presented in figure 5, shows variation between each growers fields. However it does not hold true to the secondary hypothesis, *if there is variance within the yield can this relationship be correlated to characteristic in adjacent landscapes?* The pattern which emerges out of figure 5 shows increased seed sets and subsequently assumed increased pollination in fields with adjacent disturbed landscapes, including growers C1, S1. N2 is the only field out of the three highest yielding fields which corresponds to having undisturbed surrounding landscapes. Therefore no assumptions can be drawn which correlate surrounding undisturbed landscapes to increased pollination of canola crops in this Albertan landscape within this study.

## **5.0 Discussion**

### **5.1 Addressing the Research Question**

Ecosystem services are increasingly studied but still complicated to understand (Metson, Ziter, Dancose 2013), the pollination services provided to large scale agriculture is beneficial for many crops. However, the general results in this study do not prove a significant relationship between seed set of canola and distances into the field. Therefore it was generalized that pollinator contributions to the large scale agricultural production of canola is uniform regardless of the different disturbed and undisturbed agricultural landscapes surrounding canola fields. This study was preformed to identify if there were any increases in canola dependant on its location within a field based on real world agricultural production. Therefore it did not incorporate the introduction of bees into the fields under analysis but relied on the surrounding landscapes to act as proxies for the presence of pollinators in the agricultural region. There was significant variation found in total yields of canola between the different grower's fields.

The research question was looking for any variation within seed set and if it could be attributed to surrounding landscapes. Undisturbed landscapes were identified as non-tilled soils and were predicted to increase pollinator visits to nearby agricultural fields as non-tilled soils proved beneficial habitats for solitary wild bee populations (Morandin, Winston, Abbott, Franklin 2007; Gathmann, Tschardtke 2002). Whereas disturbed landscapes surrounding canola fields were proven in previous studies to be unproductive habitat for many wild bees and therefore predicted to provide less pollinator contributions to nearby canola. Through ANOVA comparisons the variation in crop yields within different fields did not follow the patterns predicted through identified surrounding landscapes. This leads to the assumption that interactive effects from other sources in the agricultural system must play larger role in determining yields of canola. It would be beneficial to identify these effects in the next comparison of yield studies for canola, in order to help distinguish baselines for different inputs into the cultivations of this crop. This may help to establish a means to quantify specific pollinator contributions to canola or other semi pollinator dependant crops.

## 5.2 Connecting Results with Current Literature

It is apparent that the results found from this research project do not run parallel with other similar works around canola and pollinations. Largely conclusions have been drawn that pollination increases the seed set and final yields of canola crops, and in particular the species *Brassica rapa* (Garibaldi et al. 2013; Morandin, Winston 2005; Klien et al. 2010). This difference may stem from the original experimental design of this project, which did not implement managed pollinators into the landscape but simply used landscapes to act as a proxy for the abundance of pollinators in the surrounding landscape.

### 5.2.1 Variation between Growers

The aim was not to quantify any increases in yield through increases in pollinator contributions but rather to locate any significant increases in yield under normal agricultural conditions, where there was an assumed wild pollinator existence. However there are other parameters at play which cause increases and decreases in canola crop yields. Figure 5 (p.28) shows a mix of fields with both disturbed (C1, S1) and undisturbed (N1) landscapes as having significantly higher seed sets at both 20m and 200m distances into the field. Therefore no conclusive evidence can be drawn on the effects of landscape on pollinator contributions in this study and higher seed sets cannot be linked to surrounding landscape conditions.

Other parameters such as species of canola within the field can play a role in the variation of seed sets. Prior research has found that there is significant variation in pollination deficit between organic conventional and genetically modified fields of canola (Morandin Winston 2005; Aizen et al 2008). The species of canola could therefore be a contributor to variance amongst our fields. Further, insects are not the sole contributor to the pollination of crops. Abiotic components like wind can be seen as significant contributors to the pollination of large scale crops (Yasari, Patwarhand 2006; Hoyel 2007; Richards 2007). In the beginning of this study there was an importance placed on collecting plants from both the east and west edges of field in order to attribute any potential pollen transfer to prevailing winds moving west to east across Canada. However with insufficient records of plant collection on both east and west sides of all fields under analysis led this parameter to be overlooked in the final analysis. However contributions from wind may prove to be an important contributor to pollination levels and should be included in future analysis.

