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Ecological Risks and Benefits from the Novel Crop Camelina sativa (L.) Crantz (Camelina)

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Ecological Risks and Benefits from the Novel Crop *Camelina sativa* (L.) Crantz (Camelina)

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Table of Contents

Abstract

It is important to fully understand the ecological implications of introducing new crop species and genetic traits because of potential hazards such as changes in native plant populations or the establishment of aggressive weeds. Conversely, the introduction of new crops and traits could provide ecological benefits such as providing pollen and nectar forage for pollinators. *Camelina sativa* (camelina or false flax) is an oilseed crop that is being genetically engineered (GE) for the production of biofuel, bioplastics, and dietary supplements for humans and animals. Relatively little scientific information exists about camelina cultivation, reproductive biology, and ecology. The primary research goals of this thesis were to: 1) understand seed establishment, growth, and development of the crop in Connecticut, 2) identify and quantify pollinating insects present in the field, and 3) to determine the potential for camelina to become a serious weed in natural and managed landscapes. Camelina was grown at the University of Connecticut Research Farm for three years (2014-2016) and data was collected from replicated subplots, transects, and insect exclosures. Field experiments showed that low precipitation and high temperatures in the early part of the growing season negatively impact seed germination, crop establishment, and yield. Seed yield in 2014 was 531 kg/ha and in 2016 was 1096 kg/ha. Field observations suggested that pollen-mediated gene flow could occur through insect pollinators. Pollinating insects from the orders Hymenoptera, Diptera, Lepidoptera, and Coleoptera were captured while visiting camelina flowers. Experiments showed that camelina was competitive with agricultural weeds and did not require herbicides. These experiments have provided new knowledge about camelina crop development, yields, and insect interactions. These results will help government regulators, farmers, and companies make decisions regarding the future use of camelina with novel traits.

Chapter 1. Introduction to *Camelina sativa:* New Uses for an Ancient Crop

Camelina sativa (L.) Crantz (common names: camelina, false-flax, gold-of-pleasure) is an annual plant that produces seeds with a high oil content of 37-40% (Budin, 1995). However, camelina has never had the same popularity as its close relative *Brassica napus* (rapeseed) for oil production. In recent years, there has been a renaissance in camelina production for two main reasons: 1) an increasing demand for sustainable biofuels from non-food crop plants, and 2) the ability of biotechnology to introduce novel traits into camelina.

The interest in biofuels has gained momentum in developed countries because these fuels offer a possible solution to problems associated with fossil fuels including environmental pollution, finite petroleum resources, and dependence on other countries. Biofuels have the potential to emit less pollution compared with fossil fuels and, if implemented correctly, could help alleviate the rise of CO_2 levels and climate change (Bernardo et al., 2003). It is often reported that oilseed crops are the most efficient and effective biofuel source (Hill et al., 2006). Various oilseed crops are currently grown in the United States and Canada including *Brassica* species (canola, rapeseed), *Glycine max* (soy), *Carthamus tinctorius* (safflower), and *Linum usitatissimum* (flax). Camelina could join this group of large-scale, oilseed crops because of its high seed yields of up to 3320 kg/hectare (Gugel & Falk, 2006).

Renewed interest in camelina is also due to novel traits made possible by molecular biology and biotechnology. Camelina oil is high in omega-3 fatty acids and other types of lipids suggesting its potential contribution to human foods, animal feeds, dietary supplements, and industrial compounds (Betancor et al., 2015; Budin et al., 1995; Petrie et al., 2014; Usher et al., 2015). Camelina can be transformed using Agrobacterium-mediated floral-dip method. This

makes it significantly easier to modify genetic sequences compared to other oilseed crops that require more complex tissue culture methods (Bansal and Durrett, 2016).

One example of a novel trait in camelina is the expression of algal genes to generate high concentrations of long chain polyunsaturated fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in seeds (Betancor et al., 2015). Through this method, camelina can effectively replace algae as a source of essential fatty acids in the diet of marine fishes (e.g. salmon) and other organisms (Betancor et al., 2015; Petrie et al., 2014; Usher et al., 2015). Herbicide resistance and other agronomic traits have also been evaluated in experimental field trials in the US (http://www.isb.vt.edu).

Another area of research emphasis has been the modification of camelina oil for biofuels. The production of sustainable, plant-based liquid fuels has major implications for improving the environment and mitigating climate change. A study using camelina in place of mineral-diesel to power trucks showed that emissions of carbon monoxide (CO), carbon dioxide (CO_2), and smoke were significantly less from trucks powered by camelina oil (Bernardo et al., 2003).

Almost nothing is known about the potential benefits and risks of growing camelina in Connecticut or the larger Northeastern region. There is almost no information about its potential risk as a weed in agricultural fields or natural areas. There is almost no information about its potential benefits for pollinators, native insects, and non-target species. Some of most important unanswered questions about camelina include: Which environmental factors favor camelina seed germination, establishment, flowering, and crop yield? What are the potential yields in the Northeastern US under low-input field conditions? How competitive is camelina against weeds and native plant communities? Which pollinators are attracted to camelina flowers and what role do they play in gene flow? How might camelina fields support fragile

populations of bees and other insects? The research conducted for this M.S. degree was designed to answer some of these important questions.

History of the Crop Species

Camelina is an ancient crop that has been cultivated and used by humans since the Iron Age (400 B.C. – 500 A.D.). Its wild ancestors covered nearly all of Europe and Central Asia and, during the Iron and Bronze Ages, the plant was cultivated for human consumption, cooking oil, and livestock feed by the peoples of Scandinavia and Western Europe (McVay and Lamb, 2008). Camelina was cultivated in areas of Northwestern Europe and Russia in the 19th and 20th century, but by the mid-20th century (1947) production of the crop declined greatly (Frohlich and Rice, 2003). This decline in camelina production is believed to be due to the lower cost of manufacturing rapeseed oil, as well as a lack of knowledge about beneficial compounds in camelina oil. Today, European cultivation is currently restricted to relatively small areas in Germany, Poland, and Russia (Putnam et al., 1993; McVay and Lamb 2008).

Camelina is believed to have been introduced into North America by accident, most likely as weed seed mixed with flax seeds transported from Europe. Figure 1 shows its current distribution as a weed in the Northeast. The earliest herbarium sample in Connecticut herbaria showed that camelina occurred as a weed in Washington DC in 1873 (Table 1, Chickering 160166 CONN). A survey of the herbarium specimens at two university collections in Connecticut showed that most specimens were collected at the turn of the 20th century (Table 1). No specimens have been collected in Connecticut in nearly eighty years. This could either be due to the decline of broad botanical surveys or the absence of camelina populations in the area.

Future work could include a comprehensive review of camelina accessions across North America and use molecular markers to assess population dynamics over time.

Figure 1: Distribution map for Camelina sativa *in the Northeastern US and eastern Canadian provinces. Counties and provinces in blue are areas where camelina has been introduced as a weed and has shown some potential to persist over time. (Source: USDA Plants Database)*

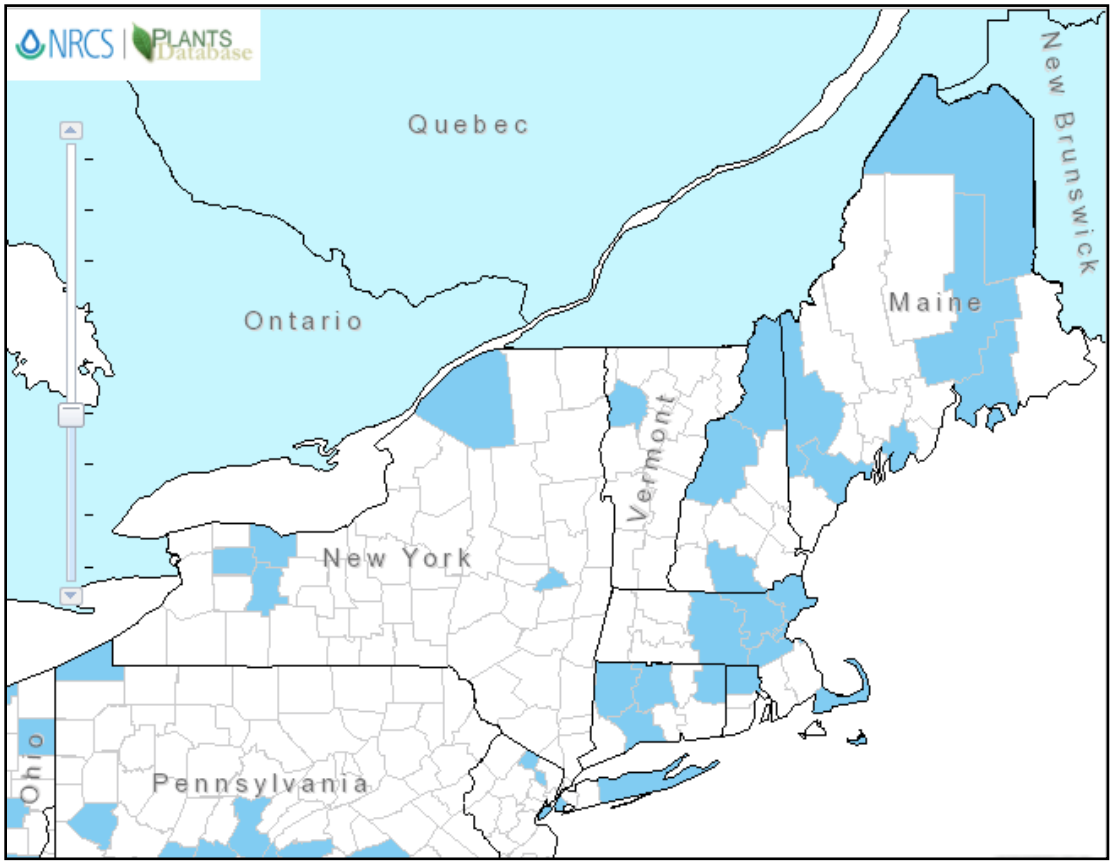


Table 1: A survey of specimens in two herbaria in Connecticut: The George Stafford Torrey Herbarium at the University of Connecticut, and the Yale Peabody Natural History Museum Herbarium. The table shows the dates and locations of Camelina sativa accessions in Connecticut. The accession from Washington DC is included to show a relatively early date for camelina as a weed.

Herbarium	Collection Location	Collection Date	Latitude/Longitude
G.S. Torrey (UConn)	Washington, DC	May 22, 1873	----------
G.S. Torrey (UConn)	Fairfield, CT	June 17, 1892	----------
Yale	Oxford, CT	July 26, 1902	41.4313 -73.1351
Yale	Southington, CT	August 1, 1902	41.6049 -72.88
G.S. Torrey (UConn)	Moosehead Lake, ME	July, 1906	----------
Yale	Waterbury, CT	July 12, 1907	41.5583 -73.036
Yale	Gaylordsville, CT	July 24, 1907	41.6465 -73.4737
Yale	Waterbury, CT	June 22, 1910	41.5583 -73.036
Yale	Southington, CT	June 21, 1911	41.6049 -72.88
Yale	Putnam, CT	May 17, 1925	41.9093 -71.8712
Yale	East Haven, CT	----------	41.2991 -72.8575
Yale	North Guilford, CT	----------	41.367 -72.717

Agronomic Production

Compared to most field crops, commercial camelina production in the U.S. began in relatively recent times. Montana State University Extension researchers reported commercial production in Montana in 2006 and 2007 when the crop covered approximately 2,833-9,713 hectares (McVay and Lamb, 2008). However, increasing interest in biofuels has spurred many experimental field trials in western and mid-western states such as South Dakota and Montana (McVay and Lamb, 2008; Davis, 2010; Grady and Nleya, 2010). Prior to our research, field trials in the Eastern US appear to have been limited to Pennsylvania (Hunter and Roth, 2010). Overall, relatively little research has been done on camelina seed yields in the US, but studies suggest that they vary from 76 kg/ha to 3320 kg/ha (Table 2). Differences in yield could be due

to many factors including crop genetics, environmental factors (e.g. soil type), and inputs (e.g. fertilizer, irrigation).

