THE BIOLOGICAL CONTROL OF SPOTTED KNAPWEED AND CONSERVATION OF ASSOCIATED POLLINATOR COMMUNITIES

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ABSTRACT

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Spotted knapweed (*Centaurea stoebe* var. *micranthos* (Gugler)) has caused significant damage to ecosystems and ecosystem services in North America, and has become recognized as a threat to natural areas in the Midwest. In the past decade, three new biological control insects have been released in Michigan: the seedhead feeding weevils *Larinus minutus*, *Larinus obtusus*, and the root-feeding weevil *Cyphocleonus achates*. Here I report the findings of seven data collection efforts related to the biological control of spotted knapweed in Michigan. Both *Larinus* species established relatively quickly and are capable dispersers, redistributing themselves on a regional level less than a decade after release. Their rate of dispersal increases with time since release. The root-feeder *C. achates* is slower to establish and has not yet dispersed. Impacts on plant communities have yet to be observed. Seeds of native plants introduced to release sites in 2011 have not yet established. In two studies investigating spotted knapweed's influence on native bee community composition and abundance, we found that while spotted knapweed is very attractive to many native bee species, sites that have greater flowering plant diversity are able to support greater bee abundance and diversity because they extend the period of floral resource availability to include times before and after spotted knapweed's bloom period.

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CHAPTER 1

LITERATURE REVIEW

Introduction

Spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos* [Gugler]) and diffuse knapweed (*Centaurea diffusa* Lam.) are plants native to Southern and Eastern Europe. In their home range, both species occur at relatively low densities, and are integrated into their grassland communities (Sheley et al., 1998). Since their introduction to Western North America in the late 1800s, however, each plant has managed to spread and become the dominant component of many grasslands and rangelands. Spotted and diffuse knapweed cause dramatic decreases in plant diversity, ecosystem functioning, and utilization of land by wild and domesticated foraging animals. The plants are also present in the Midwestern United States, where they have more recently been recognized as a problem. In Michigan and much of the Midwest, spotted knapweed is the more serious invader, threatening the ecological integrity of intact natural areas. Conventional control methods such as herbicides and mowing have been ineffective at slowing its spread. Western states, notably Montana, Colorado, and Washington have spearheaded the effort to use classical biological control to slow and reverse the invasion of spotted and diffuse knapweeds, and there has been nearly 40 years of scientific literature on the subject. In Michigan we have the opportunity to benefit from the decades of research, and can choose the most effective biological control insects available. However, it is also recognized that spotted knapweed, aka star-thistle, provides an important nectar resource to Michigan honeybees and beekeepers. Therefore, large-scale control of spotted knapweed should also include the restoration of native nectar-providing plants.

Spotted knapweed biology

The native range of spotted knapweed spreads from Southern Europe east to central Russia (Sheley et al., 1998). **S**potted knapweed was first documented in North America in Victoria, British Columbia in 1893, and was probably introduced through discarded ships ballast and as a contaminant in alfalfa seed (Sheley et al., 1998). Recent molecular data points to multiple introductions of the plant (Hufbauer and Sforza, 2008). It is a relatively short-lived perennial plant in the Asteraceae family, capable of living for up to 9 yr. In the spring it forms a basal rosette consisting of deeply lobed leaves born on short pedicels. Each leaf can grow up to 20 cm long and 5 cm wide. After growing for 1-4 yr as a rosette, it will bolt to form one or more erect flowering stems, ranging from 15 to 60 cm in height (Story and Good, 2001; Wilson and Randall, 2005). Spotted knapweed adults have sparsely hairy stems that branch alternately and sessile stem leaves that decrease in size approaching the stem apex. Depending on their size, individuals can have anywhere from one to 100 flowerheads, with each head consisting of 25 to 35 purple to pink flowers (Wilson and Randall, 2005, Sheley et al.,1998). Below each set of flowers is a whorl of leafy bracts, each with a black tint along their top margin (**Figure 1**). The bracts are tipped with soft spines, the center spine being the shortest. The flowers can bloom continuously from June through October, with some variation due to local climates and weather. The seeds of spotted knapweed are brown to black, oval, and 3 mm long. Their pappus hairs range from 1 to 2 mm, and are persistent. A large plant can produce up to 600 seeds, and production per square meter has been documented to range from 5,000 to 40,000 seeds (Sheley et al., 1998).

Spotted knapweed seedheads open after desiccation, usually two to three weeks after maturing. Because the pappus hairs are small, the seeds are only capable of spreading within one meter of the parent plant by themselves. Because of this, populations of spotted knapweed tend to spread peripherally from existing stands, unless the seeds are physically transported to a new location. Seed dispersal has been documented to occur from attachment to passing animals, excrement of deer, mice, or birds, mud on vehicles, attachment to shoes, in hay and crop seed, and along waterways (Sheley et al., 1998).

