THESIS

EVALUATION OF FACTORS THAT CONTRIBUTE TO THE EXPANSION AND CONTROL OF BRASSICA INSECT PESTS IN NORTHERN COLORADO

Submitted by

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ABSTRACT

EVALUATION OF FACTORS THAT CONTRIBUTE TO THE EXPANSION AND CONTROL OF BRASSICA INSECT PESTS IN NORTHERN COLORADO

Human modifications to landscapes in agricultural areas can both eliminate and create new habitat for various organisms. One of the ways that sites are modified is by creating changes in plant communities. These can occur from physical disturbances from activities such as plowing or road building. Introduction and spread of non-native plants also greatly affects the types of plant communities common both within and outside of agricultural fields.

The first part of this study consisted of an arthropod fauna survey on three non-native winter annual or perennial brassicaceous weeds that are presently common in disturbed sites in and around agricultural areas of Colorado: blue mustard, Chorispora tenella (L.), flixweed, Descurainia sophia (L.), and whitetop/hoary cress, Cardaria draba (L.). This survey was performed to see what insects, harmful and beneficial, might use them as bridge habitat between the winter and the growing season. Multiple locations where these plants are grown (typically in agricultural areas and disturbed sites) were chosen and were visited once a week during spring 2016 and 2017 and sampled with a sweep net. It was found that though flixweed supported the most genera, whitetop had the highest relative diversity in terms of both number of genera and evenness of numbers found. Blue mustard lacked in insect diversity. The most common phytophagous insects included false chinch bug (Nysius spp.), pale legume bug (Lygus elisus Van Duzee), diamondback moth, Plutella xylostella (L.) and western black flea beetle, Phyllotreta pusilla Horn. The most common natural enemy species present on the plants were parasitoid wasps and lady beetles though these and other beneficial insects were found in small numbers. It was concluded that these three brassicaceous weeds can might act as important ecological bridges for phytophagous insects.

In part two of this study a test was conducted on the effects of supplemental insect food and wintergreen oil, which attract certain natural enemies, to reduce damage by certain lepidopteran pests associated with brassica crops. Plantings of broccoli, cabbage, and kale were sprayed weekly with the test treatments of supplemental insect food and supplemental insect food plus wintergreen oil. Weekly surveys of the plot were made to count numbers of imported cabbageworm, Pieris rapae (L.) and cabbage looper, Trichoplusia ni (Hübner) present and to note the presence of any natural enemy species. Harvest assessments were made of leaf injury to cabbage and the number of caterpillars present/head in broccoli. There were no significant statistical differences between treatments in regards to the number of insect pests present on plants or the injury produced.

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Off-field and non-crop plants, including weeds, can have great impacts on the incidence of both pest and beneficial arthropods affecting crops (Alhmedi et al., 2011; Gurr et al., 2003; Lavandero et al., 2006; Norris and Kogan, 2005; Rodriguez-Saona et al., 2012). These effects of these off-field plants may have direct or indirect, negative and/or positive effects on insects present within agroecosystems (Gols et al., 2008, Rafay et al., 2014).

Many insects have evolved with strong relationships to particular plants (Capinera, 2005). Sometimes native plants may serve as a source of ecological movement or invasion in which pests shift from an unimportant plant to economically important hosts (Sim et al., 2017). For example, several of the most important crop pests that occur in North America are native species that have moved from non-crop host plants to cultivated crops. One of the best known examples involves the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), which was originally associated with the buffalobur, *Solanum rostratum* (Dunal), but was found moving onto potato plants by the 1850s (Wang et al., 2017). The original host of the apple maggot, *Rhagoletis pomonella* (Walsh), was hawthorn (*Crataegus* spp.) but some populations shifted to develop on the larger fruit of the apple in the mid-1800s (Sim et al., 2017). A further example of host range shift from native to cultivated plants involved the boll weevil, *Anthonomus grandis* (Boheman), a well-known pest of cotton, *Gossypium hirsutum* (L.). The boll weevil originally used *Hampea* sp., a malvaceous plant related to cotton that is native to Mexico, before moving into cotton fields early in the domestication of the crop (Pimenta et al., 2016).

In some agroecosystems, weeds may serve as alternative hosts of pests both within and external to plots and fields (Norris and Kogan, 2005). When arthropods have a wide host range they can sometimes move from weeds to crop plants causing varying amounts of crop damage (Capinera, 2005). For example, spotted-wing drosophila, *Drosophila suzukii* (Matsumura), a serious pest of small fruit crops, has been shown to develop on a wide range of fruiting plants, including many plants grown for ornamental purposes (Arno et al., 2016; Castro-Sosa et al., 2017). *Lygus* spp. plant bugs are well known for utilizing multiple plant hosts, including volunteer alfalfa, *Medicago sativa* (L.), and lambsquarters, *Chenopodium album* (L.), in the western United States (Demirel et al., 2005; Fye, 1982; Hagler et al., 2016; Mueller et al., 2005). Populations of potato leafhopper, *Empoasca fabae* (Harris), in the eastern United States build populations on a wide range of fabaceous host plants from which they later migrated into potato (Lamp et al., 1994). Both the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and the northern corn rootworm, *Diabrotica barberi* Smith & Lawrence, may use wild grasses as temporary hosts until maize is available (Oyediran et al., 2008; Oyediran et al., 2004). The onion thrips, *Thrips tabaci* (Lindeman), has been shown to utilize field bindweed, *Convolvulus arvensis* (L.), and redroot pigweed, *Amaranthus retroflexus* (L.), if onions are unavailable. Another type of thrips, the western flower thrips, *Frankliniella occidentalis* (Pergande), has been known to utilize evergreen and early flowering plants around orchards for shelter and food during the winter and early spring (Pearsall and Myers, 2000). The potato aphid, *Macrosiphum euphorbiae* (Thomas), will feed on hairy nightshade, *Solanum sarrachoides* (Sendtner), when potatoes are unavailable and might even be a superior food source for aphid reproduction (Srinivasan et al., 2008). Though the European corn borer, *Ostrinia nubilalis* (Hübner) is a wellknown pest of maize, it has a very wide host range that includes many cultivated plants and some that are considered weeds, such as mugwort, *Artemisia vulgaris* (L.), hops, *Humulus lupulus* (L.), and hemp, *Cannabis sativa* (L.) (Brindley and Dicke, 1963; Calcagno et al., 2007).

Non-crops can also enhance the survivability and effectiveness of beneficial natural enemies. While often associated with pests, weeds and non-crop plants can also be helpful in that they provide alternate habitat and food sources for natural enemies. Using a new host plant may increase food availability and alter the vulnerability of herbivores to their natural enemies (Sarfraz et al., 2009). Many weeds and non-crop plants provide pollen as well as shelter and alternative areas for hosts of parasitoid wasps (Alhmedi et al., 2011; Gols et al., 2008; Kloen and Altieri, 1990; Lavandero et al., 2006; Norris and Kogan, 2005). Syrphid flies also utilize noncrop and off-field plants in a similar manner to parasitoid wasps; as a source of pollen and oviposition location (Alhmedi et al., 2011; Gurr et al., 2003; Kloen and Altieri, 1990). Chrysopids will utilize both the pollen and nectar of non-crop plants and as a potential early source for preferred prey (Alhmedi et al., 2011; Rodriguez-Saona et al., 2012). Many lady beetles (Coccinellidae) often rely on the pollen and nectar provided by non-crop plants as a source of nutrition for survival during times or prey scarcity (Alhmedi et al., 2011; de Castro-Guedes et al., 2016; Grevstad and Klepetka, 1992; Gurr et al., 2003). The use of "beetle banks", strips of grasses planted around or in farms and gardens, in the United Kingdom has shown that these grasses provide important overwintering habitat necessary for the survival of polyphagous predatory carabid beetles (Collins et al., 2003; Lys and Nentwig, 1994). Even spiders are known to utilize non-crop plants as both physical shelter and alternative hunting ground for prey items (Kerzicnik et al., 2013; Lys and Nentwig, 1994; Mukhtar et al., 2012).