Table 2: Seed yields of Camelina sativa *from field trials in the United States, Canada, and Europe.*

Seed Yield (kg/hectare)	Location	Reference
962-3320	Western Canada	Gugel and Falk, 2006
372 – 1867	Montana	McVay and Lamb, 2008 Montana extension
76 – 2211	Colorado, Montana, Wyoming and Washington	Jewett MS thesis, pg 49-50
1120 – 1681	Pennsylvania	Penn State bulletin
1605 – 2392	Germany	Vollman et al., 1996
2017 –2242	Montana	Ehrensing and Guy, 2008, Oregon State Univ.
1009-1905	Montana	Ehrensing and Guy, 2008, Oregon State Univ.
2354 –2690	Idaho	Ehrensing and Guy, 2008, Oregon State Univ.
1004, 1158, 1148	South Dakota	Grady and Nleya, 2010 South Dakota Extension
1500	Nova Scotia	Yang, 2016

Camelina is a short-season crop adapted to the cool temperate regions of Europe, Asia, and North America. Camelina has many traits that make it an ideal candidate for oil production, crop rotation schemes, a cover crop in winter, or a crop for marginal lands (Ehrensing and Guy, 2008; Fleenor, 2011; Putnam et al., 1993). Camelina is reported to be less susceptible to diseases and pests, such as flea beetles, when compared to other biofuel crops (Gugel & Falk, 2006). Previous studies showed that camelina planted in the spring is less susceptible to weed competition than camelina grown as a winter annual (fall planting) (Crowley, 1999). However, there are many gaps in the information regarding camelina production and adaptations to various environmental factors.

It is generally expected that camelina cultivation will increase in Europe, the United States, Canada and other regions due to its high oil content, desirable lipid composition, novel traits from genetic engineering, and ability to grow in low-input farming systems. Breeding programs and genetic modifications are just beginning to reach the commercial phase of development (Betancor et al., 2015). Taking these factors together, camelina could have a strong future as a genetically engineered crop (GE) producing high-value compounds (e.g. omega-3-fatty acids) or as a part of sustainable crop production systems (e.g. winter cover crop).

Taxonomy and Biology of *Camelina sativa*

Camelina sativa (L.) Crantz is a member of the tribe Camelineae within the Brassicaceae (mustard) family. The Brassicaceae family contains many important crop species including *Brassica oleracea* (cabbage, broccoli, cauliflower, kale, Brussel sprouts, etc.) and *Brassica napus* (rapeseed, Canola). The tribe Camelineae comprises eight genera and thirty-four species, most of these from Eurasia including *Arabidopsis thaliana,* a species that has been used a model organism for plant genomics (Al-Shehbaz et al., 2006; Al-Shehbaz 2012; Sagun et al., 2016; Warwick et al., 2006). Camelina has perfect flowers with anthers that contain medium sized prolate or spheroidal to prolate pollen grains (Sagun et al., 2016).

Camelina is an annual with a short life cycle of 85-100 days. When grown in high density populations, it grows to 30-92 cm height at maturity and produces lanceolate leaves about 5-8 cm in length. The plant produces small, yellow flowers with four petals which are organized as a raceme. After pollination, the flowers create fruits called siliques that hold an average of 15 seeds per silique (Hunter and Roth, 2014). These siliques dessicate during plant maturation and eventually dehisce to release the seeds.

Camelina seeds are somewhat similar to other Brassica oilseeds such as flax and canola. They have similar elemental and chemical makeup, except for camelina's higher sulfur content (Putnam et al., 1993). This higher sulfur content most likely is used as a defense mechanism against herbivores. The seeds contain many beneficial and essential oils and compounds with an overall oil content of 28-40% by weight. The oil has high concentrations of specific fatty acids including linolenic, linoleic, and oleic acids. All of these fatty acids occur in estimated concentrations of at least 15%, with the predominant being linolenic acid (27.9-38%) (Budin et al., 1995; Frohlich and Rice, 2003). The occurrence of these oils in high concentrations is due to high concentrations of the C_{18} precursor α-linolenic acid that allows production of many long-chain polyunsaturated fatty acids (Usher et al., 2015). In addition to the natural production of oils, camelina has been genetically engineered to enhance the production of eicosapentaenoic and docosahexaenoic acids (Usher et al., 2015).

Not a great deal is known about reproductive biology of *Camelina sativa* besides the fact that it is primarily self-pollinating. Several research groups have studied outcrossing rates and insect visitation (Eberle et al., 2015; Groeneveld and Klein, 2013; Walsh et al., 2012). An experiment using a field trial with conventional and transgenic camelina documented outcrossing rates ranging from 2.8% in neighboring plants to 0.09% at 20 m distance (Walsh et al., 2012). One publication has reported the extraction of nectar from camelina flowers and indicated that nectar concentrations exceeded those of canola (Eberle et al., 2015). This suggests that insects may be attracted by both pollen and nectar resources. There is no clear evidence that insect pollinators disperse camelina pollen over time or space.

Only two previous studies have examined the insects visiting camelina fields. In a study in Germany, insects from the families *Apidae* and *Syrphidae*, as well as bees from the genera

Lasioglossum and *Hylaeus* were observed and/or collected on flowers of camelina (Groeneveld and Klein, 2013). This study also reported that the greatest seed set and reproductive capability was in open pollination when insects were not excluded (Groeneveld and Klein, 2013). This suggested that insects play a role in seed set and yield. A study in Montana and South Dakota reported high visitation by small bees and flies (Eberle et al., 2015). Significant insect visitation to the flowers could be caused by high quantities of nectar production (100 kg/ha) during anthesis (Eberle et al., 2015). However, there is no proof that insects carry camelina pollen between plants (gene flow) or increase seed production.

Potential Ecological Risks from Camelina

If the price of biodiesel becomes competitive with fossil fuels, camelina is well positioned to become a widespread crop in North America. In addition, genetic modification could lead to fields dedicated to high-value dietary supplements and other compounds. Regardless of the application, camelina cultivation should be assessed for its potential environmental risks and benefits. The two main concerns regarding the introduction of camelina fields are: 1) escape from cultivation to become serious weeds that require management, or 2) gene flow from the crop to closely-related native or non-native plants (e.g. weeds) that leads to negative impacts.

Every plant has characteristics that could contribute to its ability to act as a weed or invasive plant in specific habitats or ecoregions. When new crops are introduced, it is important to understand their potential to become weeds or volunteers in agricultural fields. The classic papers by Baker (Baker, 1965) and others (Ellstrand et al., 1999; Hulme, 2012; Keese et al., 2014; Keese, 2008; Pheloung, 2001; Roberts et al., 2010; Stone et al., 2008) have contributed to

a better understanding of weed biology and ecology. In more recent times, Weed Risk Assessment (WRA) protocols have been developed to help predict weediness or invasion (Cousens, 2008). Some of the traits or conditions linked to weediness or invasion include: plant fecundity; the method of seed dispersal; seed bank persistence; chemical physiology or morphology affecting other plants; lack of potential herbivores or pathogens; the availability of suitable habitat such as disturbed areas (Baker, 1965; Rejmanek and Richardson, 1996; Williamson and Fitter, 1996).

Since camelina has been a weed for over 100 years in North America and Connecticut, it should be analyzed through a WRA prior to widespread cultivation across the US. Davis et al. (2010) conducted weed risk assessment research using the Australian Weed Risk Assessment as a platform for the project. Camelina was grown under different disturbance regimes (herbicide, mechanical, or no disturbance). Data was collected on the emergence and seedling survival as well as plant biomass and fecundity of camelina under each of the growing conditions. The results from these experiments were entered into the Australian WRA protocol which found that camelina should either be excluded from introduction or be evaluated further before introduction depending on whether animal dispersal and hybridization with weedy relatives were answered with a 'yes' or 'no' (Davis, 2010). However, field experiments by Davis et al. (2010) showed that camelina was not very competitive and was unlikely to become a serious weed in Montana. A model of population dynamics showed that camelina populations would likely become extinct in less than six years. Thus, the authors concluded that camelina does not pose a risk.

Gene flow is a concern when new crop species are introduced or existing crops receive novel traits through breeding or biotechnology (Ellstrand et al., 2003; Ellstrand et al., 1999; Jhala and Hall, 2013; Walsh et al., 2015; Warwick et al., 2009). Gene flow is usually defined as the

movement of genes from one plant population to another through the movement of pollen or seed. In the most extreme cases, gene flow between crops and their wild relatives can result in the extinction of the wild relatives (Ellstrand et al., 1999).

Plant gene flow has been shown to contribute to species invasion (Saltonstall, 2002; Weeks et al., 2011). Invasiveness or weediness can be exacerbated by the introduction of herbicide resistance genes or genes that increase plant fitness (e.g. seed number, reproductive ability, drought tolerance). One concern is a loss of biodiversity in areas near farms due to a competitive dominance of a crop species. Alternatively, weed species might receive transgenes or beneficial traits that further increase their ability to compete with native plant populations. Camelina has several close relatives that are known to be or have been present as weeds in the Northeast. These non-native weeds include *Camelina microcarpa, Camelina rumelica,* and *Camelina alyssum* (USDA Plants Database). A few studies have examined the potential for gene flow between camelina and its close relatives (Martin et al., 2015; Seguin-Swartz et al., 2013; Walsh et al., 2012; Walsh et al., 2015). In addition, the common weed *Capsella bursa-pastoris* (Shepard's purse) can hybridize with camelina, although previous studies have reported sterility in the F2 generation (Julie-Galau et al., 2014).

Potential Ecological Benefits from Camelina

Recent declines in pollinator habitat and abundance has led to great concern about these insects as well as conservation efforts. *Apis mellifera* (honey bee) is one of the most abundant pollinating insect species on the planet and is capable of increasing the yields of 96% of animal pollinated plants (Potts et al., 2010). However, the United States has experienced a 59% decline in honey bee colonies between 1947 and 2005 (Potts et al., 2010). These insects play a vital role

in the environment, and the loss of these insects could have huge implications throughout all trophic levels and ecosystems. Not only are these pollinators critical for the ecosystem, but also for human food production. The global economic value of insect pollination in 2005 was estimated to be 170 billion USD (Potts et al., 2010). One of the proposed major drivers in the decline of pollinators is the loss of habitat or habitat fragmentation (Potts et al., 2010). Pollinators have too far to travel between pollen and nectar sources as well as too few sources of food.