Knapweed seeds can germinate at temperatures between 7 and 34 degrees C and at soil depths ranging from 0 to 5 cm, with optimal germination occurring on the soil surface. Knapweeds produce three types of seeds: active seeds, which germinate upon dispersal; dormant light-sensitive seeds, which need time and subsequent exposure to light before they begin to germinate; and dormant light-insensitive seeds which germinate after a period of time has passed. Any given plant is capable of producing all three types of seeds. This variation in seed production can help a single plant hedge its bets, and may contribute to knapweed's success (Sheley and Larson, 1996). Spotted knapweed is also capable of reproducing vegetatively through lateral shoots. While they can only spread a few cm through lateral sprouting, vegetative reproduction can multiply the number of seeds an individual is capable of producing (Sheley et al., 1998).

Spotted knapweed has been referred to by several specific epithets. The most common of these in the literature are *Centaurea biebersteinii* (Jaub. et Spach), *Centaurea maculosa* (Lam), and *Centaurea stoebe.* The current classification for diploid spotted knapweed is *Centaurea stoebe* subsp. st*oebe,* and tetraploid spotted knapweed is *Centaurea stoebe* subsp. *micranthos*

(Ochsmann, 2000; Blair et al., 2008). Treier et al. (2009) showed that the populations of spotted knapweed in North America were nearly all tetraploid, while only one of the 93 populations sampled in Europe contained any tetraploid individuals (the rest being diploid). The authors suggest that both diploid and tetraploid forms were introduced into North America, but it was the tetraploid form that became invasive. To avoid confusion, I will refer to all of these by their shared common name, spotted knapweed, unless otherwise specified.

Diffuse knapweed biology

While spotted knapweed is the subject of this thesis, much of the research on knapweed biological control has stemmed from releases targeting diffuse knapweed and so a brief discussion of its biology is warranted. Diffuse knapweed was first collected in North America in 1907 in Washington state, and was probably introduced as a contaminant in alfalfa seed (Sheley et al., 1998) While present in Michigan since 1943 (Reznicek 2011) has yet to become a significant threat in the Midwest. Diffuse knapweed can hybridize with the diploid form of spotted knapweed, and many diffuse knapweed individuals in North America contain some spotted knapweed DNA (Blair et al., 2008). Many ecological studies have been performed using diffuse knapweed, and while caution should be used when making ecological inferences between species, data observed in studies on diffuse knapweed can be useful when considering spotted knapweed biology and management.

Diffuse knapweed is a biennial or short-lived perennial plant. It shares a similar native distribution to spotted knapweed, but occupies a slighter drier range of habitats (Sheley et al., 1998). Like spotted knapweed, it starts as a basal rosette with deeply lobed leaves. In its second year of growth, it sends up a single stem ranging from 15 to 60 cm in height. This stem has many small branches, mostly on its upper half. Diffuse knapweed flowers are usually white, though they can sometimes be pink-purple. The flower heads tend to be longer and narrower than those of spotted knapweed. Each bract on the seedhead holds a long, sharp spine. Unlike spotted knapweed, diffuse knapweed is able to self-distribute its seeds; after the plant has senesced, the stem breaks off and the plant tumbles in the wind, releasing seeds as it goes (Wilson and Randall, 2005).

Knapweed impacts

Both spotted and diffuse knapweed have been shown to have significant impacts on surrounding communities and ecosystems. These plants can have direct and indirect effects on plant and animal community diversity and competition (Sheley et al., 1998). They reduce native plant diversity through competition, alteration of the abiotic environment, and possibly through allelopathy and the alteration of soil biota (Watson and Renney 1974; Ridenour and Callaway 2001; Marshall and Storer, 2008; Ortega and Pearson 2005). Diffuse and spotted knapweed are able to form nearly monotypic stands because their seeds germinate continuously, rather than all at once, which allows them to occupy all of the available niches in a given habitat and outcompete neighboring plants (Sheley and Larson, 1996).

This displacement of plant communities the most significant impact knapweeds have on ecosystems. It has many cascading effects, ranging from the reduction of total biotic diversity to the alteration of abiotic processes. Invasion by knapweeds reduces the amount of forage available to livestock such as cattle and sheep (Watson and Renny, 1974). Similarly, knapweeds reduce the amount of forage available to many types of wildlife. Hakim (1979) found 98% fewer elk pellets on land invaded by spotted knapweed as compared to uninvaded rangeland, indicating that knapweed invasion can have a significant impact on elk populations by reducing forage.

A study by Ortega et al. (2006) demonstrated decreased nesting fidelity in chipping sparrows in habitats dominated by spotted knapweed. This decrease was correlated with a reduction in grasshopper abundance. The authors speculate that this reduction in grasshopper abundance was likely caused by a loss of native plant food resources following knapweed invasion, although other less direct impacts of invasion should be considered. For example, Pearson (2009) found that spotted knapweed altered the architecture of grasslands in a way that favored web-building *Dictnya* spiders. *Dictnya* densities were 46-74 times higher in invaded grassland, and individuals built webs that were larger than those found on uninvaded grasslands. These findings led the author to estimate that predation by *Dictnya* spiders increased more than 89-fold compared to un-invaded grasslands. MacDonald et al., (2003, 2007) speculates that this predation is bound to have profound impacts on the structure of arthropod communities.