Studies have shown that some weeds and non-crop plants, notably annual and perennial species that survive over the winter can provide "green bridges" between cropping and noncropping periods for pests and other arthropods thereby allowing early access to areas containing preferred plants (den Belder et al. 2008; Chen et al., 2009). *Lygus* spp. in California are known to utilize volunteer alfalfa, *Medicago sativa* (L)., yarrow, *Achillea millefolium* (L.), and tarweed (*Hemizonia* spp.) as overwintering hosts, for both shelter and food during the winter season (Mueller et al., 2005). The brown marmorated stink, *Halyomorpha halys* (Stal), is a highly polyphagous and mobile pest insect capable of utilizing both perennial and annual plants and trees as food and shelter, giving it a broad overwintering host range (Lee and Leskey, 2015). The blueberry maggot, *Rhagoletis mendax* (Curran), has been known to utilize a variety weeds as overwintering shelter before returning to blueberry when it's mature enough (Norris and Kogan, 2005). Some weeds are known to be overwintering sites acting as shelter for grasshopper eggs, and as early season sources of food (Capinera, 2005). As stated previously, the onion thrips is capable of utilizing multiple hosts for food, but it may also utilize these same plants as a form of shelter during the winter (Schwartz et al., 2014). The rice water weevil, *Lissorhoptrus oryzophilus* (Kuschel), will use dallisgrass, *Paspalum dilatatum* (Poir), and other weeds for protection during the winter until rice has been planted (Norris and Kogan, 2005).

Some insect pests of cultivated brassicaceous crops use wild and weedy hosts to provide a "green bridge" or bridge habitat that allows survival between growing seasons. Flea beetles (Chrysomelidae) are known to use a number of wild brassicaceous plants as overwintering habitat, including plants such as charlock mustard, *Sinapis arvensis* (L.) and white mustard *Sinapis alba* (L.) (Andersen et al., 2006; Kloen and Altieri, 1990). The diamondback moth is known to utilize flixweed and spider flower, *Cleome hassleriana* (Chodat), as bridge habitat between the winter and spring planting seasons (Niu et al., 2014;

Talekar and Shelton, 1993). Lygus bugs have been shown to utilize a number of different wild brassicaceous plants, such as flixweed, black mustard, *Brassica nigra* (L.) and shortpod mustard, *Hirschfeldia incana* (L.) as alternative bridge habitat for shelter, food, and oviposition (Demirel et al., 2005; Mueller et al., 2005). Shepards' purse, *Capsella bursa-pastoris* (L.) and field pennycress, *Thlaspi arvense* (L.) are both wild annual mustards that may act as reservoirs for the Swede midge, *Contarinia nasturtii* (Kieffer) both during cropping and non-cropping periods (Chen et al., 2009).

Crops in the family Brassicaceae (commonly referred to as mustards, crucifers, cabbage family plants and, for many species, simply brassicas) have a very long history of use by humans as animal feed, condiments, vegetables used in human diets (Mitich, 1996) and as a source of edible and industrial oils (Lamb, 1989). The earliest evidence of brassica cultivation dates back to 3,000 BCE to the ancient Sumerian civilization with the farming of *Brassica oleracea* (L.) (Mikic, 2018). *Brassica oleracea*, has been very intensively modified by humans and is the source of crops that include kale, cabbage, Brussels sprouts, cauliflower, broccoli, collards, and kohlrabi. Independently, brassica cultivation developed in China, where Chinese cabbage,*Brassica rapa* (L.) subsp. *pekinensis* (Lour) has been grown for over 1,600 years (Sun, 2015). More recently, oilseed brassicas have become increasingly important agricultural crops, notably canola. These are derived from one or more brassicaceous species, including *Brassica napus* (L.), *Brassica. campestris* (L.), *Brassica juncea* (L.).

Brassicaceae have also been used extensively on vegetation diversity studies and pest research (Altieri and Gliessman, 1983). Plants in the family Brassicaceae produce defense related chemical compounds known as glucosinates (Gols et al., 2008). After a plant has tissue damage, myrosinases catalyze the hydrolysis of glucosinates into (iso)thiocyanates and nitriles that assist in the defense against herbivorous insects (Gols et al., 2008). Glucosinates are antifeedants for many polyphagous insects but most pests of Brassicacae crops use glucosinates as attractants or feeding stimulants (Lamb, 1989). Some insects attracted by glucosinate compounds include certain flea beetles in the genus *Phyllotreta* (Kloen and Altieri, 1990; Tansey et al., 2015), some seed bugs of the genus *Nysius* (Demirel and Cranshaw, 2006; Pivnick et al., 1991), certain aphids (Lamb, 1989), diamondback moth, *Plutella xylostella* (L.), and imported cabbageworm, *Pieris rapae* (L.) (Gols et al., 2008).

Generalist herbivores are usually more sensitive to high levels of glucosinates than specialist insects. This is because generalists tend to produce enzymes that can break down a wide range of allelochemicals, whereas specialists have evolved to break down specific plant compounds that are associated with their diet (Gols et al., 2008). However, there are some generalist herbivores, such as certain *Lygus* spp., that are commonly associated with Brassicaceae (Cripps et al., 2006a; Cripps et al., 2006b; Lamb, 1989).

There are 37 genera of Brassicaceae endemic to North America, comprising of over 600 species, most of which are primarily found in the western regions of North America (Cripps et al., 2006b). In addition, there have been several species that have been introduced, both purposefully and accidentally, including several that are now considered to be important weeds. In the Great Plains area, that includes eastern Colorado, very commonly encountered brassicaceous weeds include flixweed, *Descurainia sophia* (L.) (Demirel et al., 2005; Mitich, 1996; Ritter et al., 2010), whitetop, *Cardaria draba* (L.) (Cripps et al., 2006a; Cripps et al., 2006b; Puliafico et al., 2008), and blue mustard, *Chorispora tenella* (L.), also known as purple mustard (Derksen et al., 2002; Schwartz et al., 2014; Swan, 1971).

Blue mustard is a broad-leaved annual mustard originally from Eurasia and its highly adaptive nature has allowed it to invade North America (Derksen et al., 2002; Swan, 1971).

Part of the reason it is so adaptive is because it can make use of soil lacking certain nutrients, such as phosphorus (Yin et al., 2005). It is known to be a competitor with winter wheat crops (Swan, 1971) and in Colorado is a common plant found growing in disturbed areas of roadsides. It grows as a winter annual and is often among the very first herbaceous plants to flower in spring.

Flixweed is another winter annual in Colorado, native to Western Asia and Africa, which has been in North America since at least the 1700s (Derksen et al., 2002; Mitich, 1996). Flixweed normally survives the winter as a rosette on the soil surface, and in the spring grows rapidly, sometimes developing up to 15 branches and flowers in late spring (Mitich, 1996). It is common along roadsides, but is also present in some crops, notably alfalfa, and has developed resistance to some herbicides (Landau et al., 2017).

Whitetop, also known as hoary cress, is a perennial mustard originally from Western and Central Asia and the Mediterranean and Eastern regions of Europe that was introduced to North America in the 1800s (Cripps et al., 2006b) (Puliafico et al., 2008). It is known for invading areas disturbed by human activity and may reproduce through both rhizomes and seeds (Cripps et al., 2006b). This plant is considered a Class B noxious weed species by the Colorado Department of Agriculture and is presently spreading rapidly across the state (Colorado.gov 2018). The insects associated with whitetop are well known in Europe, but have not been as well studied in the United States (Cripps et al., 2006a; Cripps et al., 2006b), and remain essentially unstudied in Colorado.