Camelina shows great potential to be a forage (nectar and pollen) resource for insects. It could also act as a pathway between other forage habitats and ameliorate the monocultures that large-scale agriculture can create. Camelina is capable of producing large amounts of nectar sugar for insects. A study in the Midwestern US showed 100 kg/ha of nectar sugar (Eberle et al., 2015).

Cover crops are of interest in sustainable agriculture because of their benefits to soil fertility, soil stability, weed management, and water management. Camelina can be used as a cover crop or in a multiple cropping system due to genetic varieties optimized for spring and winter seeding. The fact that camelina can be grown as a winter cover crop creates the potential for additional farm income as well as providing the benefits of a cover crop.

Research Justification and Objectives

Camelina sativa has the potential to become an oilseed crop of great economic and agricultural importance in the United States and the Northeast region. As our requirements for alternative fuels increases, it is expected that the area used for biofuels crops will increase. Furthermore, camelina could be used as a winter cover crop to prevent soil erosion or planted to

provide nectar and pollen to beneficial insects. Thus, it is important to understand the potential impacts positive and negative impacts. The major objectives for this research program were to better understand camelina growth and development, reproductive biology, ecological benefits, and potential negative impacts. These objectives and their respective chapters are summarized as follows:

1. Characterize the growth, development, and yield of camelina from seeding to harvest with emphasis on the effects of temperature and precipitation. (Chapter 2)
2. Identify the insects that visit camelina flowers and determine their abundance with respect to temporal events such as flowering. Determine the role of insects on camelina seed set. (Chapter 2)
3. Evaluate the competitive ability of camelina against agricultural weed species common to Connecticut's agricultural fields. (Chapter 2)
4. Determine the ability of camelina seed to overwinter in a seed bank at two soils depths. (Chapter 3)

Chapter 2. Camelina Development, Insect Pollinators, and Weed Competition.

Introduction

Camelina sativa (L.) Crantz (camelina, false-flax) is a Brassica oilseed crop that can produce a wide variety of products from biofuels to dietary supplements. Research also suggests that it can provide nectar and pollen for pollinators, or function as a winter cover crop (Eberle et al., 2015; Groeneveld and Klein, 2014). Camelina's agronomic traits, economic value, and close relationship to *Arabidopsis thaliana* have promoted rapid advances in genetic engineering, genomics, and lipid metabolism research (Bansal and Durrett, 2016; Vollmann and Eynck, 2015; Moser, 2010). Studies have shown that camelina oil can be converted to biodiesel at reasonable cost (Fröhlich and Rice, 2005) and emit low amounts of carbon dioxide and other pollutants (Bernardo et al., 2003). Camelina has been genetically engineered (GE) to express algal genes that generate high concentrations of long chain polyunsaturated fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in seeds (Betancor et al., 2015). Thus, camelina can effectively replace algae as a source of essential fatty acids in the diet of marine fishes and other organisms (Betancor et al., 2015; Petrie et al., 2014; Usher et al., 2015). Herbicide resistance and other agronomic traits have also been evaluated in experimental field trials in the US (http://www.isb.vt.edu).

Insect Visitation

Camelina is generally classified as a self-pollinating crop species, but several studies have shown outcrossing through pollen-mediated gene flow (Walsh et al., 2012; Walsh et al., 2015). A field study in Canada reported gene flow decreasing with distance from 0.78% at 0.2 m distance to 0.001% at 20 m, but did not identify the mechanism for pollen dispersal (Walsh et al., 2015).

Gene flow is an important consideration in ecological risk assessments prior to the release of GE crops (Dale et al., 2002; Ellstrand et al., 1999; Keese et al., 2013). Our preliminary experiments showed that pollinating insects were abundant during camelina flowering and might contribute to pollen-mediated gene flow. To the best of our knowledge, only two studies have assessed pollinators in camelina fields. A study of camelina in South Dakota and Minnesota identified insects in six taxa (Eberle et al., 2015). A study in Germany reported pollinators from five insect orders, but insect visitation did not consistently increase seed yield (Groeneveld and Klein, 2014). There is currently no direct evidence that insect pollinators disperse camelina pollen over time or space, or contribute to pollen-mediated gene flow.

Camelina as a Weed

Camelina is a short-season crop adapted to the cool temperate regions of Europe, Asia, and North America. Many references mention its ability to grow on marginal agricultural land, use less fertilizer, tolerate drought stress, and have fewer insect pests compared to other Brassicas such as rapeseed or canola (Bansal and Durrett, 2016; Ehrensing and Guy, 2008; Francis and Warwick, 2009; Gugel and Falk, 2006; Jewett, 2013; McVay and Lamb, 2004; Zubr, 1997). These same traits have been noted in invasive plant species and weeds, so they could contribute to camelina's ability to persist as a non-native weed in North America (Barney and DiTomaso, 2008; Raghu et al., 2010). The development of GE camelina has triggered questions about long-term ecological impacts and weed risk assessment (WRA) research (Davis et al., 2011, Cousens, 2008). WRA is especially important when novel traits could increase plant fitness (e.g. nitrogen use efficiency, salt tolerance), volunteers in agricultural fields, weed populations, or invasives in natural areas (Cousens, 2008; Warwick et al., 2009). Davis et al

(2010) reported that a qualitative WRA approach would prohibit introduction of conventional (non-GE) camelina in the US, but their field studies in Montana coupled with population dynamics models suggested that conventional camelina was unlikely to become a serious weed (Davis, 2010). Additional research in other geographic regions and other genotypes would strengthen WRAs and predictions about GE camelina.

In the US, regulatory decisions about the commercialization of GE crops are based upon case-by-case analysis of potential 'plant pest risks' including new weeds, weed management challenges, and invasive species (National Academy of Science, 2016). The need for robust ecological risk assessments provides a rationale for studying camelina crop development, pollen dispersal, and weed competition in the Northeastern US. The major research goals were to: 1) characterize camelina crop development and seed yield in the Northeastern US, 2) identify insects that could be involved in pollen-mediated gene flow, and 3) understand the competitive ability of camelina against agricultural weed communities.

Materials and Methods

Camelina cultivation

Camelina sativa 'SO-40' (Sustainable Oils, California, USA) was grown at the University of Connecticut Plant Science Research Farm in Storrs, Connecticut for three years (2014, 2015, 2016) in a 0.32-hectare plot located at 41º80'N, 72º23'W. The farm is within the Level III 59 Northeastern Coastal Zone and Level IV 59c Southern New England Coastal Plains and Hills ecoregion (US Environmental Protection Agency, https://archive.epa.gov/wed/ecoregions/web/html/new_eng_eco.html, Last accessed June 23, 2016). The plot had a 3-8% West to East slope with a Paxton and Montauk fine sandy loam soil

type with a soil pH of 5.8 (http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx). A weather station at the research farm (http://newa.cornell.edu/, Storrs Research Farm) provided information about precipitation and temperature. Growing degree days (GDD) was calculated as the mean of maximum and minimum temperatures recorded in each day minus the base temperature (10º C). Urea fertilizer (46-0-0) was applied at 168 kg/hectare before seeding in 2014 and 2015; fertilizer (15-15-15) was applied at 643 kg/hectare in 2016. No herbicides or insecticides were applied.

Camelina seed 'SO-40' were sown on at 6.27 kg/hectare (May 6, 2014), 6.5 kg/hectare (May 5, 2015), or 7.7 kg/hectare (April 29, 2016). Laboratory tests showed about 95% germination rate each year. The experimental design included random placement of 20 0.5m x 0.5m subplots in the field using geospatial coordinates and a random number generator. Camelina development in subplots was observed 12-16 times between seeding and harvest with data recorded on: number of camelina plants present, date of first open flower, date of last open flower, percent camelina plants with open flowers, and percent plants with siliques. The flowering stage was defined as a plant with at least one open flower and siliques; fruiting was defined as siliques present without any open flowers. Harvest occurred when 75% of plants had mature siliques. The above-ground portions of camelina plants and weeds were removed from 10 subplots at harvest on July 22, 2014 and 20 subplots on July 23, 2015 and (date), 2016. Camelina plants were dried and data collected on the following traits: plant biomass (gdw); number of intact, half, or missing siliques; number of seeds per plant; seed biomass per plant. Weeds were removed from each subplot, identified to species level, dried, and weighed.

Insect pollinators

The abundance and function of insect pollinators was studied through two experiments: 1) insect exclosures, and 2) sweepnet transects. For the insect exclosure experiment, five 1m x 1m x 1m cubes (insect exclosures) were built from polyvinyl chloride tubing (PVC) and placed at equal distances across the field before flowering. The PVC cubes were covered with netting (Outback travel net, Mombasa Brand, Arlington, Texas) to exclude flying insects. These insect exclosures were placed on plants just before flowering began and removed just after flowering had completed. This approach minimized the amount of shading for plants within the structure. At harvest, 20 camelina plants were collected from inside each insect exclosure and 20 plants were collected from adjacent control plots where insects had access to the camelina flowers. These adjacent control plots were approximately 1 meter to the east of each exclosure to minimize the effect of the structures. Camelina plants were analyzed for biomass (gdw), silique number, seed number per silique, total seed number, and seed mass.

Sweepnet transects were conducted to identify and quantify pollinating insects in 2014 and 2016. No sampling was conducted in 2015 due to crop failure. Four sweepnet transects were conducted twice per day (10:00 am and 3:00 pm) on seven days occurring before, during, and after flowering when the weather was favorable for insect activity (no precipitation, little or no wind). Sweepnet transects were conducted between days 32-65 after seeding (early June-early July). Insects were collected using a sweepnet (BioQuip, catalog number 7312MS, Rancho Dominguez, California) across the top third of the camelina plants while walking in a "W" pattern (four diagonal transects) across the field. Insects were transferred to plastic bags, frozen, and sorted into functional groups. Insects were identified in the lab using established references (Borror et al., 1976; Gullan and Cranston, 2010; Xerces Society, 2011).

Weed competition

Subplots were used to study weed populations and the competitive ability of camelina. Data was collected from 10 subplots in 2014 and 20 subplots in 2015 and 2016. No herbicides were applied to the soil. Weeds in the subplots were harvested on the same day as the camelina crop. The species of each individual weed was identified and the number of individuals in each species was recorded. Weeds were dried and dry weight biomass recorded.

4. Statistical Methods

Data analysis was conducted using Microsoft Excel, SAS (version 9.1, SAS Institute, Inc., Cary, NC), and SigmaPlot (version 11.0, SYSTAT, Chicago, IL). The first step was to analyze the data for homogeneity of variances and normality using SAS.