Spotted knapweed has a significant effect on soil microbial abundance and diversity. The arbuscular mycorrhizal fungi (AMF) communities in knapweed invaded soils differed significantly from those of adjacent uninvaded soils, and the hyphal lengths in invaded soils were reduced by 24%, suggesting an overall reduction in AMF abundance (Mummey and Rillig, 2006). Similarly, Broz et al. (2007) found a dramatic reduction in both fungal abundance and diversity in soils with a high density of spotted knapweed compared to those with a low density of spotted knapweed. Because of the strong influence soil fungi and bacteria have on plant processes, this reduction of fungal abundance and diversity is likely to be playing a part in spotted knapweed's increased competitive ability (Callaway et al., 2004).

In Michigan, spotted knapweed is able to invade intact native ecosystems including dry sand prairies, dry-mesic prairies (Kost, 2004), mesic sand prairies (Kost and Slaughter, 2009), dry-mesic Southern forests (Lee, 2007), oak barrens (Cohen, 2001), oak-pine barrens (Cohen, 2000), and open dunes (Albert, 1999), where it is often considered a primary threat to biodiversity. Open dunes are important habitats for the Lake Huron locust (*Trimerotropis huroniana* [Walker]), the piping plover (*Charadrius melodus* [Ord]), and pitcher's thistle (*Cirsium pitcheri* [Torr. ex Eat.]), all of which are species of special concern (Albert, 1999).

A large proportion of monotypic spotted knapweed stands in Michigan occur on marginal land such as old fields, gravel mines and roadsides, where plant diversity is lower (MacDonald et al., 2003, 2007). However, one could speculate that such populations contribute to regional seed banks and thus may be an indirect threat to natural habitats. As knapweed densities increase on marginal lands, the propagule pressure on intact habitats will be even stronger. This could potentially lead to the colonization and domination of these natural areas by spotted knapweed, and a further reduction of ecosystem integrity.

At the Pictured Rocks National Lakeshore in Northern Michigan, Marshall and Storer (2008) found that spotted knapweed invasion of open dune habitats had adverse effects on native plant communities and altered insect community composition. Native dune plants are specially adapted to the constantly changing structure of sand dunes. Spotted knapweed has been able to colonize dunes systems, stabilizing the sand and facilitating invasion by other exotic plants. This change in the plant community and dune stability has altered the insect community, increasing the abundance of ants and weevils.

In addition to the biotic alterations they cause to ecosystems, knapweeds can alter abiotic processes as well. Spotted knapweed doesn't accumulate large amounts of plant litter, and thus habitats where it is dominant tend to have more open ground between plants (Emery and Gross 2006). Lacey et al. (1989) demonstrated that this leads to increased runoff and sediment yield under normal rainfall conditions. This can lead to the loss of topsoil, which could further alter plant communities. It also has the potential to adversely affect water quality and increase the sediment levels in reservoirs (Lacey et al., 1989).

The lack of leaf litter between knapweed plants also makes it hard for fire to carry through monotypic stands (Emery and Gross 2005). This could be expected to lead to an alteration of natural fire regimes on which many Michigan ecosystems depend, and facilitate further invasion by knapweeds and other exotic species.

Plant invasion theory

The success of invasive plant species has sparked a surge in research attempting to explain what mechanisms enable a given plant species to become invasive. The classical explanation for invasive success is that if a given species is able to escape the herbivores, parasites, and diseases that limited it in its native range, then it will have an increased competitive advantage in the introduced range**.** This is known as the Enemy Release Hypothesis**,** and it has been supported by a number of field experiments (Cappuccino and Carpenter, 2005; Carpenter and Cappuccino, 2005, Jogesh et al, 2008). An extension of this idea, the Evolution of Increased Competitive

Ability (EICA) was proposed by Blossey and Notzold (1995). According to the EICA hypothesis, exotic plants that have escaped their natural enemies can allocate less energy to the production of defenses such as chemicals and tough leaf tissues. This enables them to grow more vigorously and to allocate more energy to reproduction. Over time, this should lead to the evolution of plants that are more vigorous, fecund, and less resistant to disease and herbivory. While the prediction of more vigorous growth in the introduced range has been supported by a number of studies (Jakobs et al., 2004; Blossey and Notzold, 1995; Ridenour et al., 2008), evidence that this increased growth is derived from the evolutionary loss of defensive mechanisms has been difficult to obtain. One exception is the findings of Broz et al. (2009), who observed increased fecundity and decreased expression of genes related to defense in North American populations of tetraploid spotted knapweed compared to their European tetraploid counterparts. Contrasting these results, Ridenour et al. (2008) demonstrated that while North American populations of spotted knapweed were larger and more competitive than their European counterparts, they were also better defended against generalist predators, countering the predictions of the EICA hypothesis.