Weed plants are often considered most important as competitors with crops for nutrients, water and sunlight, but some may also help maintain populations of certain arthropods including some that are crop pests. They may also act as an alternative food source for pests, reducing the likelihood of a major pest attack on crops (Rafay et al., 2014). A greater understanding of the overwintering and pre-crop fauna that might use invasive weeds as "bridges" could lead to a better understanding of the role weeds play in agro ecosystems (Rafay et al., 2014) and their potential role in supporting certain pest and beneficial arthropods (Kloen and Altieri, 1990; Puliafico et al., 2008; Sarfraz et al., 2009). Understanding these communities could help develop more efficient and well informed integrated pest management strategies regarding these plants in the Front Range of Colorado.

This study had three primary objectives. The first was to determine what arthropod taxa comprise the communities on whitetop, flixweed, and blue mustard in the region. The next was to see which of these specific individual plant communities had the most relative diversity, determine what the most abundant insects in these communities were, and how their abundance varies between the different plants. The third objective was to assess the importance of these three invasive weeds in providing habitat for pest insects and their natural enemies the bridge the period between growing seasons of cultivated brassicas.

Materials and Methods

Study sites involved areas found to support large patches of one or more of the target host weeds (blue mustard, flixweed, whitetop). Most of these were located in areas disturbed by human activity, such as waste areas along roadsides and edges of farm fields where these weeds are particularly well adapted (Cripps et al., 2006a; Demirel et al., 2005). All were located within Larimer County, Colorado.

In 2016, 26 field sites were included in field surveys. Surveys began on 4 April. Collections were made weekly, weather permitting. Each of the different hosts were sampled from the point when they were large enough to sample with a sweep net. In 2016, sampling started with blue mustard on 7 April and collection continued until the plants died or deflowered on10 June. In 2017, 27 sites were included in the survey with sampling beginning on 17 April and ending on 27 June.

Specimens were collected with a standard steel frame sweep net with a muslin bag of 34.3 cm diameter. At each site, collections were made using between 10-30 sweeps (Koch et al., 2016), sweeping the net back and forth from left to right (with each sweep on each side counting as one sweep), 180^{o}, then right to left (Ritter et al., 2010). Individual sweeps were spaced so that there was minimal disturbance during sampling of the sampled area. The different number of sweeps used at a site were based on the size of the plant patches.

Collected specimens transferred from the collecting net to a plastic bag then returned to the lab where they were immediately placed in a freezer for storage. These were later removed for identification and enumeration. Identifications were made to the species level where possible. Due to the stage or condition of some of the samples, only genus or family level identifications were possible. Upon identification specimens were stored in vials with a 75% alcohol solution.

To establish if there were difference in the species diversity found on the three targeted host plants, a Shannon-Weiner diversity index was calculated. This is one of several methods used to quantify species diversity and measures both species diversity and the evenness of the distribution of the genera. This index uses the calculation $H=-\sum(pi \times \ln pi[pi=ni/N)$ where pi (the proportion of species) is calculated by dividing *ni* (the number of specimens from each taxa) by N (the total number of collected specimens) multiplied by the natural log. $\sum$ is the sum of the calculations and *H* is the biodiversity index calculated (Chalcraft et al., 2009; Li et al., 2017; Mhlanga et al., 2015).

Results

Field Season 2016

During the 2016 field season, a total of 59 samples were collected at the 26 sites. Among all collections, a total of 34 different arthropods were identified (with some only to the family level) representing 26 families in 9 orders (Tables 1-3). Overall, the most abundant phytophagous insects in this field season were false chinch bugs, *Nysius* spp., pale legume bugs, *Lygus elisus* (Van Duzee) and the seed bug *Melacoryphus lateralis* (Dallas). Wasps in the family Braconidae were the most abundant of the natural enemy species present in samples.

On blue mustard (Table 1), false chinch bugs were the most abundant phytophagous insect collected. Other potential pest species found were western black flea beetles, *Phyllotreta pusilla* (Horn), diamondback moths, *Plutella xylostella* (L.), and pale legume bugs. Among natural enemies only a very low number of braconid wasps were recovered. No bees or other notable pollinators were found in the samples.

On flixweed (Table 2), very high numbers of false chinch bugs, and pale legume bugs were collected. The diamondback moth was present in low numbers as was the western tarnished plant bug *Lygus hesperus* (Knight). No western black flea beetles were collected. The seed bug *Melacoryphus lateralis* Dallas, an insect native to the western US that feeds on a variety of vegetation and seeds, was commonly collected. Among natural enemy species a *Zelus* sp. assassin bug and the convergent lady beetle, *Hippodamia convergens* (Guérin-Méneville), were the most abundant predators. Three families of parasitoid wasps were also collected during this study with the most common being braconid wasps. No notable pollinators were found amongst flixweed in the 2016 season.

On whitetop (Table 3), the most abundant phytophagous insects were false chinch bugs, pale legume bugs, and the western black flea beetles. The most common predators or parasitoids found in whitetop were convergent lady beetles and braconid wasps. Aside from a few syrphid flies, no pollinator species were collected in whitetop patches during the 2016 field season.

A Shannon-Weiner index calculated for the insects collected on these plants in 2016 was slightly higher (1.36) on whitetop compared to flixweed (1.14) and blue mustard (0.89). These are all values that indicate moderately low diversity. The somewhat greater diversity of insects present on whitetop may be related to its more prolific and later flowering in comparison to blue mustard and flixweed. The highest number total insects were recovered from flixweed, but these were heavily skewed by the presence of large populations of false chinch bugs and pale legume bugs. These decrease the evenness of the recorded taxa, which is considered in the ShannonWeiner index. The earliest flowering and maturing species, blue mustard, showed the lowest diversity in this season.

Field Season 2017

During 2017, a total of 131 collections were made at 27 sites. A total of 39 arthropods were identified down to either the genus or species level representing 2 classes, 8 orders, and 34 families (Tables 1-3). By far the most abundant phytophagous insects collected during the 2017 field season were false chinch bugs, followed by western black flea beetles and diamondback moth larvae. The most abundant predator was the convergent lady beetle. A variety of parasitoid wasps were collected as well. In contrast to 2016, bees were collected during this field season.

In the arthropod community associated with blue mustard (Table 1), the most abundant phytophagous arthropod was the false chinch bug followed by the western black flea beetle and *Melanoplus* spp. grasshoppers. The most commonly occurring predators or parasitoids were ichnuemonid wasps and a variety of syrphid flies. A few halictid bees were collected in the genera *Halictus* and *Lasioglossum*.

False chinch bugs were again the most abundant phytophagous arthropod on flixweed (Table 2) and was often present in very high numbers. Other phytophagous species noted were western black beetles the diamondback moths. Numbers of *Lygus* spp. collected were much lower than in 2016. The most abundant predators and parasitoids included convergent lady beetles, various carabid beetles and ichneumonid wasps. A few pollinator species were collected including *Hylaeus* spp. of the colletid bees, syrphid flies, and honey bees, *Apis mellifera* L.

False chinch bugs were also the most common insect associated with whitetop (Table 3) although in lower numbers than were found on flixweed. The other most common phytophagous species collected included western flea beetle, diamondback moth and *Melanoplus* spp. grasshoppers. The most commonly collected predatory arthropods were the convergent lady beetle and the insidious flower bug, *Orius insidiosus* (Say). Three families of bees were collected from whitetop in 2017 including the honey bee, haliticids in the genus *Lasioglossum* spp., and colletids in the genus *Hylaeus* spp.