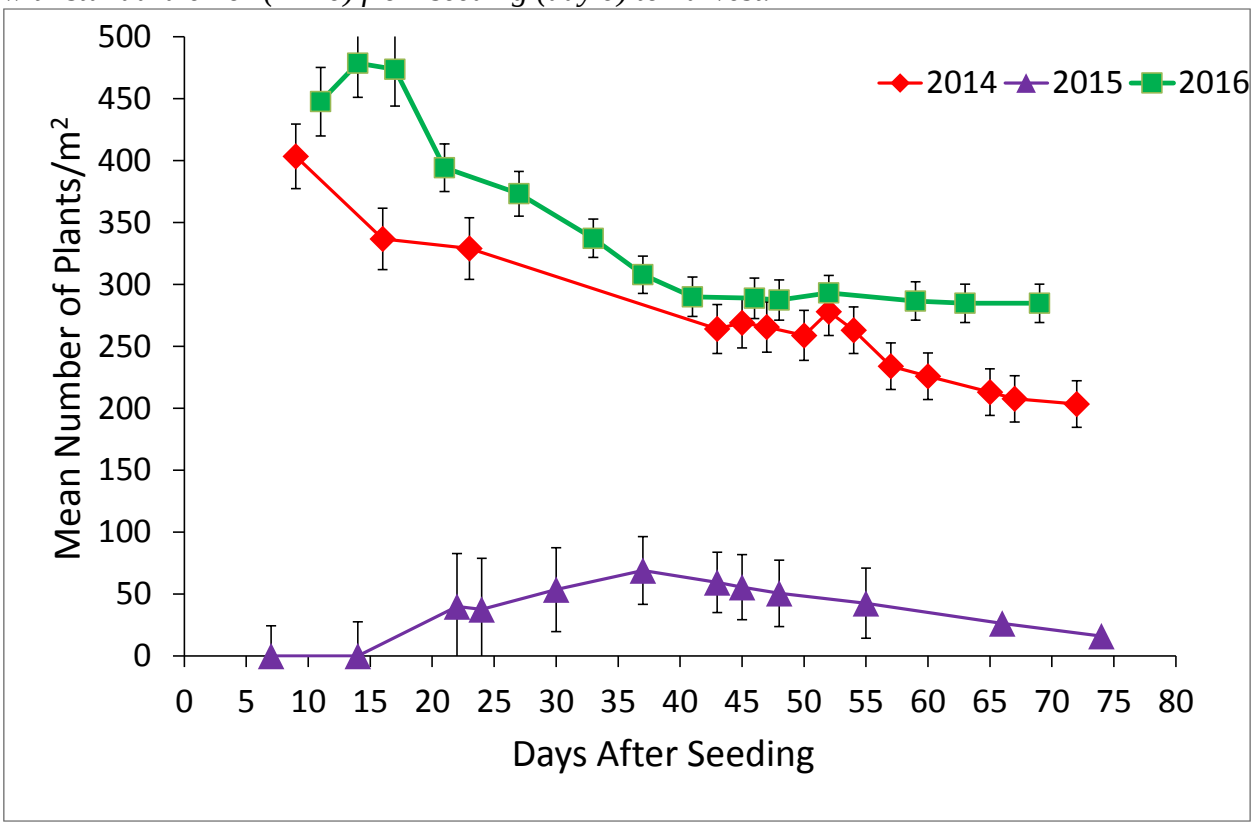
Analysis of data was conducted using paired t-tests and ANOVA (SAS PROC T-TEST, PROC ANOVA). A paired t-test was conducted to compare seed biomass per plant inside and outside the insect exclosures. Correlations between the number of camelina plants flowering and the number of pollinators were assesssed using SigmaPlot to create a Pearson's correlation 'r' between the two variables. Pearson's correlations were also created using SigmaPlot to find what effects, if any, the presence of weeds had on the number of camelina plants present. Two diversity and evenness indexes were calculated for the weed species in the field: Margalef's Diversity index and Simpson's Dominance index. Margalef's Diversity index was defined as $D_{mg} = (S - 1) / \ln (N)$ and Simpson's Dominance index was defined as $D = \Sigma \{[n_i (n_i - 1)] / [N (N - 1)]\}$. The value for N in Margalef's equation was determined by the total number of weed species in the Northeastern US (237) (Uva et al., 1997).

Results and Discussion

Crop Development and Yield

Camelina 'SO-40' was planted in the same location for three years between April 29-May 6 at a rate of 6.3-7.7 kg seed/ha. Observation of plants in 20 subplots began after seeding and continued until harvest. Seedlings were first observed at 3-7 days after planting, with first true leaves at days 13-15 (Table 1). Based on a paired t-test, crop population density varied between years (Figure 1). The number of camelina plants/m^2 was greater in 2016 than in 2014 or 2015 ($p < 0.0001$), and the density was greater in 2014 than in 2015 ($p = 0.002$). Crop density declined from seeding to harvest (self-thinning) in 2014 by 50% and in 2016 by 40%. However, a different pattern was observed in 2015 with an increase in plant number to day 37 followed by a gradual decline to harvest. The maximum population densities in 2014 (264 plants/m^2) and 2016 (307 plants/$m^{2)}$) were much higher than in 2015 (69 plants/m^2). The crop was considered a failure in 2015.

Figure 1. Self-thinning in camelina populations. Mean number of camelina plants/m2 are shown with standard error (n=20) from seeding (day 0) to harvest.



Key developmental events occurred at about the same plant age in all three years (Table 1). For example, first true leaves were observed at 13-15 days and anthesis began at 42-44 days (Table 1). In general, crop failure in 2015 did not alter the timing of key developmental events. In 2014 and 2016, flowers were observed on 90-94% of camelina plants and the period of flowering (one or more open flowers/day) lasted for 16-23 days (Figure 2). However, only 18% of plants produced flowers in 2015.

Biomass, seed, and silique yield varied between years (Table 2). Crop failure in 2015 was reflected in the nearly twenty-two fold decrease in seed number/plant from 2014 to 2015

(Table 2). As a result of crop failure, crop yield was not calculated and insect studies were not conducted in 2015. 2014 and 2016 were clearly the more successful years of growth over 2015. In 2014, plants grew well achieving strong biomass and seed production in the plants. Plants achieved even higher biomass and seed production levels in 2016 compared to those in 2014 (Table 2). However, plants from 2016 had lower numbers of siliques per plant compared to 2014. The overall seed yield was higher in 2016, likely due to an increase in plant density over the previous two years as well as an increase in the number of seeds being produced per plant and therefore, the number of seeds being produced per silique.(Figure 1). 2014 and 2016 show the positive yields that can come from growing camelina in the northeastern United States under low-input conditions. Only fertilizer was used at the beginning of the growing season, yet good yields were achieved.

Figure 2. Percent camelina plants in with one or more open flowers (anthesis).

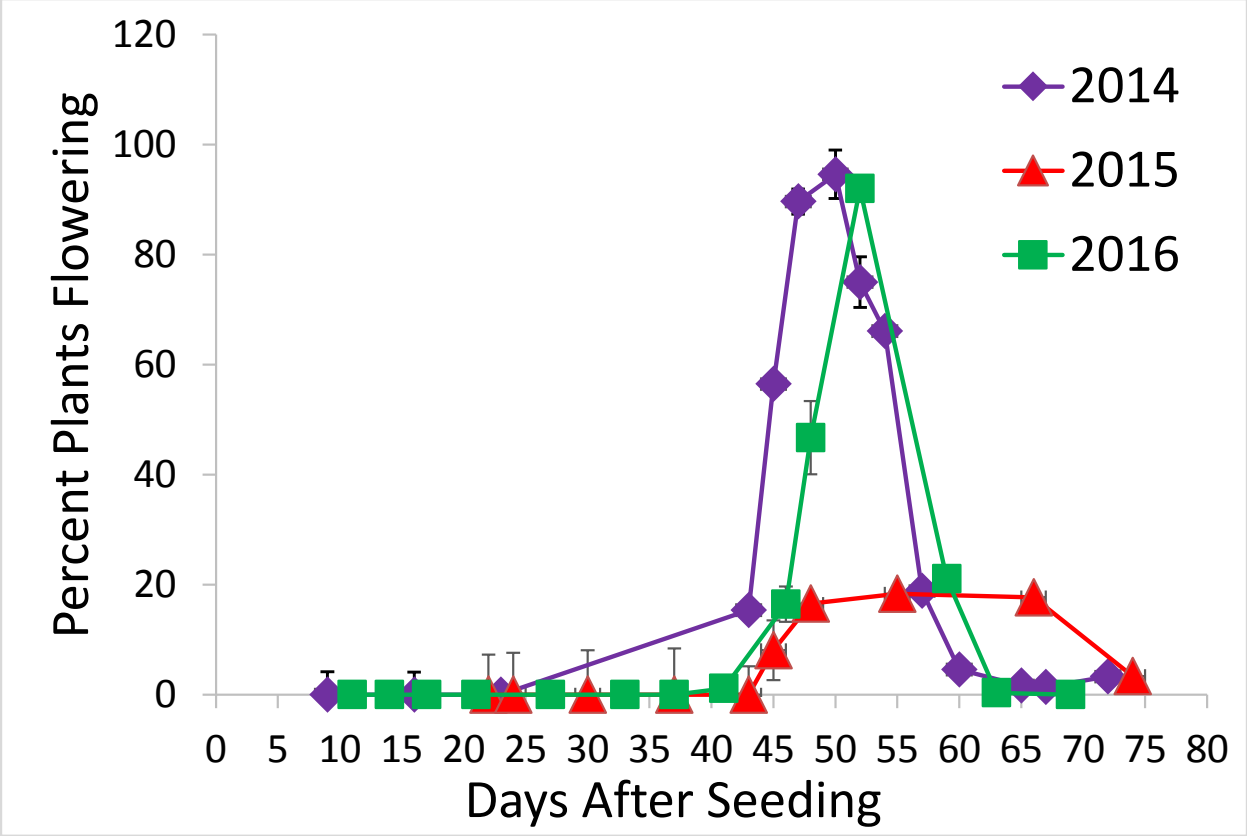


Table 1: Temporal events in crop development with cumulative growing degree days (GDD) and number of days after seeding. The number of days to first seed germination was recorded based on observations across the entire field. All other information was recorded based on observation of plants inside subplots. Not applicable=NA.

Developmental Event	2014		2015		2016	
	# of Days after Seeding	Cumulative GDD	# of Days after Seeding	Cumulative GDD	# of Days after Seeding	Cumulative GDD
First observed germination	2	3.5	2	31.9	2	0.6
First true leaves	13	84.5	15	210.0	14	41.4
First anthesis	42	382.0	44	585.1	43	444.0
Peak anthesis (>90%)	50	502.5	NA	NA	52	573.4
End of anthesis	65	797.2	70	1038.1	63	801.0
Harvest date	80	1115.9	80	1266.8	88	1371.0

Camelina seed biomass in subplots was used to estimate a crop yield of 531.6 kg/hectare in 2014 and 1096.6 kg/hectare in 2016. These values fell within the range (372-1867 kg/hectare) reported for Montana (McVay and Lamb, Montana State Univ. Extension, 2006) and exceeded the value cited for 'low yielding' plots (500 kg/ha) in Colorado field trials (Jewett, 2013). The yield in 2016 was only slightly lower than reported for Sustainable Oils cultivars grown in South Dakota (1104-1158 kg/ha) or other cultivars grown in Pennsylvania (1120 - 1681 kg/ha) (Hunter and Roth, 2010). Thus, commercial production of camelina is feasible in the Northeastern US.