The discovery of root exudates +/- catechin and 8-hydroxyquinoline in spotted and diffuse knapweed, respectively, has led to another potential explanation for the success of some invasive plants: the Novel Weapons Hypothesis. This hypothesis proposes that some exotic plants produce chemicals that plants and animals in their introduced range have never been exposed to before, and are thus susceptible to. These could function in the form of compounds that are toxic to naïve herbivores, or as allelopathic chemicals that are toxic to plants that are not adapted to co-existing with them (Callaway and Ridenour, 2004). Knapweeds have been a key player in the debate over the Novel Weapons Hypothesis, and many studies have attempted to demonstrate the allelopathic properties of +/- catechin (Callaway and Aeschehoug, 2000; Bais et al., 2002; Perry et al., 2007; Thorpe et al., 2009). While many experiments have suggested that allelopathy is taking place, this phenomenon is very difficult to prove, and results have not always been consistent (ex. Bais et al., 2002; Reinhart and Rinella, 2011). Several studies measuring the amount of +/- catechin in soils from spotted knapweed-dominated sites found that the chemical was rarely detected, and when it was, it occurred at levels far below those that have been found to be phytotoxic (Blair et al., 2005; He et al., 2009; Perry et al., 2007). Perry et al. (2007) observed a pulsing in the levels of +/- catechin; that is, for most of the year there was none found, but on one sampling day there was a very high level present. It could be that spotted knapweed only produces the chemical sporadically, which could still give the plant a competitive advantage but would make it very difficult to measure the levels of +/- catechin present in the field. It may also be that knapweed root exudates modify the soil biota to the plant's advantage.

It is difficult to provide definitive evidence showing whether the EICA, the Novel Weapons Hypothesis or the Enemy Release Hypothesis best explains the success of knapweeds in North America. The fact is that these are not mutually exclusive ideas, and the true explanation probably includes all three. Because of the large range and many habitats that spotted knapweed has come to occupy in North America, it can be problematic to make broad generalizations about the plant. For instance, while spotted knapweed has been shown to invade and dominate rangeland in Montana and Colorado, displacing other plant species, Reinhart and Rinella (2011) observed a different pattern in Shenandoah National Park, Virginia. In grassy balds, which are relatively rare habitats, spotted knapweed has become established and integrated into the plant community without decreasing the overall species richness, abundance, or evenness. In these habitats, the knapweed plants collected produced an average of 143 seeds per plant, compared to an average of 2000 seeds per plant observed in the Western U. S. (Reinhart and Rinella, 2011). Additionally, greenhouse experiments showed no evidence of allelopathy in these populations of spotted knapweed. While these findings do not eliminate concerns over the potential harm spotted knapweed can cause, they demonstrate the variability of ecological impacts that different populations of the plant can have in different locations.

There is a large degree of genetic variation within the North American population of spotted knapweed, and there is evidence that it hybridizes with diffuse knapweed (Hufbauer and Sforza, 2008). Such genetic variation could lead to disparate ecological responses by both knapweed and the plant communities it interacts with. In addition, variation in the amount of disturbance, whether natural or human-induced, is also bound to cause discrepancy in the effect spotted knapweed has on a plant community. Knapweed is a plant that thrives in disturbed habitats (Watson and Renney, 1974)**,** and it may be difficult to know how much disturbance is happening in a given community without careful observation. The success of knapweeds can also be influenced by factors such as the availability of soil nutrients and the composition of the plant community being invaded. Recent studies and field observations support the hypothesis that knapweeds perform better in low-nutrient soils relative to other plants (Seastedt et al. 2007; Hooper and Johnson, 1999; Knochel et al., 2010a), though variability in these results again point to the difficulty of making broad generalizations about knapweed ecology.

Spotted knapweed management

Concern about the spread of knapweeds and other invasive plants has led to the development of many different management strategies. These include prevention, cultural methods, conventional chemical control, and biological control. Because knapweeds are capable of quickly establishing large populations, the most effective method of control is to keep them from spreading in the first place. Preventative methods include keeping seeds from spreading to new locations, and the removal of colonizing individuals before they have a chance to spread. This approach necessitates the cooperation of many people and organizations working to stop new outbreaks.

Mowing can be an effective method of controlling spotted knapweed. It is important that the plants are cut while they are flowering, but before they have a chance to produce seed. If the plants are cut before the flowers open, there will be a second production of flowers later in the year (Story et al., 2010). Fire can kill adult plants, reduce the number of viable seeds in the seedbank and help native plants that are adapted to fire. Burning in mid to late-spring is the most effective. However, knapweed doesn’t carry fire well by itself, so seeding with grasses prior to burning can be helpful (MacDonald et al., 2007; Emery and Gross 2005). Burning in the summer can reduce knapweed densities, but it also has a detrimental effect on warm-season grasses (Emery and Gross, 2005). Late-spring burning can inhibit the growth of early-flowering native forbs (Rosberg, 2001), so caution must be used if providing resources for pollinators is a goal of restoration.

Spotted knapweed biological control

A successful plant management plan should consider all available control options, but many techniques tend to be labor-intensive, expensive, and unable to provide long-term control. They have been largely inadequate when it comes to stopping the further spread of knapweeds. Biological control offers an alternative that is relatively inexpensive, does not require much labor, and can be self-perpetuating. While most of the biological control efforts directed at knapweeds have involved specialized insect herbivores, species of fungi (*Fusarium spp.)* have also been identified as effective control agent (Caesar et al., 2002).