A Shannon-Weiner index indicated that in the 2017 field season both whitetop (2.18) and purple mustard (2.17) had higher diversity of arthropods relative to flixweed (1.06). Again, the total number of insects collected were greatest on flixweed but the evenness of species abundance was highly skewed by the occurrence of very large numbers of a few species (false chinch bugs, western black flea beetles, diamondback moths). Higher values were recorded in 2017 on whitetop 2.18 vs. 1.36) and, particularly, blue mustard (2.17 vs. 0.89), which may be due to the greater period of bloom that extended sampling a couple weeks later in the second study year.

Table 1.1 Arthropods collected during sampling of blue mustard (*Chorispora tenella*), Spring 2016 and 2017, Larimer County, Colorado. During the 2016 field season 18 samples of blue mustard were collected from 9 sites between 12 April and 20 May. During the 2017 field season 48 samples of blue mustard were collected from 17 sites between 17 April and 15 June.

	Total Number Collected	
Taxa (Order, Family, Genus/species)	**2016[a]**	**2017[b]**
Orthoptera		
Acrididae		
Chortophaga viridifasciata (Dee Geer)	1	1
Melanoplus spp.	0	19
Hemiptera		
Aphididae	0	1
Cicadellidae		
Circulifer sp.	0	1
Macrosteles quadrilineatus (Forbes)	0	3
Nabidae		
Nabis alternatus (Parshley)	1	0
Lygaeidae		
Nysius spp.	114	70
Miridae		
Lygus elisus (Van Duzee)	2	3
Orthotylus sp.	1	4
Anthocoridae		
Orius insidiosus (Say)	0	1
Berytidae		
Jalysus spinosus (Say)	1	0
Pentatomidae		
Thyanta custator (McAtee)	0	1
Coleoptera		
Chrysomelidae		
Phyllotreta pusilla (Horn)	12	57

Cassida rubiginosa (Müller)	0	1
Carabidae		
Cicindela sp.	0	1
Amara sp.	0	1
Coccinellidae		
Hippodamia convergens (Guérin-Méneville)	0	1
Neuroptera		
Chrysopidae		
Chrysoperla plorabunda (Fitch)	0	1
Hymenoptera		
Braconidae	2	0
Ichneumonidae	0	4
Platygastridae	0	1
Halictidae		
Halictus sp.	0	5
Lasioglossum sp.	0	10
Formicidae		
Formica spp.	1	0
Lepidoptera		
Plutellidae		
Plutella xylostella (L.)	12	4
Diptera		
Chironomidae		
Chironomus sp.	0	2
Orthocladius sp.	0	2
Asilidae		
Nannocyrtopogon sp.	0	1
Syrphidae		
Paragus sp.	0	3
Eupeodes sp.	0	1
Sphaerophoria sp.	0	1
Toxomerus sp.	0	1
Tephritidae	0	1
Sciaridae	0	1
Araneae		

Salticidae	0	1

[a] Sample totals are derived 18 samples that collected arthropods using a total of 420 sweeps of a sweep net with a muslin bag of 34.3 cm diameter.
[b] Sample totals are derived 48 samples that collected arthropods using a total of 1056 sweeps of a sweep net with a muslin bag of 34.3 cm diameter.

Table 1.2 Arthropods collected during sampling of flixweed (*Descurainia sophia*), Spring 2016 and 2017, Larimer County, Colorado. During the 2016 field season 29 samples of flixweed were collected from 15 sites between 7 April and 10 June. During the 2017 field season 42 samples of flixweed were collected from 13 sites between 14 May and 27 June.

	Total Number Collected	
Taxa (Order, Family, Genus/species)	**2016[a]**	**2017[b]**
Ephemeroptera		
Baetidae		
Callibaetis ferrugineus hadeni (L.)	1	0
Orthoptera		
Acrididae		
Chortophaga viridifasciata (Dee Geer)	1	0
Melanoplus spp.	2	8
Hemiptera		
Reduviidae		
Zelus sp.	11	0
Nabidae		
Nabis alternatus (Parshley)	4	2
Lygaeidae		
Lygaeus kalmii (Stal)	1	0
Melacorphyus lateralis (Dallas)	77	0
Nysius spp.	993	844
Miridae		
Leptoterna sp.	1	0
Lygus elisus (Van Duzee)	470	18
Lygus hesperus (Palisot de Beauvois)	7	0
Orthotylus sp.	7	11
Cicadellidae		
Macrosteles quadrilineatus (Forbes)	0	1
Anthocoridae		

Orius insidiosus (Say)	2	2
Berytidae		
Jalysus spinosus (Say)	0	1
Pentatomidae		
Thyanta custator (McAtee)	0	1
Rhopalidae		
Harmostes reflexus (Say)	0	3
Coleoptera		
Chrysomelidae		
Phyllotreta pusilla (Horn)	0	54
Carabidae		
Cicindela sp.	0	5
Amara sp.	1	5
Pterostichus sp.	0	1
Coccinellidae		
Hippodamia convergens (Guérin-Méneville)	5	6
Neuroptera		
Chrysopidae		
Chrysoperla plorabunda (Fitch)	1	1
Hymenoptera		
Braconidae	7	1
Ichneumonidae	5	5
Platygastridae	0	1
Chalcidoididea	3	0
Platygastridae	0	1
Colletidae		
Hylaeus spp.	2	3
Apidae		
Apis mellifera (L.)	0	1
Formicidae		
Formica spp.	25	9
Tapinoma sessile (Say)	0	1
Lepidoptera		
Plutellidae		
Plutella xylostella (L.)	9	37
Diptera		
Chironomidae		
Chironomus sp.	0	17
Orthocladius sp.	0	2
Bibionidae		
Bibio sp.	2	0
Asilidae		
Nannocyrtopogon sp.	0	2
Sarcophagidae		
Scathophaga stercoraria (L.)	1	1

Sepsidae		
Sepsis sp.	4	2
Syrphidae		
Sphaerophoria sp.	1	1
Toxomerus sp.	0	3
Tephritidae	2	1
Tachinidae	1	0
Araneae		
Salticidae	0	4
Lycosidae	0	1

[a] Sample totals are derived 42 samples that collected arthropods using a total of 862 sweeps of a sweep net with a muslin bag of 34.3 cm diameter.
[b] Sample totals are derived from 29 samples that collected arthropods using a total of 745 sweeps of a sweep net with a muslin bag of 34.3 cm diameter.

Table 1.3 Arthropods collected during sampling of whitetop/hoary cress (*Cardaria draba*), Spring 2016 and Spring 2017, Larimer County, Colorado. During the 2016 field season 11 samples of whitetop were collected from 7 sites between 22 April and 7 June. During the 2017 field season 33 samples of whitetop were collected from 8 sites between 16 May and 27 June.