Poor crop development in 2015 prompted analysis of two environmental variables: precipitation and growing degree days (GDD). Figure 3 shows cumulative precipitation for each year compared to a 50-year average (1963-2013). In 2015, there was no rainfall for 13 days after seeding, lower total precipitation up to day 42, and fewer rainfall events compared to other years (Figure 3). However, a paired t-test showed that precipitation over the growing season in 2015

was higher than in 2014 and 2016 ($p<0.0001$). Thus, the pattern in 2015 was delayed rainfall and few events followed by higher levels of rainfall later in the growing season after day 45. While precipitation in all three years was significantly different from the 50 year average ($p<0.0001$), precipitation in 2014 and 2016 were not significantly different from each other ($p = 0.4095$). These results suggested that precipitation after seeding is critical to seed germination and the early stages of camelina development. Future experiments should incorporate direct measurements of soil moisture and its effect on seed germination.

Since temperature plays a role in crop development, the pattern of cumulative GDD from planting (day 0) to harvest (approximately 80 days) were compared with each other and a 50-year mean (Figure 4). GDDs in 2015 were significantly higher than 2014, 2016, or the 50-year average ($p < 0.001$). Thus, 2015 was warmer than 2014 or 2016. Although all of the significant events in the growth and development of the crop occurred at about the same number of days after seeding (Table 1), temperatures as shown through cumulative GDD was greater (Figure 4). Cumulative GDD for early developmental events such as seed germination or first true leaves were much higher in 2015 than in 2014 and 2016 (Table 1). For example, the first true leaves in 2015 occurred at 210 GDD compared to 84 GDD in 2014 and 41 GDD in 2016. Anthesis began at roughly the number of days (±2 days), but the GDD in 2015 greatly exceeded the GDD in 2014 and 2016 (Table 2). GDD in 2015 were significantly higher than in 2014 ($p < 0.0001$) and GDD in 2014 were significantly higher than 2016 ($p < 0.0001$), therefore GDD in 2015 was also higher than 2016 ($p < 0.0001$). When comparing GDD values for these years with the 50-year average, all three years had significantly higher GDD values ($p < 0.0001$) Differences in GDDs were relatively small at the time of harvest with 2014 < 2015 < 2016.

It is reasonable to assume that these environmental variables, at least in part, inhibited crop establishment, development, and yield in 2015. Drought has been linked to poor stand establishment in camelina fields in South Dakota (Eberle et al., 2015) and reduced yields in Western Canada (Gugel and Falk, 2006). While some publications (Bansal and Durrett, 2016; McVay and Lamb, 2008; Yang et al., 2016) emphasized camelina drought tolerance relative to canola and other oilseed crops, field trials in Connecticut and other regions suggest that low moisture and high temperatures in the early part of the growing season can negatively affect establishment and yield.

Figure 3. Cumulative precipation in 2014, 2015, and 2016 with the 50 year average shown for comparison.

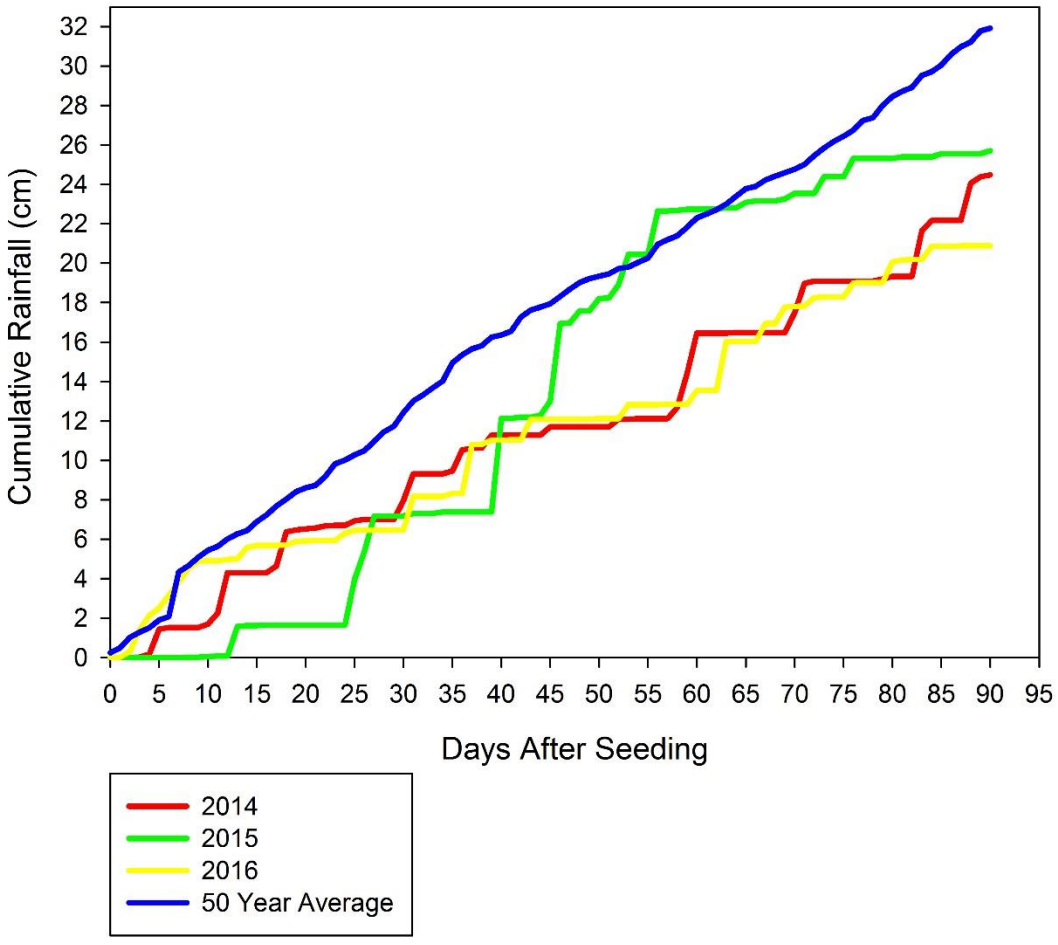


Figure 4. Cumulative growing degree days for 2014, 2015, and 2016 with a 50 year average shown for comparsion. Chapter 1 and 2 in the handbook.

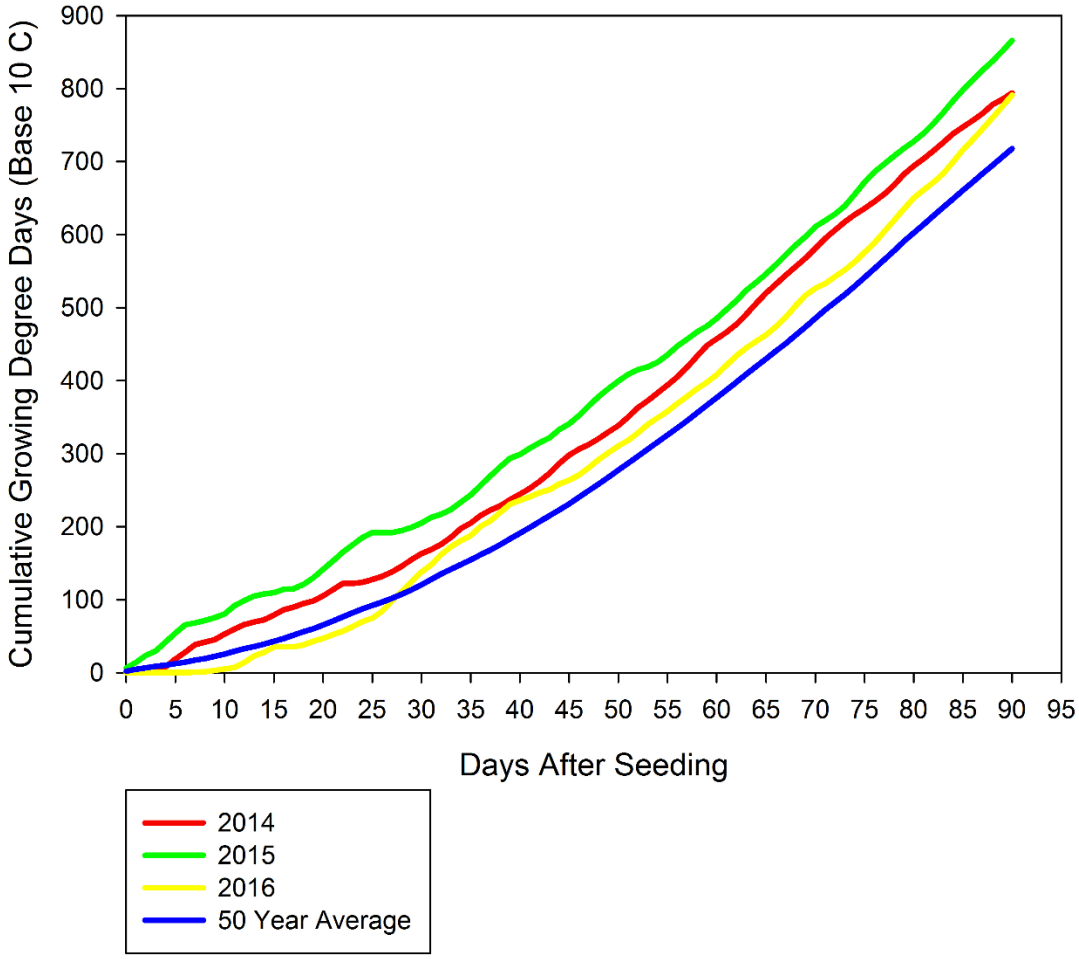


Table 2: Characteristics of harvested camelina plants. Mean values are shown with standard deviations. Letters indicate differences determined by Fisher's LSD. P=0.05. Not applicable=NA.

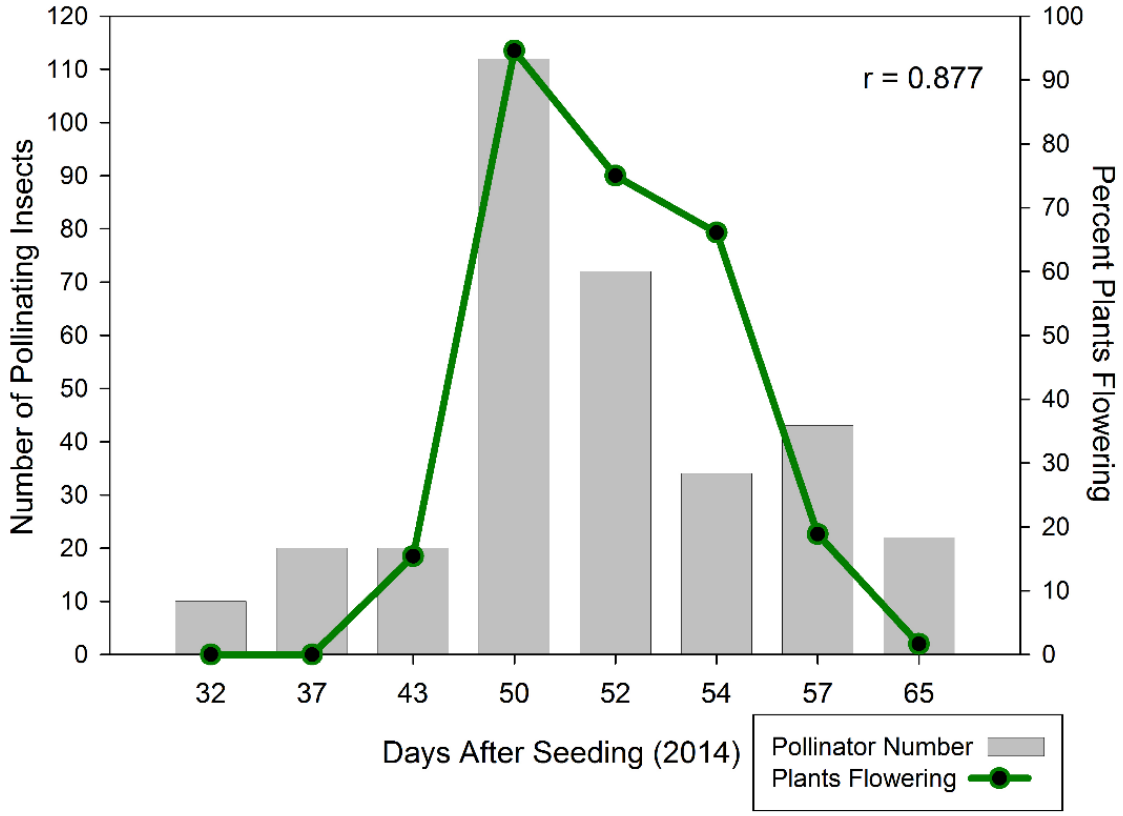
	2014	**2015**	**2016**
Plant biomass (mg)	640±560 b	140±170 c	1036±887 a
Number Intact Siliques / Plant	17.9±16.2 a	3.6±5.7 c	17.4±9.9 b
Number Seeds / Plant	151.5±160.3 b	7.9±17.8 c	NA
Seed Mass / Plant (mg)	150±150 b	10±20 c	180±129 a