The effort to slow and reverse the invasion of diffuse and spotted knapweeds has led to one of the longest ongoing classical biological control programs in North America (Bourchier and Crowe, 2011). In total, 13 insects have been introduced into North America to control spotted and diffuse knapweeds. Eight of these are seedhead-feeders, including four flies (*Urophora affinis* [Frauenfeld], *U rophora quadrifasciata* [Meigan], *Chaetorellia acrolophi* [White and Marquardt], *Terellia virens* [Loew]), one moth (*Metzneria paucipunctella* [Zeller]), and three beetles (*Larinus minutus* [Gyllenhal], *Larinus obtusus* [Gyllenhal], *Bangasternus fausti* [Reitter]). The other five control insects are all root-feeders. Three of these are moths (*Pterolonche inspersa* [Staudinger], *Agapeta zoegana* [Linnaeus], and *Pelochrista medullana* [Staudinger]) and two are beetles (*Cyphocleonus achates* [Fahraeus] and *Sphenoptera jugoslavica* [Obenberger]) (Wilson and Randall, 2005).

As spotted knapweed has become more prevalent in the Midwest, the introduction of control insects has occurred in many Midwestern states such as Minnesota, Wisconsin, North Dakota, Missouri, and Arkansas. Because these introductions are happening decades after the first introductions in Montana, Midwestern states are able to benefit from the years of research and observation that was conducted on the effectiveness of control insects in the West. Drawing from the accumulating scientific literature on knapweed biological control, Michigan State University has identified three insects as being the most effective control agents: The seedhead-feeding weevils *L. minutus* and *L. obtusus*, and the root-feeding weevil *C. achates*. These three insects constitute new introductions to Michigan, and they are also part of the control programs that have been implemented in neighboring states. In addition to the weevils, the seedhead-feeding flies *U. affinis* and *U. quadrifaciata* were introduced to Michigan in 1995, and have become widely established wherever knapweed occurs in the state (Lang et al., 2001, Landis and Sebolt, unpublished data). However, the flies alone have not had a significant impact on knapweed populations, and additional control agents are necessary to slow the spread of the plant.

Biology of *Larinus minutus*

The lesser knapweed flower weevil, L. *minutus* (**Figure 2**), is a small black weevil in the subfamily Lixinae. They are 4 to 5 mm long, and like most members of their family have a protruding snout. Their larvae are white, C-shaped grubs with brown head capsules (Wilson and Randall, 2005). The native range of *L. minutus* is from Southern Europe through the Mediterranean region, and east to the Caucus Mountains. Here the weevils are found in association with *C. maculosa*, *C. diffusa*, *C. grenaria*, and *C. calcitrapa* (Jordan, 1995).

In host-specificity tests conducted on 72 plant species occurring in nine families, Jordan (1995) found that the adult weevils feed only on plants within the subtribe Centaureinae. For oviposition and larval development, they rely exclusively on several closely related members of the genus

Centaurea. Because there are no members of the genus *Centaurea* native to North America, it is predicted that *Larinus minutus* will be confined to invasive knapweeds.

Adults of *L. minutus* spend the winter in the soil litter near the base of their host plant. In the spring, they emerge and feed on newly grown plant tissue. The females need to feed on knapweed flowers for their ovaries to develop. Once the flowers appear, usually in mid to late June, the weevils mate and lay their eggs throughout the summer. The female lays from one to five eggs at a time in the flowerhead, and can lay up to 130 eggs in a season. The larvae hatch three days after oviposition, and immediately begin feeding on the pappus hairs. They then migrate downward and begin feeding on the seeds. The larvae are aggressive and will often eat each other and other insects in the same seedhead (Wilson and Randall, 2005). Field trials in diffuse knapweed found that larvae consumed all of the seeds in an infested flowerhead (Kashefi and Sobhian, 1998). After the larvae have finished feeding, usually after three instars occurring over four weeks, they construct a pupal chamber using the material left in the seed head. After about two weeks in the chamber, the pupae’s metamorphosis is complete, and they emerge as adults. These adults feed vigorously on the remaining green knapweed vegetation and flowers. As the plants begin to senesce, the new generation of weevils moves down to the soil litter at the base of the plants in preparation for winter (Wilson and Randall, 2005).

Biology of *Larinus obtusus*

The biology and life-cycle of *L. obtusus* is in all practical senses the same as *L. minutus*. The two weevils are difficult to tell apart (**Figure 3**). The most reliable characteristic to distinguish the two species is the lack of a red-tinted tibia in *L. obtusus* (Wilson and Randall, 2005).

Host specificity testing was conducted using 78 species of plants representing different levels of relatedness to spotted knapweed. After these tests, it was concluded that *L. obtusus* is specific to plants in the genus *Centaurea* (Groppe et al., 1992).