	Total Number Collected	
Taxa (Order, Family, Genus/species)	**2016**	**2017**
Orthoptera		
Acrididae		
Chortophaga viridifasciata (Dee Geer)	0	4
Melanoplus spp.	0	30
Hemiptera		
Nabidae		
Nabis alternatus (Parshley)	0	2
Lygaeidae		
Melacorphyus lateralis (Dallas)	5	0
Nysius spp.	197	132
Miridae		
Lygus elisus (Van Duzee)	54	4
Lygus hesperus (Knight)	5	1
Cicadellidae		
Draeculacephala sp.	0	1
Anthocoridae		
Orius insidiosus (Say)	2	5
Berytidae		
Jalysus spinosus (Say)	0	1
Pentatomidae		
Thyanta custator (McAtee)	1	0
Rhopalidae		
Harmostes reflexus (Say)	0	1
Coleoptera		
Chrysomelidae		
Phyllotreta pusilla (Horn)	23	73
Acanthoscelides sp.	1	6
Cassida rubiginosa (Müller)	2	1
Coccinellidae		
Coleomegilla maculata (Dee Geer)	1	0
Harmonia axydris (Pallens)	0	1
Hippodamia convergens (Guérin-Méneville)	2	6
Neuroptera		

Chrysopidae		
Chrysoperla plorabunda (Fitch)	0	1
Hymenoptera		
Tenthredinidae		
Euura mucronata (Smith)	1	0
Braconidae	5	0
Ichneumonidae	0	3
Platygastridae	2	0
Chalcidoididea	2	1
Colletidae		
Hylaeus spp.	0	2
Halictidae		
Lasioglossum spp.	0	3
Apidae		
Apis mellifera (L.)	0	6
Formicidae		
Formica spp.	5	1
Tapinoma sessile (Say)	2	0
Trichoptera		
Hydropsychidae		
Cheumatopsyche analis (Banks)	1	0
Lepidoptera		
Noctuidae		
Schinia sp.	1	0
Plutellidae		
Plutella xylostella (L.)	8	35
Diptera		
Chironomidae		
Chironomus sp.	0	8
Bibionidae		
Bibio sp.	0	1
Syrphidae		
Sphaerophoria sp.	1	0
Toxomerus sp.	1	0
Calliphoridae		
Calliphora sp.	0	2
Phormia sp.	1	0
Tephritidae	1	1
Drosophilidae	0	5
Araneae		
Saltcidae	0	4

[a] Sample totals are derived 11 samples that collected arthropods using a total of 289 sweeps of a sweep net with a muslin bag of 34.3 cm diameter.

[b] Sample totals are derived 33 samples that collected arthropods using a total of 696 sweeps of a sweep net with a muslin bag of 34.3 cm diameter.

Discussion

More taxa in general (39 vs. 34) and more predatory arthropods were found in the 2017 field season than in the 2016 field season. Two reasons for this may be a matter of time and location. Collections lasted for a week longer in the 2017 field season, allowing more time for various arthropods to emerge. And, although there was only one more total site in 2017 (27) than in 2016 (26), there were differences in some of the study areas between the season where areas used in 2016 were of poor quality and alternate locations were substituted in 2017. Collecting from other locations may have diversified the arthropod genera caught.

Another reason may be due, in part, to weather differences between the survey periods of the two years. In the 2016 field season, the average high was 23.8 degrees Celsius and the average amount of precipitation was 3.3 centimeters, while in 2017 it was 24.4 degrees Celsius with 4.1centimeters of precipitation, according to data the Colorado Agricultural Meteorological Network (CoAgMet 2017). The Colorado Agricultural Meteorological Network noted that it also rained more often and started earlier in the 2017 field season (CoAgMet 2017). Since arthropods are ectothermic, they are more directly affected by climate changes, notably temperature differences (Buchholz et al., 2013). Changes in climate can alter species range, predation and parasitic behavior (Eigenbrode et al., 2015), and it can even cause metabolic changes in plants (Harvey, 2015). In addition, this earlier influx of precipitation in 2017 may have produced longer and more extensive periods of bloom amongst the studied plants resulting in some differences in numbers and types of insects present during sampling.

Blue mustard is known to provide shelter for pierid butterflies (Chew, 1977) and various thrips (Pearsall and Myers, 2000; Schwartz et al., 2014). While no pierid butterflies were found in this study, blue mustard, in both 2016 and 2017, supported a small population of diamondback moths, another brassica specialist. With the exception of some ichneumonid wasps, few biocontrol agents were found during the surveys. No notable pollinators were collected.

Flixweed has been known to provide shelter for diamondback moth (Niu et al., 2014; Sarfraz et al., 2009; Talekar and Shelton, 1993) and lygus bugs (Demirel et al., 2005; Ritter et al., 2010). In both 2016 and 2017, diamondback moths and lygus bugs were found commonly with the most common *Lygus* sp. in this study being the pale legume bug. False chinch bugs were also found in great abundance. Carabid and coccinellid beetles proved to be among the most abundant predatory insects. A variety of parasitoids were collected as well. With the exception of a honey bee and a few syrphid flies, few pollinators were found.

Whitetop has been reported to host diamondback moth (Cripps et al., 2006b; Puliafico et al., 2008), flea beetles (Puliafico et al., 2008), lygus bugs (Cripps et al., 2006b), and false chinch bugs (Cripps et al., 2006a). All three of these insects were found in moderate to abundant numbers over the course of this study. The minute pirate bug and a variety of coccinellid beetles were the most abundantly collected predatory arthropods. When it comes to pollinators, three different kinds of bees were collected and a couple of syrphid flies.

Few studies have been undertaken with regard to which natural enemies utilize these invasive brassicaceous plants but some studies have shown that certain parasitoids might utilize flixweed to parasitize diamondback moths and lygus bugs (Demirel et al., 2005; Niu et al., 2014; Niu et al., 2015). Blue mustard, in addition to hosting a variety of parasitoid wasps, also appeared to host a variety of syrphid flies as well. Flixweed also contained a variety of parasitoid wasps, as well as carabid and coccinellid beetles. It should be noted that all these predators and parasitoids occurred in small numbers.

Studies have shown that what humans consider weeds can often be useful as both shelter and food for both pest and beneficial arthropods (Gols et al., 2008; Kloen and Altieri, 1990; Rafay et al., 2014). Furthermore studies have shown that weeds often play an important role as bridge habitat between growing seasons while arthropods wait for their preferred plants (Mueller et al., 2005; Niu et al., 2014; Norris and Kogan, 2005; Rafay et al., 2014). The three weeds and their arthropod communities chosen for this study were whitetop, flixweed, and blue mustard. These three weeds appear to have yet to have their insect fauna communities examined and compared side by side in the northern Front Range of Colorado, the southern Rocky Mountains and the Great Plains region of the US.

The survey performed in this study suggests that the non-native weeds surveyed attracted, and acted as ecological bridges for several phytophagous species, including some that are significant pests of cultivated brassicas. However, these plants, in general, did not seem to host many natural enemy species or pollinators. Furthermore, there was a range in the importance of the 3 surveyed weedy mustards as early season hosts for insects associated with brassicaceous crops. The earliest of these, blue mustard, was noted to support relatively few arthropods and numbers of crop pest species were low. Conversely, flixweed supported a more diverse taxa, including large numbers (hundreds) of false chinch bugs and pale legume bugs. This plant likely is very important as a host that provides bridge habitat for those two pests and is also an early season host of western black flea beetle. Whitetop also can be an important overwintering host for false chinch bugs, western black flea beetles, and pale legume bugs.

These plants may also be important in early season populations of natural enemy species. Lady beetles and parasitoid wasps were found in greater abundance on flixweed than the other two plants. Lesser numbers of predators and parasitoids were found on whitetop and blue mustard suggesting these plants provide less value as bridge habitat between growing seasons of cultivated brassicaceous crops.

Growers of cultivated brassicas should consider the locations of these non-crop weedy brassicas when planning plantings of susceptible crops. Species that seem to be heavily utilized by crop pest species and provide early season bridge habitat, such as flixweed, could increase risks to nearby crops. Although this study focused on just 3 winter annual/perennial brassicaceous weeds it did demonstrate the importance of expanding studies of the insect fauna associated with weed species that occur in agricultural settings. The diversity they may bring to agricultural landscapes may have considerable importance in the populations of insects that occur in crops by serving as ecological bridge habitat between growing seasons or as important alternative hosts for both crop pest and natural enemy species.