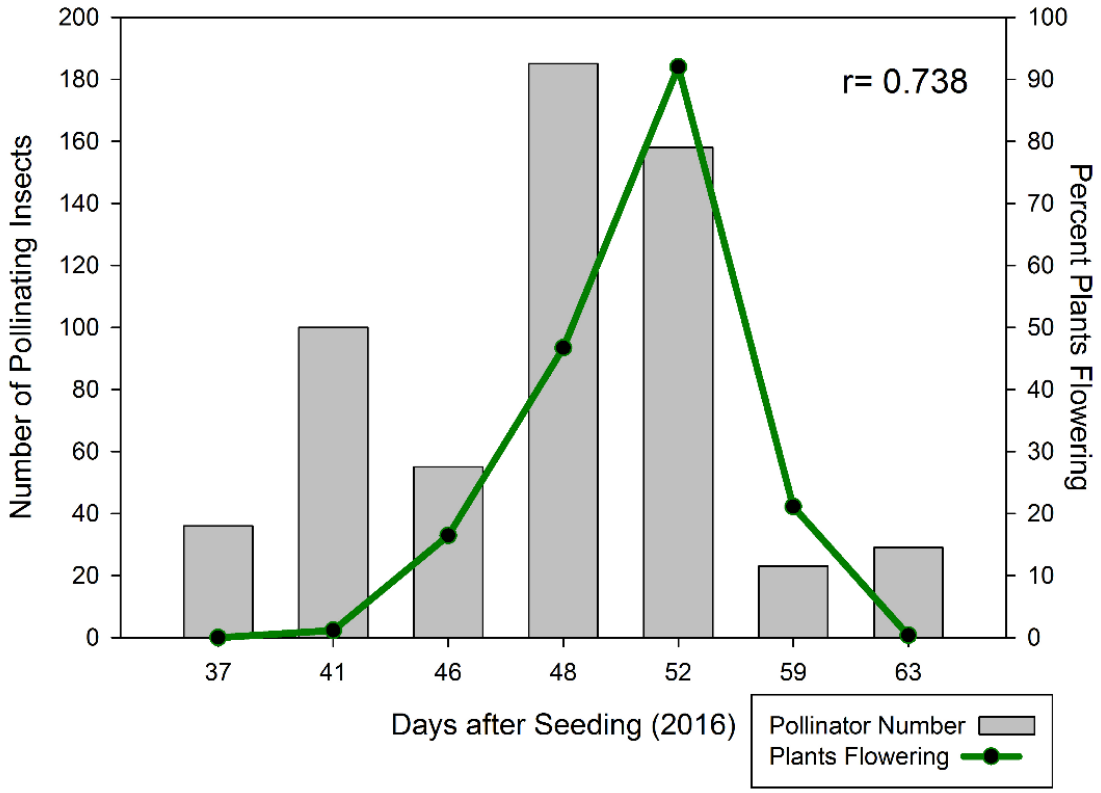
Insect Pollinators

Sweepnet transects were conducted in 2014 and 2016 to document the insect taxa present and the number of pollinators visiting camelina flowers. The number of pollinators changed over time with a temporal match between the maximum number of pollinators and the highest percent of camelina plants in flower (Figure 5). For example, the peak of flowering (95% at 50 days after planting) in 2014 coincided with 110 individual pollinating insects collected in combined sweepnet transects (8 transects/day). Both years showed a strong correlation between the number of pollinating insects collected and the percent camelina plants flowering. However, the number of pollinating insects at the peak of flowering was greater in 2016 (185 insects) compared to 2014 (110 insects). This could be due to the slightly higher density of plants and flowers per square meter in 2016 compared to 2014. Pollinators were largely absent or in very low numbers before and after flowering. Thus, these results suggest that camelina attracts

pollinators and provides some kind of food resources for these insects. Honey bees and native insect pollinators provide a crucial service to many agricultural crops and wild plants, but can also contribute to pollen-mediated gene flow from transgenic crops (Cresswell et al., 2002; Mallory-Smith et al., 2015; Potts et al., 2010). Substantial declines in honey bee stocks and native pollinators have been documented in the US leading to conservation efforts (Cameron et al., 2011; Potts et al., 2010).

Figure 5. Percent camelina plants in flower and the total number of pollinating insects collected in sweepnet transects in 2014 and 2016. Pearson correlation coefficients are given for each year.





Sweepnet transects (n=56 in each year) across the camelina field revealed a diverse group of insect taxa known to act as pollinators of flowering plants (Table 3) (Borror et al., 1976; Gullan and Cranston, 2010; Xerces Society, 2011). The pollinators collected were in four orders: Hymenoptera, Diptera, Coleoptera, and Lepidoptera. Members of the Hymenoptera order were the most abundant pollinators in both years. The Hymenoptera included honey bees (*Apis melifera*), mining bees (Andrenidae), sweat bees (Halictidae), and bumble bees (*Bombus* spp.) with leaf cutter bees (Megachilidae) having the lowest abundance (Figure 6, Figure 7, Table 3). Twice as many honey bees were collected in 2016 than 2014, but they represented approximately the same percentage of all pollinators in both years (33%) (Figure 6, Table 3). The number of bumble bees was approximately the same in each year (Table 3). In the Diptera, syrphid flies (Syrphidae) were more abundant than other fly taxa, comprising 24.6% and 33.1%

of the entire pollinator population in 2014 and 2016 respectively. The twofold increase in syrphid flies between 2014 and 2016 was similar to the increase in honey bees in 2016. Few Lepidoptera and Coleoptera were collected from the flowers, but this may have been due to the sampling method and the behavior of these insects in the field (e.g. more difficult to capture than bees).

The abundance of pollinators indicates that camelina attracts these insects and provides some resources in the Connecticut agricultural landscape. This result is similar to that reported for Mid-Western states and Germany (Eberle et al., 2015; Groeneveld and Klein, 2014). Honey bees made up a much larger percent of the pollinators in Connecticut (approximately 30%) compared to the two Mid-Western states (3%). Conversely, camelina in Germany had higher percent honey bees (47%) than Connecticut (Groeneveld and Klein, 2014). A study on gene flow between transgenic and conventional peanut plants reported that 50% of the pollen samples taken from bees included transgenic peanut pollen (Hu et al., 2015). Thus, bees and other pollinators in Connecticut might contribute to pollen-mediated gene flow between camelina fields (crop-to-crop), to closely-related wild plants (crop-to-wild), or to weedy relatives (crop-to-weed).

Table 3. List of insect taxa and their abundance in 2014 and 2016.

Order	**Family, Genus, or Species**	**Common Name**	**Number of individuals (2014)**	**Number of individuals (2016)**
Coleoptera	Cantharidae	Soldier Beetles	0	5
	Elateridae	Click Beetles	0	1
Diptera	Agromyzidae	Leaf-miner flies	36	0
	Anthomyiidae	------	4	67
	Bombyliidae	Bee flies	2	12
	Drosophilidae	------	1	5
	Muscidae	------	11	0
	Syrphidae	Syrphid flies (hover fly)	84	186
	Tachinidae	Tachinid flies	2	0
Hymenoptera	Andrenidae	Mining bees	40	12
	Apidae		99	191
	Apis mellifera	Honey bees	88	175
	Bombus spp.	Bumble bees	11	16
	Halictidae	Sweat bees	24	50
	Megachilidae	Leaf cutter bees	3	0
Lepidoptera	Erebidae	-------	1	0
	Pieridae	--------	3	0

Figure 6. Percent pollinators in taxonomic groups in 2014 and 2016.