Biology of *Cyphocleonus achates*

The knapweed root weevil, *C. achates* (**Figure 4**), is a medium-sized beetle, averaging 13 to 15 mm in length. It has brown and grey mottling, large black eyes, and a short, thick snout (Wilson and Randall, 2005). Larvae are white grubs averaging 13 mm. *C. achates* is in the subfamily Cleoninae. It is one of ten species in its genus, all of which occur in Eurasia. The native range of *C. achates* is South-eastern Europe and Asia Minor, where it is associated with spotted knapweed and, to a lesser extent, diffuse knapweed (Stinson et al., 1994). In host-specificity tests conducted on 71 plants, Stinson et al. (1994) showed that while *C. achates* adults will lightly browse on a variety of plants in Asteraceae, members of the genus *Centaurea* are required for oviposition and larval development.

Larval *C. achates* overwinter in the root cortex of its host plant. In the spring the larvae resume feeding on the root tissue, and after passing through four stadia, they pupate in the root and emerge as adults. This occurs from mid-July through early September, with the majority of individuals emerging in August (Wilson and Randall, 2005). After emerging from their pupal chambers and chewing through the root wall, the adults climb to the top of their host plants and sit on the open flowers, presumably to harden their newly-formed exoskeletons in the sun. They then commence feeding on the leaves of their host (Stinson et al., 1994). During the night and when temperatures are low, the weevils hide near knapweed root crowns, buried just below the soil surface. On warm days, however, they climb to the top of the plants to feed and search for mates. Reproduction begins soon after emergence, and continues throughout the summer. Females will copulate several times with one or more males before seeking out a suitable site for ovipostion. They are drawn to large plants, because these are usually the ones with the largest root-crowns, and thus the most food for their larvae. During laboratory testing, Stinson et al. (1994) showed that larvae required large roots to survive to adulthood. Because of this, *C. achates* prefer spotted knapweed, which as a perennial has larger roots than diffuse knapweed. Once the female has found a suitable plant, she will excavate through the soil to the root and chew a hole into it. She then backs out of her tunnel, turns around, and backs into the hole and deposits one egg into the hole. Finally she crawls out, turns around again, re-enters the hole head-first, and encases the egg with soil particles held together with a secretion. Upon exiting, she fills the tunnel in with soil, and searches for a new site. The number of eggs a female lays in her lifetime is variable, with averages around 40, but some individuals laying up to 100 eggs (Stinson et al., 1994). The eggs take 10 to 12 days to develop. Upon hatching, the larvae mine through the root to the cortex, where they stay until pupation. The larvae feed throughout the fall, and usually molt into their second instar before beginning their dormancy at the onset of winter (Stinson et al., 1994).

Biological control results

The first biological control insect to be introduced in the United States for knapweed biological control was *U. affinis*, in 1973 (Wilson and Randall, 2005). Its cogener *U. quadrifasciata* followed in 1980, and together these have become the most widely spread insects associated with knapweeds. They can be found almost anywhere knapweeds occur in North America. The two flies seem to occupy the same niche, and it has been shown that *U. affinis* is the more competitive of the two. Because of this, *U. affinis* tends to displace *U. quadrifasciata* and is more abundant (Smith, 2004). *Urophora* spp. have been shown to substantially reduce seed production, but their impact has not been enough to control either species of knapweed (Wilson and Randall, 2005; Myers et al., 2009). However, even though they don't independently reduce knapweed densities, they may be important players when their effects are added to those of other insects.

The results of biological control releases in North America have varied in the degree of their success, but a clear pattern seems to be emerging. Diffuse knapweed is somewhat more susceptible to control by single natural enemy releases—namely *L. minutus*—while the more robust spotted knapweed may require the additive effects of both root herbivores and seed predators to halt its spread.

Overall, seedhead predators have been successful in controlling diffuse knapweed, and *L. minutus* has been called the "silver bullet" insect responsible for reducing diffuse knapweed populations in British Columbia (Meyers et al., 2009). After monitoring rosette density, seedhead production per plant, and seeds per seedhead from 1997 to 2001, Seastedt et al. (2003) calculated a decline from 5000 seeds/m^2 to less than 100 seeds/m^2. They also observed a reduction in diffuse knapweed density from 8.3% of absolute cover in 2000 to 1.9% cover in 2001. The authors believe that *L. minutus* contributed significantly to both of these reductions, both because of their consumption of seeds within occupied seedheads, and because of vegetative herbivory by adult weevils. Density and seedhead reductions were the strongest near the release sites, and at a nearby field without insect introductions, the density of diffuse knapweed increased over the same time period.

While seedhead predators have been fairly successful in controlling diffuse knapweed, they haven’t been as effective as independent controls of spotted knapweed. After monitoring the effects of seedhead predators on spotted knapweed at two sites in Western Montana, Story et al. (2008) observed that by 2006 seed production had decreased by 91.6 - 93.8% since the introduction of seedhead predators began in 1973. Furthermore, the soil seedbank in areas where seedhead predators have been established is significantly reduced compared to seedbanks in areas where there are fewer seedhead predators. This reduction in the viability of the seedbank is supported by Knochel and Seasedt (2010), who found that just 6 yr after *L. minutus* establishment, the subsample of seeds recovered from the soil contained no viable individuals. However, in spite of strongly reduced seed production, spotted knapweed has persisted at sites with seed predators for many years without showing significant density declines. Story et al. (2008) speculate that this may because of the perennial nature of spotted knapweed. Even if their sexual reproduction is severely limited, they can often persist via asexual production of rosettes through root collars.