### **5.2.2 Variation between 20m and 200m Distances**

Foraging distances into the field was presumed to play a role in the abundance of pollination which was contributed to canola. Fields with abundance undisturbed landscapes would be presumed to house larger quantities of pollinators. Therefore it was assumed these pollinators would have the ability to travel farther into neighbouring canola fields and contribute to higher pollination at 200m distances into the field. These assumptions were supported by Morandin and Winston 2005 study which used measured distances of 20m, 200m and 500m which effectively divided the average 800 m<sup>2</sup> canola fields into six plotted areas [our study divided the fields into four to better fit our analysis into our time restraints]. After finding no significant difference between the 20m and 200m plotted areas our results matched those found in Morandin and Winston's findings, "there was no relationship between distance from hedgerows and pollinations deficit...no relationship between seed deficit and distance from hedgerow or edge" (2005). Having found similar conclusions around pollination variations within the field as previously performed studies helps to solidify assumptions that wild pollinator contributions within canola fields do not play a role in increasing or decreasing final seed sets of plants. The degrees to which insects contribute to final yields of canola can fluctuate and depend on more parameters than insect abundance within field landscapes (Morandin Winston 2005; Potts et al. 2010).

### **5.3 Recommendations**

The canola fields used in the study fall under the scope of large scale agriculture. Part of the analysis of this large scale crop made use of pseudo-replicates of the canola plants and associated seed sets. This meant all of the 160 plants were pooled into their specific field plots at either 20m or 200m which reduced the sample size significantly and would appear to reduce the power of variables used. Pseudo-replication was used after running the ANOVA using independent plant variables, the replication was justified as it produced the same results as those found in the independent variable test using N=160 plants. The result being there is no significant difference in the yield of seed sets per canola plant between 20m and 200m distances into a field. The correlations set between the plants in each field meant the individual plants could no longer be seen as a random effect in each field. Therefore any correlation found could contribute to a greater understanding of how the distance itself effected the seed sets of each plant. Pseudo-replication specifically



decreases the degree of heterogeneity of what regulated the environmental conditions in this research, this could affect the sensitivity of the experiment (Hurlbert 1984). However with similar results with both independent and pooled plants the sensitivity of the experiment did not prove to be reduced and this method of sampling could be used in future studies of intensive agricultural variables.

This research project lead to a greater understanding of how landscapes play a role in autogamous agricultural crops. Further it touched on the how canola yields do not have a heavy reliance on nearby habitat parameters and corresponding pollinator abundances in sustaining healthy yields. Not finding significant changes in yield corresponding to the distance the plants are grown into the fields, nor finding evidence of any benefits from surrounding landscapes leads to further research possibilities.

Most studies including this one, look at the impacts of specific individual drivers. Our research looked at the independent driver of distance into the field as a parameter for pollinator contributions. Potts et al. called upon more research to be done which looks at the interactive effects in an agricultural environment which may lead to increases or decreases in pollinator services (2010). Interactive effects such as wind, pollinator and landscape contributions to autogamous or semi autogamous crops like canola can further the knowledge the agricultural industry needs in order to support sustainable agriculture into the future. For example final comparisons in relation to seed set and the effect of prevailing winds as a vector for pollination. *Brassica napus ssp.* has been found to be readily pollinated by wind when grown in particularly dense stands such as those found in Albertan (Hoyle 2007, Richards 2001). Underlying assumption may be set in accordance to Canada's prevailing winds travelling west to east.

Agricultural production is an interactive system which makes use of different herbicides, pesticides, irrigation techniques and machinery (Kohler, Verhults, Van Klink, Kliejn 2008). There is a need to understand pollinator's role within the whole system of agriculture. Understanding pollinator's holistic role in agricultural systems can help to improve the health of crops into the future and has the potential to decrease the negative effects felt from intensive agricultural expansion. Further quantification of pollinator contributions may lead to increased mosaics of land uses known to help increase pollinator abundance (Osborne, Williams 2000). Further this may demystify the stigma around the current decrease in pollinator populations.