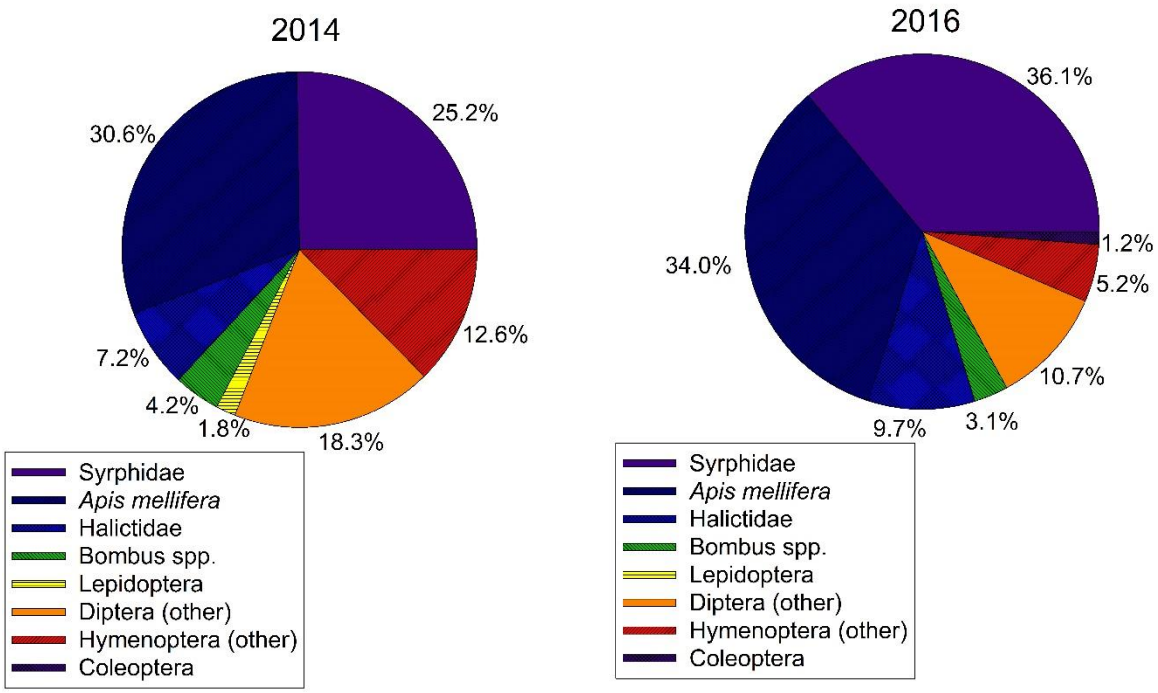
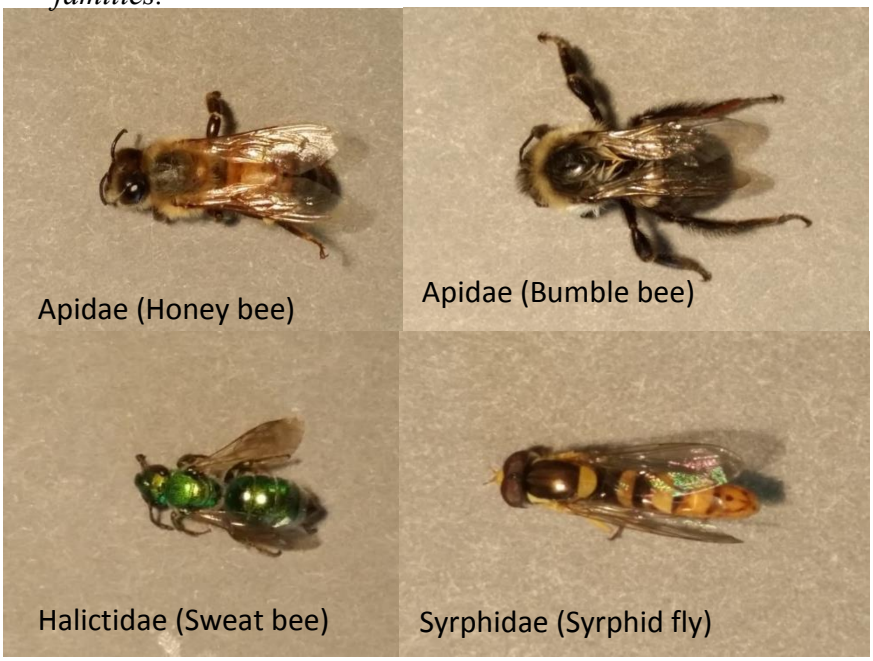
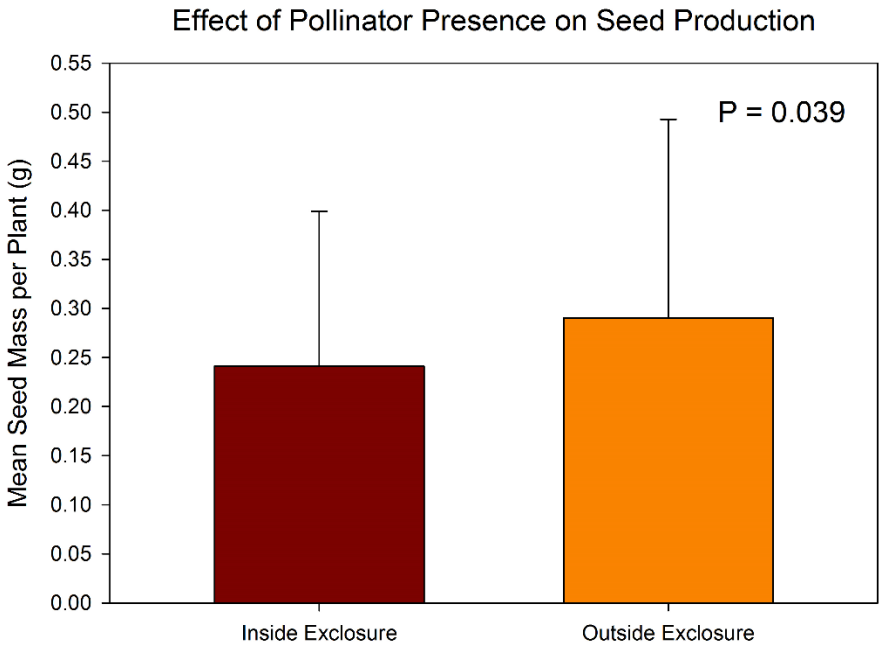


Figure 7. Photographs of insects from four major insect families.



Insect exclosures were employed to determine the effect of insects and pollinators on seed set and crop yield. An ANOVA ($p = 0.051$) with Fisher's LSD and Tukey multiple means comparison tests lettering was conducted on the seed mass produced per plant between 2014 and 2016 . The test resulted in no significant differences between years, therefore data on seed mass in 2014 and 2016 was combined. Camelina seed yield was increased by insect visitation (Fisher's LSD, $p = 0.039$) (Figure 8). A Tukey multiple comparison test was utilized in addition to Fisher's LSD as it offers a more conservative approach. This test also showed significant differences in seed mass per plant between the two treatments. Although our range of data shown by standard deviation was large, a generous sampling size ($n=200$) allowed differences to become clear. This result suggested that insects (e.g. pollinator taxa captured in sweepnet transects) might play a role in pollination, seed set, yield, or pollen-mediated gene flow. The effect of the insect exclosures differed from that previously reported where no significant difference was observed on protected plants compared to those exposed to wind and insects in Germany (Groeneveld and Klein, 2013). This shows that insect pollinators are likely to transfer pollen between individual camelina plants. This would also provide a reason for camelina to provide nutrients such as pollen and nectar to the insects. The difference between treatments could be caused by outside factors such as shading from the nets. However, every precaution was taken to minimize usage of the net and keep the two treatments as balanced as possible.

Figure 8. Seed biomass per camelina plant with or without exposure to insect visitation. Mean (n=20) and standard deviation are shown for combined data from 2014 and 2016. P value as determined by Fisher's LSD.



Crop: Weed Interaction

Weed species, abundance, and biomass were recorded for three years in field subplots. Eight species of weeds were identified in the subplots (Table 4) and were typical of agricultural fields in the Northeastern US (Uva et al, 1997). In all three years, the field had approximately the same number of weed species and the weed population was dominated by one species, giant foxtail. Giant foxtail was the most abundant weeds in all three years. Margalef's and Simpson's Dominance indices were calculated to characterize diversity in the weed populations (Nkoa et al., 2015). In 2014, the Margalef's index was $D_{mg} = 1.646$ and the Simpson's index was $D = 0.644$. In 2015, the Margalef's index was $D_{mg} = 1.646$ and the Simpson's index was $D = 0.700$. Margalef's index suggests that species diversity, in terms of the number of species, was the same in 2014 and 2015. Simpson's index suggested that the distribution of weed species was uneven in both years with dominance by one or two species such as giant foxtail. An ANOVA was

conducted to determine differences in weed biomass/m^2 between years. The total weed biomass was similar in all three years: 465.2±133.2 g/m^2 in 2014; 526±163.2 g/m^2 in 2015; and 583.4±283.9 in 2016 ($p = 0.375$). The similarity in weed biomass across years is surprising given the very low number of camelina plants in 2014. Regression analysis was conducted to identify correlations between weed biomass (total weed biomass/plot, biomass/ weed species), camelina biomass, and number of camelina plants. A paired t-test was conducted to analyze differences in camelina plants/m^2 between years. Results showed that the mean number of camelina plants/m^2 at each time point that data was collected was significantly different between years ($p < 0.0001$), so analysis was done separately for each year. In 2014, there was a slight negative correlation between the number of ragweed and camelina biomass ($R= -0.633$). In 2015, there was a slight negative correlation between the number of giant foxtail plants and the number of camelina plants ($R= -0.626$). However, the overall conclusion was that camelina fields in the Northeastern US could produce substantial yields (e.g. >1,000 kg/ha) with weed competition and without herbicide applications.

Table 4. Weed species, biomass, and abundance. Means and standard deviations are shown for 20 subplots (2015, 2016) or 10 subplots (2014).

Species	**Common Name**	**2014**		**2015**		**2016**	
		Mean grams dry weight	**Mean Number of plants/m^2**	**Mean grams dry weight**	**Mean Number of plants/m^2**	**Mean grams dry weight**	**Mean Number of plants/m^2**
Setaria faberi	Giant foxtail	0.84±0.04	390±162	0.42±0.01	998±513	0.72±0.22	628±361
Ambrosia artemisiifollia	Common ragweed	1.42±0.09	64±51	1.04±0.04	34±34	1.49±0.38	87±123
Chenopodium album	Lambs quarters	0.21±0.03	17±20	0.90±0.02	71±34	0.38±0.25	55±28
Amaranthus powellii	Green amaranth	0.05±0.02	1±2	0.73±0.06	16±41	1.02±0.54	7±26
Oxalis acetosella	Wood sorrel	0.33±0.10	10±16	0.32±0.02	16±22	0.18±0.08	2±2
Plantago lanceolata	Narrow leaf plantain	0.53±0.10	2±3	0.21±0.02	2±8	0±0	0±0
Polygonum pensylvanicum	Pennsylvania smartweed	1.53±0.48	3±8	0.68±0.12	60±151	1.55±0.31	21±111
Silene latifolia	White campion	0.17±	8±15	0±0	0±0	0.92±0.63	1±4

Conclusions

This study showed that camelina can produce substantial yields in the Northeastern United States with an external input limited to one application of fertilizer prior to seeding. Variations in weather between years showed that camelina seeds require rainfall or irrigation for good seed germination and seedling development. Other studies have shown relatively high levels of cold hardiness and this would probably allow earlier planting dates in the Northeast when soil moisture and rainfall might be higher (Davis et al., 2013; Davis, 2010; Ehrensing and Guy, 2008; Jewett, 2013; Putnam et al., 1993). Earlier planting dates might also allow better competition with weed populations, although weeds did not reduce yield in this study. A high percentage of self-thinning (40-50%) occurred and future research could examine ways (e.g. reduce weed competition, modify soil fertility) to maintain higher plant densities up to harvest.

There was a strong temporal match between camelina flowering and the presence of pollinating insects. Sixteen different insect taxa were collected suggesting that camelina attracts and supports a wide variety of pollinators. Thus, camelina could benefit the environment by providing forage resources for native and non-native pollinators, many of which are in decline due to factors such as colony collapse disorder or habitat fragmentation.

Chapter 3. Persistence of Camelina Seed in Seed Banks in Connecticut

Introduction

The soil seed bank is important when considering the persistence of crop species as weeds in the agricultural environment, understanding plant population genetics, or measuring gene flow (Legere, 2005; Thompson et al., 1993; Walsh et al., 2013). This is because dormant seeds in the soil seed bank can act as a vital bridge between generations and allow new populations to develop years after the species has been lost from that location. Most of the world's noxious and destructive weeds have a viable seed bank that allows them to persist (Thompson et al., 1993).

Many factors impact the length of time that seeds can remain in the seed bank. Some of these factors include seed size, shape, and the vertical distribution of the seeds in the soil (Bekker et al., 1998; Thompson et al., 1993). Studies have found that seeds that are larger and deeper in the soil have greater longevity or viability in the soil seed bank. There are two categories of seed banks: transient and persistent. Seeds in a transient seed bank only persist for a maximum of one year before either germinating or losing viability. Persistent seed banks contain seeds that are one-year-old or greater and these species are often associated with a secondary seed dormancy (Thompson and Grime, 1979). Crop species tend to have transient seed banks rather than

persistent seed banks, and they are more likely to have primary dormancy rather than secondary dormancy (Warwick and Stewart, 2005).