Story et al. (2008) also suggest that spotted knapweed recruitment and density share a non-linear relationship, and once a critical threshold of seed production is reached, knapweed density will quickly decrease. This hypothesis is supported by data comparing stem density to the number of seeds in the seedbank. The authors believe that this threshold may be near 160 seeds/m^2, although they acknowledge that that estimate may not be very precise, and there is likely to be significant regional variation. Based on seedbank comparisons between sites that have had seedhead predators for more than 30 yr and sites that have only had seedhead predators for 10 yr, the authors believe that the introduction of seedhead predators alone could take up to 20 yr to reduce spotted knapweed densities. This timeline is in contrast to the 6 yr effect that Knochel and Seastedt (2010) observed, implying that there are likely other factors at play. Regardless of amount of time it takes seedhead predators to independently decrease spotted knapweed abundance, introducing additional stressors such as root herbivores and increased plant competition through seeding should speed the process (Seastedt et al., 2007; Jacobs et al., 2006).

In studies conducted in vitro, in common gardens experiments, and through field observations, *C. achates* has emerged as the most promising root-feeding control insect. The weevil was first introduced into Western Montana in 1988, and since then has been released in Colorado, Minnesota, Arizona, California, Indiana, Idaho, Nebraska, Nevada, Oregon, South Dakota, Utah, Washington, Wisconsin, and Wyoming (http://www.biocontrol.entomology.Cornell. edu.weedfeed/Cyphocleonus.html). In field sites where the weevil has become established, decreases of both diffuse and spotted knapweed have been observed (Jacobs et al., 2006; Seastedt et al., 2003; Clark, 2001; Story et al., 2006; Smith, 2004). Story et al., (2006) monitored spotted knapweed density at two *C. achates* release sites in Western Montana from 1993 to 2004. At the first site, a total 325 weevils were released between 1989 and 1990, and at the second site, a total of 694 weevils were released between 1991 and 1992. By 1998, the populations had grown to estimated levels of 90,776 and 97,173, respectively. The density of spotted knapweed declined significantly at both sites (99% and 77%, respectively). At six nearby control sites lacking C. achates, spotted knapweed populations showed no significant decrease. This is especially striking given that six other biological control insects (*A. zoegana, U. affinis, U.*

quadrifasciata, M. paucipunctella, L. obtusus and L. minutus) were present at the control sites. While the authors acknowledge that there may have been synergistic effects between some of the other insects present and *C. achates*, these results point to *C. achates* being a key control agent of spotted knapweed biological control (Story et al., 2006).

The ability of *C. achates* to impact spotted knapweed was demonstrated experimentally by Corn et al., (2006). The authors transplanted rosettes of spotted knapweed to an outdoor experimental plot, and separated them into groups of 20 plants each. To these groups they introduced treatments of two, eight, or zero pairs of adult *C. achates*, and plant vigor and mortality were monitored from 2001 to 2003. The experiment was then replicated from 2002 to 2004. The plants exposed to the root-feeding weevils showed significantly decreased vigor and increased mortality in the first replication. In the second replication plant vigor was again decreased in plots with the weevil, but mortality was similar across treatments. This outcome is believed to be a result of drought conditions over the summers of 2002 and 2003. In order to confirm that *C. achates* can impact spotted knapweed in years with normal precipitation, Corn et al. (2007) performed an experiment that included treatments of watering and exposure to *C. achates*. The results led the authors to conclude that while drought may decrease spotted knapweed growth, the weevil can significantly reduce knapweed growth regardless of precipitation.

The stand densities of spotted knapweed at 13 sites were assessed by Clark et al. (2001) before the release of *C. achates* and another root-feeder, *Agapeta zoegana*, between 1991 and 1993, and again post-release (1997 and 1998). Spotted knapweed showed a significant decline at all 13 sites, but the level of decline was not correlated to the occurrence of root-feeders. These results perplexed the authors, but they may be explained by observations made by Corn et al. (2006):

“…our experiments show that current C. achates numbers do not necessarily reflect the history of attacks and their long-term effects. This may explain why Clark et al (2001) found declining spotted knapweed 5–7 yr after the release of C. achates and A. zoegana, but no correlation between declining populations of knapweed and establishment of these biological control agents.”

In a common garden experiment, Knochel et al. (2010b) exposed spotted knapweed to either *C. achates* alone, *L. minutus* alone, or a combined treatment of both insects together. It was found that *C. achates* had an impact on the fitness of spotted knapweed, because of both root herbivory by the larvae and the consumption of rosettes by adult insects. This is significant because rosette herbivory was predicted to be a key component of knapweed control by Myers and Risley (2000). *L. minutus* was also able to reduce knapweed fitness independently through herbivory by adult insects. Importantly, it was shown that the belowground effects of *C. achates* and the aboveground effects of *L. minutus* are additive, meaning that together they decrease growth and flower production more than either does independently.