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CHAPTER 2: EVALUATION OF SUPPLEMETAL FOOD AND ATTRACTANTS OF INSECT NATURAL ENEMIES TO ASSIST MANAGEMENT OF LEPIDOPTERAN PESTS OF CRUCIFER CROPS IN THE NORTHERN FRONT RANGE OF COLORADO

Many arthropods used for biological control of crop damaging insects and mites may have omnivorous habits at some point in their life cycle. This can include nectar, honeydew, and/or pollen which are used by many species, particularly during the adult stage (Messelink et al., 2014; Wade et al., 2008a; Wade et al., 2008b). The diversification of agro-ecosystems with floral plants is commonly advocated to provide essential nutrients and resources to parasitoids and predators (Winkler et al., 2010). Non-crop plants and weeds can not only provide shelter and a place to oviposit for beneficial insects, but during various life stages or times of prey scarcity they can also become another source of food, via feeding on pollen or plant sap (Capinera, 2005; Gurr et al., 2003).

These supplemental food needs may also be provided by applications or use of artificial foods typically derived from protein based foods (yeast, whey, and soy) and sugars (sucrose, pollen and honey mixtures, etc.) that mimic honeydew, protein-based food sprays has been considered a possible means of providing essential nutrition for predators and parasitoids at various life stages (Patt et al., 2012; Wade et al., 2008b). The origins of predator food sprays for arthropods can be traced back to the 1940's-1950's when research was on going into techniques for the laboratory mass rearing of natural enemies (Wade et al., 2008b). Since then, food augmentations has been used to both rear and attract a number of natural enemies. Artificial sources of food appear to be of importance to parasitoids. One 2015 study found that, when provided with a commercial sugar solution, the parasitoid *Aphytis melinus* (DeBach) increased is population size by twofold (Tena et al., 2015). Another study found that when provided with a mix of honey, sugar, and date sugar that *Bracon hebetor* (Say) was exceptionally more fecund and lived longer (Ashraf et al., 2017). The aphid parasitoid *Aphidius ervi* (Haliday) appears to have an increased feeding duration and greater longevity when provided with a glucose-fructose mixture (Azzouz et al., 2004). A 2008 study showed that an artificial source of protein can increase both the fecundity and longevity of the bollworm parasitoid *Ichneumon promissorius* (Erichson) (Wade et al., 2008a).

Lacewings (Neuroptera: Chrysopidae) are one of the most voracious of the entomophagous insect predators used for biological control, with wide distribution and range of diet (Ye et al., 2015). As a species that has alternate food needs in the adult stage (e.g., pollen, nectar, honeydew) they have often been used for research regarding supplemental food sprays. A 2014 study found that an artificial diet high in protein and carbohydrates increased development time, fecundity, and pupal weight of *Chrysoperla externa* (Hagen) (Haramboure et al., 2016). It has been found that an artificial diet consisting of a mixture of pollen and honey increased the fecundity, hatching success, and larval survival of *Mallada boninensis* (Okamoto) when compared to insect only diets (Rahman et al., 2017). A study published in 2015 found that an artificial diet consisting of chicken egg, beer yeast powder, honey, sucrose, trehalose, vitamin C, potassium sorbate, and water increased the development and oviposition rate of the lacewing *Mallada basalis* (Walker) (Ye et al., 2015).

Most coccinellids, also known as lady beetles, prefer to prey on aphids, psyllids and other hemipteran pest insects (Ali et al., 2016). Studies have shown that providing man-made sources of protein could benefit various lady beetles. It has been shown that supplementing the diet of

Propylea japonica (Thunberg) and multicolored Asian lady beetles, *Harmonia axydrids* (Pallas) with a mixture of shrimp, beef, beef liver, and egg yolk led to a much lower pre-oviposition period (Ali et al., 2016). A study published in 2016 using yellow meal worm, *Tenebrio molitor* (L). powder to enhance artificial diets for *Coleomegilla maculata* (De Geer) found that using the insect powder food supplement increased fecundity, egg viability, and larval survival (Rojas et al., 2016). Lady beetles also feed on a variety of sugar sources and frequently visit insectaries, floral and extra-floral, and honeydew coated plant surfaces (Seagraves et al., 2011). Studies since the 1960's have shown an increased number of coccinellid adults in fields associated with sucrose sprays (Ewert and Chiang, 1966). A 2010 study showed that total coccinellid densities of seven spotted lady beetles. *Coccinella septempunctata* (L.), convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, and multicolored Asian lady beetles were 50-77% higher in soybean plots treated with a sugar spray (Seagraves et al., 2011).

Spiders play an important role as part of the predatory arthropod fauna in different ecosystems and are known to play in important role in pest suppression in agriculture. The formulation of artificial diets has increased spider survivability. A combination of milk, egg yolk, and soybean liquid has been shown to increase the survivability of *Hibana velox* (Becker), *Cheiracanthium inclusum* (Hentz), and *Trachelas volutus* (Gertsch) during periods of low prey density (Amalin et al., 2001). A study published in 1999 showed that a mix of sucrose and soybean liquid contained carbohydrates and other sugars attributed to the increased energy and long term survivability of *Hibana velox* (Amalin et al., 1999). In one study a mixture of sucrose, baker's yeast, and soy flour promoted the growth and development of prey limited *Hibana futilis* (Banks) (Patt et al., 2012).

Semiochemicals are a chemical or mixture of chemicals that act as messengers among organisms. This includes chemicals that alter interactions among organisms of the same species (pheromones) and among organisms of different species (allelochemicals) (Rodriguez-Saona et al., 2012). Most plants produce a unique blend of volatile compounds (Rodriguez-Saona et al., 2011) called herbivore induced plant volatiles. Herbivore-induced plant volatiles (HIPVs) are a group of allelochemicals released when herbivores cause damage that elicits a response from plant defense pathways (Mallinger et al., 2011). HIPVs resulting from herbivore-plant interactions play an important role in foraging, oviposition, and orientation processes of predators and parasitoids (Kergunteuil et al., 2012) and it has long been believed that the use of HIPVs in natural or synthetic form could be used to manipulate and increase populations of natural enemies in a particular area (Mallinger et al., 2011). Recent research in biological pest control has examined the possibility of using the qualities of HIPVs to attract natural enemies to protect crops from pests (Rowen et al., 2017).

Plant essential oils (EOs) are a major type of botanical product used for controlling arthropods because they often affect behavior and physiology. Plant EOs have been used to protect crops and stored products from insects for over 150 years (Machial et al., 2010). They have low environmental persistence and mammalian toxicity and are readily available for purchase in large quantities at affordable prices (Zhang et al., 2013). EOs have the potential to be an effective resource as pest control agents as they often contain strong plant volatiles (Kim et al., 2016). Plant volatiles have been shown to both attract and repel insects (McCormick et al., 2014) and have proven to be acutely toxic to a variety of insects. (Machial et al., 2010). One commonly used EO is wintergreen oil, which is extracted from the leaves of *Gaultheria* *fragrantissima* (Wall). The main component of wintergreen oil is methyl salicylate (Kim et al., 2016).

Methyl salicylate (MeSA) is a volatile compound that is commonly emitted from several plant species when they are being fed upon (Rodriguez-Saona et al., 2012) and, when applied to crops, have the potential to increase activity of some natural enemies of phytophagous plant pests (Lee, 2010). MeSA has been shown to attract predatory mites in laboratory studies (James, 2003). Numerous studies have shown that lacewings are attracted to MeSA both in the lab and in the field (Baldacchino et al., 2010; James, 2003; Mallinger et al., 2011; Rodriguez-Saona et al., 2012). Predatory beetles, such as carabid beetles (Lee, 2010), and predatory coccinellids (Lee, 2010; Mallinger et al., 2011; Rodriguez-Saona et al., 2012; Rodriguez-Saona et al., 2011) are also known to be attracted to the compound. Studies have shown that various syrphid flies are also drawn to this volatile compound (Lee, 2010; Mallinger et al., 2011; Rodriguez-Saona et al., 2011; Rowen et al., 2017). MeSA is known to attract various types of parasitoid wasps, some of the most important biological control agents in many agricultural settings (Lee, 2010; Mallinger et al., 2011; Rodriguez-Saona et al., 2012; Rowen et al., 2017). Tachinid flies, another important group of parasitoids, also seem to be attracted to MeSA (Rodriguez-Saona et al., 2011). Hemipteran predators, including the big-eyed bugs (*Geocoris* spp.) and predatory mirids have also been known to show some attraction to MeSA (Rodriguez-Saona et al., 2011). Because of this evidence of natural enemy attraction to MeSA, it is marketed as a method for enhancing biocontrol on a commercial level (Rodriguez-Saona et al., 2012).