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## Appendix B

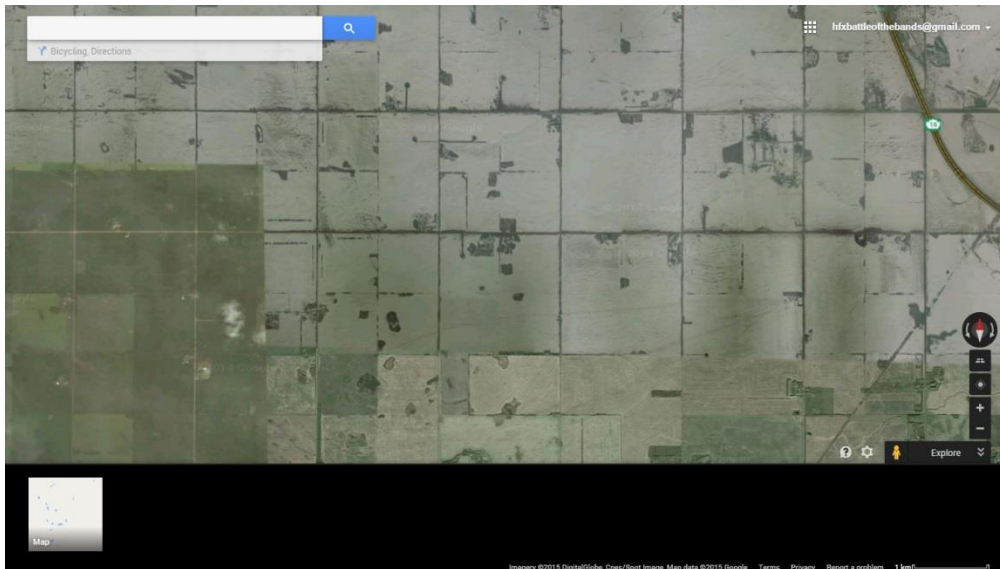


Figure 10: C1 visual representation of disturbed landscape surrounding fields of Canola in central Alberta. Majority of landscape consumed by intensive agricultural practices. Surrounding fields include: canola, barley and wheat.

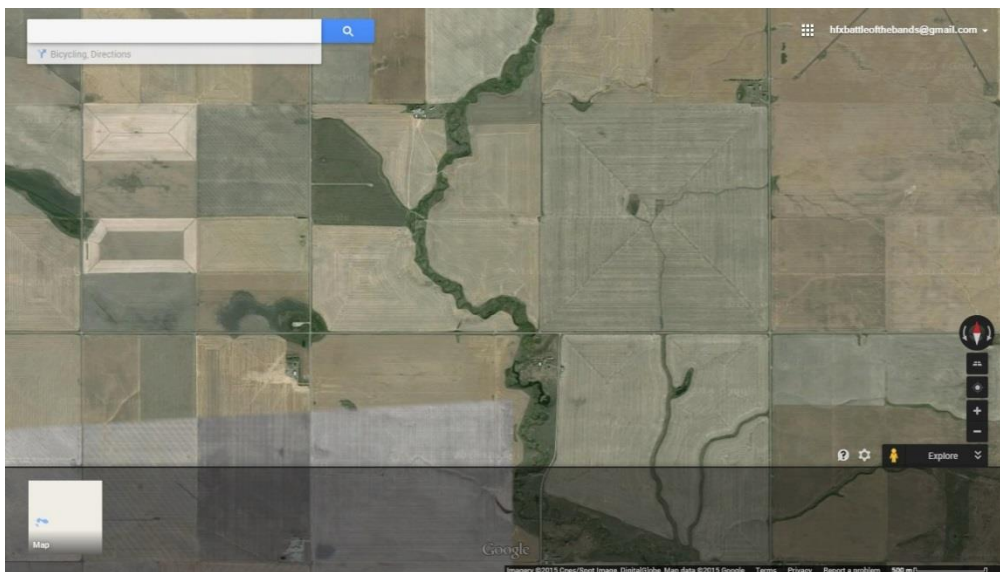


Figure 11: S1 Visual representation of disturbed landscape around fields of canola in southern Alberta. Surrounding fields include: canola, wheat and peas

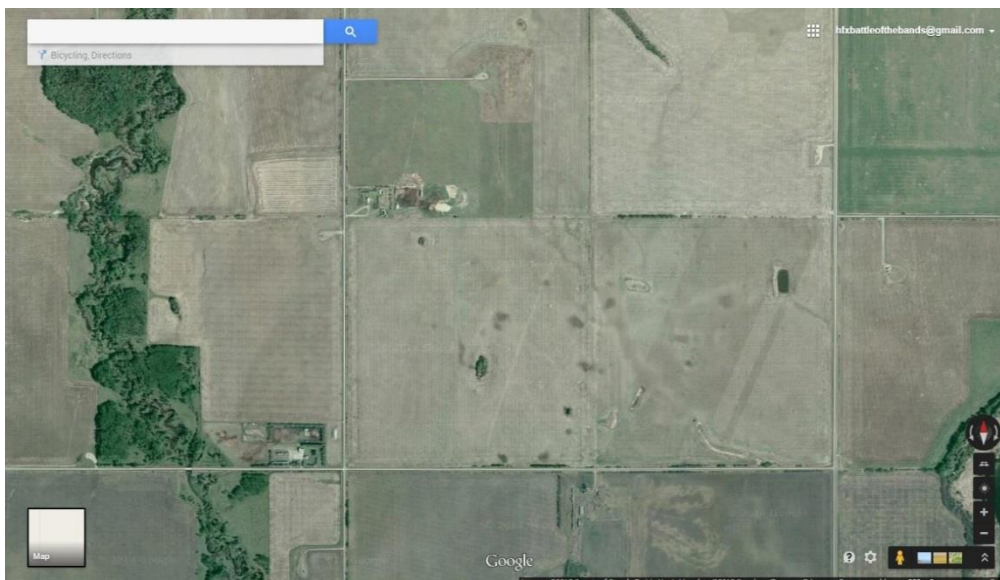


Figure 12: N2 visual representations of disturbed landscape surrounding canola field in northern Alberta. Surrounding landscape includes: canola and wheat.