Camelina has received attention as a successful low-input crop that can grow well on marginal agricultural land (Putnam et al., 1993). Marginal lands are lands that have relatively poor water supply, poor soil quality, pollution, or excessive slopes for most agricultural practices. Camelina is adaptable and can be grown various conditions. It also possesses some traits commonly associated with weed species such as prolific seed production, the ability to grow on infertile soils, and no requirement for specialist pollinators.

Another common characteristic of weedy species is persistence in the seed bank. However, little information is available about the persistence of camelina seed in the soil seed bank. Some reports suggest that seed dormancy is absent in camelina, but most of them provide no evidence to support this claim (Ehrensing and Guy, 2008; Fleenor, 2011; Gugel and Falk, 2006; Hunter and Roth, 2010; Robinson, 1987). There are only two experiments on seed dormancy in camelina (Davis, 2010; Walsh et al., 2013). One study in western Canada showed 50% seed decay after 25-66 days and 99% seed decay after 161-438 days depending on the depth and location of camelina seed burial (Walsh et al., 2013). This suggested that camelina is unlikely to be weedy or invasive in agricultural areas due to a lack of persistence in the soil seed bank (Walsh et al., 2013). However, this study tested only two camelina cultivars (Calena and CN101985). Overall, both studies concluded that camelina seeds do not persist in the soil seed bank (Davis, 2010; Walsh et al., 2013). However, differences might be observed in other cultivars and genetic lines.

Several species closely related to camelina have shown potential for primary and secondary seed dormancy. *Brassica napus* (canola, rapeseed) has shown signs of seed

dormancy. When canola fruits shatter naturally they release seeds that show very little dormancy. However, buried seeds can enter secondary dormancy (Munier et al., 2012). Seed dormancy has also been demonstrated in *Capsella bursa-pastoris* (Shepherd's purse), a plant in the same taxonomic tribe as camelina. Secondary dormancy rates as high as 39% were observed in *Capsella* (Toorop et al., 2012). Secondary dormancy was much stronger than the primary dormancy and occurred easily in many of the seeds (Toorop et al., 2012). If plant species closely related to camelina have primary and secondary dormancy, perhaps some cultivars and genetic lines might show these traits.

The goal of this experiment was to determine if camelina 'SO-40' could persist in the soil seed bank over the winter in Connecticut. If camelina seeds persist in the seed bank over the winter, there would be a higher potential for weediness. Although it is possible that certain varieties or genetic lines could over-winter as plants aboveground (act as winter annuals), the existence of viable seeds in the soil would suggest that camelina has some chance of establishing volunteer or weedy populations over time.

Materials and Methods

Three replicate plots were arranged in a field at the University of Connecticut Plant Science Research Farm in Storrs, Connecticut during Fall 2014. The farm is within the Level III 59 Northeastern Coastal Zone and Level IV 59c Southern New England Coastal Plains and Hills ecoregion (US Environmental Protection Agency, https://archive.epa.gov/wed/ecoregions/web/html/new_eng_eco.html, Last accessed June 23, 2016). The plot had a 3-8% West to East slope with a Paxton and Montauk fine sandy loam soil type with a soil pH of 5.8 (http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx).

The experimental design consisted of 100 seeds of *Camelina sativa* (L.) Crantz 'SO-40' (Sustainable Oils, California, USA) in 36 mesh bags mixed with 5 grams of sand. Sand was mixed with the seeds in the mesh bags to provide separation between the seeds and better water drainage. The seed bags were buried in the field at two depths of 3 cm or 10 cm. A protective covering made of wood and poultry wire was placed over each plot to deter any damage from animals such as birds. Every month throughout the winter period (November-April), three bags from each burial depth were to be exhumed and seeds analyzed for germination and viability.

Two methods were used to test the seeds for viability. The first test was to take half of the seeds (50 seeds) out of the exhumed bag and run a germination test. This test consisted of placing seeds just below the surface of growing media (Fafard 3B Mix, Sun Gro Horticulture, Agawam, MA) in a 13 cm potting container. The pots were then placed in a mist chamber at the Floriculture Greenhouse at the University of Connecticut. After one week, the number of germinated plants was counted and a percent germination was calculated out of the 50 seeds. A plant was considered to have germinated when a hypocotyl with two cotyledon appeared above the media surface. The second test used to determine seed viability was the tetrazolium (TZ) assay that is often used in addition to germination tests. Fifty seeds were removed from each of the exhumed bags and stained using a 1% tetrazolium solution made from 2,3,5 triphenyl tetrazolium chloride. Seeds were first soaked in a 10% bleach solution for 10 minutes. Seeds were then incubated with 1% tetrazolium solution for 48 hours in the dark and rinsed three times with distilled water (Verma et al., 2013). Seeds were observed using a light microscope and those that turned red (due to the production of formazan) were deemed to be respiring and therefore viable. This germination and viability procedure was to be repeated once every month as seeds were exhumed from the burial location.

Results and Discussion

Seed bags were collected at after the first month of burial (November). When the bags were opened, only seed coats and the remnants of plant roots were observed. No intact seeds were retrieved from the bags meaning that the tetrazolium (TZ) viability test could not be conducted. The germination test could be was conducted by removing all materials from the buried mesh bags and placing them in the pots. No seed germination was recorded from the contents of any of the exhumed bags. In order to confirm these results, several more bags were prematurely removed from the soil plots. The contents of these bags were examined, but no intact seeds were identified and no germination was observed.

Although this was an unexpected result, it was informative. The presence of only seed coats and plant roots suggested that the seeds germinated and then died when they could not reach the soil surface. This is consistent with a few other reports that camelina has no seed dormancy (Fleenor, 2011; Walsh et al., 2013).

One limitation in this experiment was that only one cultivar of camelina (SO-40) was studied. 'SO-40' is a variety which is typically sown in the spring. In contrast, other camelina varieties are reported to grow as winter annuals. These lines should be studied for the presence of transient seed dormancy. The closely-related species, *Brassica napus*, provides evidence that different varieties of the same species can exhibit differences in seed dormancy (Legere, 2005). These differences in dormancy can cause differences in seed persistence in the soil. In addition, a closely-related weed species, *Capsella bursa-pastoris*, has shown dormancy as high as 39% in some varieties (Toorop et al., 2012). This leads to speculation about whether certain cultivars or varieties of camelina might express some form of primary or secondary dormancy.

The lack of dormancy in camelina ‘SO-40’ suggests that long term persistence of camelina as a weed population is unlikely through a transient seed bank. Further experiments are needed to determine the role of seed banks as a bridge for camelina populations to escape cultivation (Legere, 2005).

Conclusions

Seeds of Camelina ‘SO-40’ were not able to survive burial for one month. It is likely that the seeds germinated and died in the bags. Thus, seed banks are unlikely to provide a mechanism for camelina to escape cultivation and become a weed.

Chapter 4. Summary of Results and Future Directions

The body of literature about camelina production practices, reproductive biology, ecology, and gene flow is relatively small. This is particularly true for the Northeastern United States because most previous studies have been conducted in the western United States, Canada, or Europe. However, a substantial body of research has been produced on camelina biotechnology and genetic modification for biofuels and other valuable compounds.

This study provided new information about camelina growth, development, yield, and reproductive biology in the Northeastern United States. This was the first study in this region. The production methods were typical of low-input agriculture (no herbicides, pesticides, or irrigation) although fertilizers were applied prior to seeding. Results in two years (2014 and 2016) showed that camelina can produce substantial yields (>1,000 kg/ha) in the Northeastern US similar to other parts of the globe. However, results from 2015 showed that low rainfall and high temperatures after seeding can lead to poor seed germination, crop development, and competition with weeds. Thus, irrigation could be beneficial when there is little rainfall at the beginning of the growing season.

In years with favorable conditions for germination and growth, camelina produced a good seed yield without weed management. However, this competitive ability was reduced under hot, dry conditions with poor camelina seed germination and seedling development. A seed bank study showed that the seeds of Camelina ‘SO-40’ lacked dormancy and did not overwinter or persist in the seed bank. This reduces the likelihood that camelina could become an agricultural weed or a volunteer in fields. However, future studies should investigate the possibility that ‘SO-40’ seeds can germinate in the fall and overwinter as plants.

New information was obtained about the insect families that visit camelina flowers and have the potential to contribute to pollen-mediated gene flow. Insects from 16 different families of pollinators were collected from the flowers of camelina. The most abundant species was *Apis mellifera* (honey bee) although syrphid flies were also abundant. The attraction of pollinators to the crop suggests that it can provide important resources such as nectar and pollen. Further studies are needed to identify the resources used by each pollinator species.

Further studies are needed to understand the implications of introducing camelina as a novel or genetically engineered crop. Despite the handful of experiments over the past decade, camelina biology and ecology is poorly understood. Of particular interest is the role of insects in camelina pollination. These experiments confirmed that a diverse group of insect taxa visit the flowers of camelina (Eberle et al., 2015; Groeneveld and Klein, 2013). However, it is not known if the insects visit to consume the nectar and pollen produced by camelina or it they contribute to pollination and gene flow. One experiment that needs to be conducted is the identification and quantification of camelina pollen on the bodies of pollinating insects. This would shed light on the potential of these insects to actually transport pollen from over space and time. This study could be done through a procedure called pollen acetolysis (Jones, 2014). In this laboratory procedure the insect is destroyed leaving only the pollen grains that were on or in the insects. This could be a critical next step in understanding the ecology and pollination of camelina. Additional studies are warranted because long-distance pollen dispersal could negatively affect the production and marketing of organic or conventional camelina.

Future studies should focus on the competitive ability of camelina in agricultural fields. If camelina is not highly competitive with agricultural weeds, then there is a smaller chance of it becoming a weed in natural or disturbed areas. One exception to this scenario would be if an

herbicide resistance trait was introduced to the camelina genome. In that case, herbicide-resistant camelina would be competitive under selection pressure with herbicides. This phenomena has been observed in herbicide-resistant canola that occur as widespread weeds. A small greenhouse study on the competitive ability of camelina with a common agricultural weed *Setaria faberi* (giant foxtail) was conducted for this thesis. However, greenhouse conditions in the winter of 2015-2016 could not be optimized to successfully grow camelina and giant foxtail in pots. Thus, the experiment was terminated. The potential for camelina to persist as a winter annual has not been determined for the Northeastern region. Thus, a variety of camelina genotypes should be tested over multiple years with different winter temperatures to determine their persistence. Experiments should be conducted to determine the synchrony of flowering between camelina and closely-related weeds. This would help determine the potential for crop-to-weed gene flow. Species that do not have synchronous flowering would not be able to participate in pollen-mediated gene flow. Finally, pollen viability and longevity needs to be assessed. The longer the pollen of camelina remains viable, the greater the risk of pollen-mediated gene flow in the landscape by either insects or wind.

In summary, these experiments have provided new knowledge about the camelina biology, ecology, and potential as a weed species. These results will help government regulators, farmers, environmental managers, and companies make decisions regarding the future use of camelina with novel traits. In addition, these experiments have contributed important baseline information for future risk assessment and gene flow research.

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