In addition to attracting natural enemies, MeSA has also been shown to kill, repel, and trap certain kinds of pests. Studies have shown that the MeSA plant volatile can be used against the northern house mosquito, *Culex pipiens pallens* (L.) to kill and control it (Ma et al., 2014). It has been found that MeSA is toxic to the spotted wing drosophilia *Drosophila suzukii* (Matsumura) (Kim et al., 2016). When used in traps, MeSA was proven to be successful at capturing western yellowjacket, *Vespula pensylvanica* (Saussure) and European paper wasps *Polistes dominulus* (Christ).

MeSA is known to repel various types of aphids including the hop aphid, *Phorodon humuli* (Schrank), the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), and the soybean aphid, *Aphis glycines* (Matsumura) (Lee, 2010; Mallinger et al., 2011; Rodriguez-Saona et al., 2012). MeSA has also been shown to be effective at repelling and limiting the growth of major lepidopteran pests such as the corn ear worm *Helicoverpa zea* (Boddie) and cabbage caterpillars (*Pieris* spp.) (James, 2003; Rodriguez-Saona et al., 2012). Wintergreen oil, of which MeSA is one of the major compounds, when sprayed on traps showed a 60-85% reduction in the number of brown marmorated stink bug, *Halyomorpha halys* Stal, captured in traps (Zhang et al., 2014). Under greenhouse conditions, it has also been shown that MeSA applications to plants can reduce the growth rate of tobacco hornworms *Manduca sexta* (L.) (Rowen et al., 2017).

The focus of this study is to evaluate whether applications of supplemental foods used by natural enemies and/or wintergreen oil can be demonstrated to help in the management of crop pests. Specifically, the study focused on crucifer crops and the two most common lepidopterous pests associated with these crops in Colorado.

Methods and Materials

Treatments evaluated in this trial involved two supplementary insect foods, a standard "Feeding Attractant for Predators" (Rincon-Vitova Insectaries©, Ventura, California) and the same product with the addition of wintergreen oil. The ingredients of these supplemental insect foods included soy flour, calf milk replacer, sucrose, and nutritional yeast.

The study took place at Colorado State University's Agricultural Research, Demonstration and Education Center (ARDEC) South Research Station northeast of Fort Collins, Colorado. Individual plots used in the study consisted of 9 rows spaced 76.2 cm apart and 5.8 m in length. Each plot was subdivided into 3 row blocks where one of three different cultivated brassicas were grown; kale, cabbage, or broccoli. A border of Brussels sprouts was placed along the entirety of the treatment area to reduce field edge effects. Each treatment consistent of four replications (4 plots), which were arranged in a randomized complete block with 4 replications.

Kale	Kale	Kale	Cabbage	Cabbage	Cabbage	Broccoli	Broccoli	Broccoli
W	W	W	W	W	W	W	W	W
F	F	F	F	F	F	F	F	F
C	C	C	C	C	C	C	C	C
W	W	W	W	W	W	W	W	W
C	C	C	C	C	C	C	C	C
F	F	F	F	F	F	F	F	F
F	F	F	F	F	F	F	F	F
C	C	C	C	C	C	C	C	C
W	W	W	W	W	W	W	W	W
W	W	W	W	W	W	W	W	W
C	C	C	C	C	C	C	C	C
F	F	F	F	F	F	F	F	F

5.8m

76.2cm

Figure 2.1 A layout of the randomized block design used in this study. Each individual plot consisted of 9 rows, 3 each of kale, cabbage or broccoli. Rows within plots were 5.8 m long and the total study area of a plot was 52 $m^{2.}$. Plots marked “F” represents plots receiving applications of the supplemental food supplement "Feeding Attractant for Predators" supplied by RinconVitova Insectaries. Plots marked “W” received similar applications of this food supplement to which wintergreen oil was added. Plots marked with “C” represents untreated check plots.

Treatments involved use of supplementary food alone, supplementary food plus wintergreen oil, and an untreated check. Treatments began on the 18 July, 2017 and the last application took place on 6 September. Treatments were applied twice a week, weather permitting, using a hand pumped compressed air Craftsman© sprayer. A mix of two and one third cups insect food to one gallon of water ratio was used. A gallon of this mixture was uniformly applied over all plants of a treatment, an area of 52 m^2. The study area was furrow irrigated on a weekly basis.

Once a week, starting on 24 July 2017, eight plants in each replicate (e.g., 8 plants of kale, cabbage and broccoli) were examined for all phytophagous species. The only species present consistently were lepidopteran pests, specifically the imported cabbageworm, *Pieris rapae* (L.) and the cabbage looper, *Trichoplusia ni* (Hubner). Numbers of eggs, larvae and pupae were recorded during each plant evaluation. Notes on other insect taxa found during the study were also taken.

At harvest, 8 September 2017, evaluations were made to assess whether the treatments affected any injury produced by the lepidopteran larvae that were present on the plots. Broccoli was evaluated by examining the total number of larvae present in the harvested head. Cabbage injury was assessed by examining lepidopterous to cabbage heads and giving each a damage rating (on a 1-5 scale) based on the amount of head injury that could be contributed to feeding by imported cabbageworm and/or cabbage looper. No harvest evaluations of kale were taken since hail damage that occurred late in the course of the study prevented accurately evaluating lepidopterous leaf injury.

Results

Over the course of this study, a total of 519 imported cabbageworm larvae (Table 1) were observed along with 97 cabbage loopers (Table 2). A total of 161 cabbage whites were observed in insect food plots, 164 in wintergreen oil plots, and 194 in check plots. 32 cabbage loopers were found in insect plots, 30 in wintergreen oil plots, and 35 in check plots.

Treatments[a]	7/24	8/1	8/14	8/25
	Sampling Date[b]			
Untreated Check	26	69	47	52
Supplemental Insect Food	31	44	41	45
Supplemental Food + Wintergreen Oil	17	55	34	58

Figure 2.2 Total number of imported cabbageworm, *Pieris rapae* (L.), larvae found in plots of cultivated brassicas (kale, cabbage, broccoli) that received one of three different treatments: supplemental insect food spray, supplemental insect food plus wintergreen oil, and an untreated check. Within each plot Lepidoptera larvae were sampled on 8 plants in each section of kale, broccoli and cabbage. This resulted in the sampling of a 24 plants/plot and 96 plants/treatment/date). Sampling took place on four dates between 24 July and 25 August, 2017. The study was conducted at the Colorado State University Agriculture Research, Demonstration and Education Center (ARDEC) south facility in Larimer County, Colorado

[a] Treatments involved biweekly applications of Rincon-Vitova Insectaries “Insect Food” or “Insect Food” with wintergreen oil beginning 18 July.

[b] No significant differences were found by date.

Treatments[a]	7/24	8/1	8/14	8/25
	Sampling Date[b]			
Untreated Check	6	21	5	3
Supplemental Insect Food	5	15	9	3
Supplemental Food + Wintergreen Oil	6	12	8	4

Figure 2.3 Total number of *Trichoplusia ni* (Hübner) larvae found in plots of cultivated brassicas (kale, cabbage, broccoli) that received one of three different treatments: supplemental insect food spray, supplemental insect food plus wintergreen oil, and an untreated check. Lepidoptera larvae were sampled on 8 plants in each plot on four dates between 24 July and 25 August, 2017. The study was conducted at the Colorado State University Agriculture Research, Demonstration and Education Center (ARDEC) south facility in Larimer County, Colorado.

[a] Treatments involved biweekly applications of Rincon-Vitova Insectaries “Insect Food” and “Insect Food” with wintergreen oil beginning 18 July.

[b] No significant differences were found by date.

Counts of imported cabbageworm eggs (Figure 2.4) totaled 163 for imported cabbageworm insect food plots, 183 in wintergreen oil plots, and 154 in check plots. There were 41 cabbage looper eggs (Figure 2.5) in the insect food plots, 51 in the wintergreen oil plots, and 46 in the check plots.

	Sampling Date[b]			
Treatments[a]	7/24	8/1	8/14	8/25
Untreated Check	92	57	5	0
Supplemental Insect Food	86	63	14	0
Supplemental Food + Wintergreen Oil	111	58	13	1

Figure 2.4 This figure shows the total number of number of *Pieris rapae* (L.) eggs found in plots of cultivated brassicas (kale, cabbage, and broccoli) that received one of three different treatments: supplemental insect food spray, supplemental insect food plus wintergreen oil, and an untreated check. Lepidoptera larvae were sampled on 8 plants in each plot on four dates between 24 July and 25 August, 2017. The study was conducted at the Colorado State University Agriculture Research, Demonstration and Education Center (ARDEC) south facility in Larimer County, Colorado.

[a] Treatments involved biweekly applications of Rincon-Vitova Insectaries "Insect Food" and "Insect Food" with Wintergreen oil.

[b] Significant differences were found between treatments on 8/14 and 8/25. No significant differences between treatments were found on other dates.

	Sampling Date[b]			
Treatments[a]	7/24	8/1	8/14	8/25
Untreated Check	8	29	7	3
Supplemental Insect Food	5	27	7	3
Supplemental Food +Wintergreen Oil	5	32	14	4

Figure 2.5 This figure shows the total number of *Trichoplusia ni* (Hübner) eggs found in plots of cultivated brassicas (kale, cabbage, broccoli) that received one of three different treatments: supplemental insect food spray, supplemental insect food plus wintergreen oil, and an untreated check. Lepidoptera larvae were sampled on 8 plants in each plot on four dates between 24 July and 25 August, 2017. The study was conducted at the Colorado State University Agriculture Research, Demonstration and Education Center (ARDEC) south facility in Larimer County, Colorado.

[a] Treatments involved biweekly applications of Rincon-Vitova Insectaries "Insect Food" and "Insect Food" with Wintergreen oil.

[b] No significant differences were found by date.

No major significant differences between the total numbers of lepidopteran larvae found in untreated check plots compared to plots receiving biweekly applications of supplemental food or in wintergreen oil/supplemental food plots (Figure 2.1, 2.2 & 2.3). This was true for both the imported cabbageworm and the cabbage looper individually. There were also no major differences found between the number of imported cabbageworm eggs or cabbage looper eggs (Figure 2.4 & 2.5).

Post-harvest larvae counts on heads of broccoli showed lower levels of cabbageworms in heads collected from wintergreen oil/supplemental food plots with an average of 2.5 a head, when compared to check plots with an average of 2.9. Standard insect food plots actually showed an increase with an average of 3.2 larvae. Cabbage loopers showed the same average number (0.1) in insect food and check plots but none were found on plots with the wintergreen oil/supplemental food treatment (Figure 2.6). A simple statistical test showed no significance There were no major numerical differences between the number of imported cabbageworm larvae found in insect food plots compared to check plots and none in wintergreen oil/supplemental food plots. There were also no major difference between the number of cabbage loopers found in insect food plots compared to check plots or in wintergreen oil/supplemental food plots when compared to check plots.

Treatments[a]	*Piers rapae* Larvae[b]	*Trichoplusia ni* Larvae[b]
Check	2.9	0.1
Supplemental Insect Food	3.2	0.1
Insect Food/Wintergreen oil	2.5	0.0

Figure 2.6 This figure shows the average number of *Pieris rapae* (L.) and *Trichoplusia ni* (Hübner) larvae found in heads of broccoli that received one of three different treatments: supplemental insect food spray, supplemental insect food plus wintergreen oil, and an untreated check. Lepidoptera larvae were sampled on 5 heads of broccoli from each plot on 8 September The study was conducted at the Colorado State University Agriculture Research, Demonstration and Education Center (ARDEC) south facility in Larimer County, Colorado.

[a] Treatments involved biweekly applications of Rincon-Vitova Insectaries "Insect Food" and "Insect Food" with Wintergreen oil.

[b] No significant differences were found between average number or larvae in broccoli heads in each plot.

Ratings of damage to cabbage (Figure 2.6) by lepidopteran larvae averaged 2.9 (on a scale of 1 to 5) was given for plants in the untreated check plots and the plots receiving supplemental foods. A damage ratings of 2.8 was given for plots that contained the wintergreen oil. There were no significant differences between insect food and check plots. There were also no significant differences in insect food plus wintergreen oil plots when compared to check plots.

Treatments[a]	Average Damage Rating of Cabbage in Each Treatment[b]
Check	2.9
Supplemental Insect Food	2.9
Insect Food and Wintergreen Oil	2.8

Figure 2.7 This figure shows the average damage rating caused by lepiopterous pests on cabbage heads that received one of three different treatments: supplemental insect food spray, supplemental insect food plus wintergreen oil, and an untreated check. Damage ratings were given to 5 heads of cabbage from each plot on 19 August. The study was conducted at the Colorado State University Agriculture Research, Demonstration and Education Center (ARDEC) south facility in Larimer County, Colorado.

[a]Treatments involved biweekly applications of Rincon-Vitova Insectaries “Insect Food” and “Insect Food” with Wintergreen oil

[b]No major differences were found between average damage ratings between plots.

Discussion

The purpose of this study was to test the ability of insect food spray mixed with wintergreen oil, whose main chemical component is MeSA, to act as a control for lepidopteran pests and compare it against a standard insect food spray and a check. Previous studies have shown wintergreen oil to be effective at attracting natural enemies and controlling some pests (James, 2003; Mallinger et al., 2011; Rodriguez-Saona et al., 2011).

Studies performed previously suggest that supplemental insect food and essential oils may be effective at both luring, repelling, and even killing certain kinds of arthropods in some crop systems. This study, however, found no significant differences between check plots, plots with supplemental insect food, and plots with wintergreen oil in terms of both number of larvae and eggs. The only difference found was in the larvae damage ratings for cabbage, which found less damage done to those in wintergreen oil plots than those in others.

One reason this study might differ from others is that one standard concentration of insect food and wintergreen oil mixture was used for two different insects. Most previous studies have focused the effects of oils in one insect. The concentration needed to affect or reduce levels of pests might differ between pest species. Another factor might be the size of the data set. This study was relatively short. A longer study period with more data might be able to provide more pertinent information to insect food and essential oil studies. Furthermore, there may be relatively few natural enemies that affect lepidopteran insects in the studied crop. Insect food supplements and MeSA attractants have largely been found to effectively augment the activity of coccinellids, chrysopids, and syrphids. These species were rarely observed in plots during this study and most of these largely are predatory on taxa other than Lepidoptera, notably aphids. Another reason might have had to do with persistence as brassicaceous plants have waxy leaves and some of the treatments simply may not have stayed on the plants.

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