Concerns over biological control

The mention of biological control can often raise concerns amongst scientists and the public alike (Story, 1992). The most obvious concern is that organisms introduced to control an exotic plant species could begin to consume non-target species of plants. This issue has the potential to become a real problem, and its possibility should not be brushed away lightly (Louda et al., 2005). Because of the potential for biological control agents to feed on non-target plants, APHIS (Animal and Plant Health Inspection Service) mandates extensive testing on any organism being brought into the United States for biological control. *L. minutus, L. obtusus,* and *C. achates* were tested for host-specificity on more than 70 plants each (Stinson et al., 1994 Jordan 1995), and it was found that they are very host specific, and require spotted knapweed, diffuse knapweed, or a very close relative within the genus *Centaurea* (all of which are exotic to North America [Susanna et al., 2011]) to reproduce. While it is possible that they could occasionally browse on a small subset of non-target species, without the presence of their preferred host they would not be able to reach numbers high enough to have an impact.

Pearson and Calloway (2008) observed the presence of *U. affinis* creating a new winter food source for white-footed mice, which lead to increased mice populations and increased consumption of desirable native seeds, and perhaps an overall negative impact on the plant community as a whole. While indirect impacts of biological control insects are definitely a concern, it should be noted that the *Urophora* flies have been acknowledged as a poor control agent by themselves, and more effective control agents have been shown to lead to a decrease in knapweed densities (Corn et al., 2006, 2007; Jacobs et al., 2006; Seastedt et al., 2003; Clark et al., 2001; Story et al., 2006, Smith, 2004).

A final issue that has been raised with biological control is that low levels of herbivory can lead to an over-compensatory response by knapweeds, and potentially increase its competitive ability (Calloway et al., 2000; Myers et al., 1990, Thelan et al., 2005). This effect may exacerbated by soils with high nutrient availability. However, these studies were conducted with control insects that have not proven to be very effective, such as the root-mining moth *A. zoegana*, or by clipping vegetation to simulate herbivory. Studies conducted with *C. achates* and *L. minutus* showed while there was a correlation between soil nutrient levels and the degree to which herbivory decreased knapweed growth, the combined presence of these two insects lead to significant declines in spotted knapweed fitness no matter what the soil nutrient levels were (Knochel and Seastedt, 2010c).

While there are potential risks to introducing biological control agents, they should be weighed against the toll invasive plants take on ecosystems and the services they provide society. In many cases, the benefits of successful biological control are more substantial than the costs. If extensive research is done before introduction to determine host- specificity, and the agents chosen are effective enough to control the target plant within a reasonable timeframe, then biological control with insects is a desirable option.

Ecology of knapweed biological control and restoration

The large degree of variation in the success of knapweed biological control has led to a number of projects investigating factors that could be affecting the outcome of natural enemy introductions. Soil nutrient availability, plant and insect phenology, plant competition, and the re-colonization of knapweed sites by new species are all pieces of the successful biological control puzzle.

Phenology and biological control

Knowing how host plant phenology influences the efficacy of biological control insects is an important aspect of understanding and effectively implementing biological control. Because of the large range spotted and diffuse knapweeds occupy in North America, there are bound to be large amounts of variation in the phenology of the plants based on latitude and altitude. Even though this could lead to differing results in biological control, there has been little research done in this area.

One notable exception is the work done by Bourchier and Crowe (2011) investigating the interaction between the phenology of spotted knapweed and the occurrence of *U. affinis* and *L. minutus*. It has been demonstrated that *U. affinis* attack seedheads early in the growing season, before the head has fully developed and opened (Berube 1980). On the other hand, *L. minutus* oviposits on flowers once they have already opened (Kashefi and Sobhian, 1998). Due to these differences, changes in flowering times could alter the abundances of these control species, and perhaps the effectiveness of control at the site. The authors showed that the presence of multiple *U. affinis* larvae in a flowerhead negatively affect the developmental success of *L. minutus* larvae. Conversely, *L. minutus* will consume solitary *U. affinis* larvae. Despite this negative interaction, both species have been able to persist because they occupy different temporal “niches.” If the flowering phenology of the plant is shifted to earlier in the season, it would be expected to favor *L. minutus*, and if it is shifted to later flowering, it would be expected to favor *U. affinis*. This could help explain the differences in *L. minutus* abundances at different sites.

In addition to variations in temperature and moisture, phenology could be affected by other factors such as soil resource levels and plant competition. Notzold et al. (1998) demonstrated experimentally that increased plant competition delayed the flowering of purple loosestrife (*Lythrum salicaria* [Linnaeus]), another invasive plant that tends to form monocultures. While these results cannot be applied to knapweeds without caution, they hint that there may be an important interplay between plant competition levels and flowering time, which in turn could influence the efficacy of seedhead feeders.

In a study investigating the interaction between seedhead insects, plant phenology, and mowing, Story et al. (2010) showed that it is important to take flowering time into account if mowing is to be used as a control method in conjunction with biological control. The authors observed that if spotted knapweed was mowed during the bolting budding stages, new flowers were formed later in the summer, after the oviposition period of most control insects had waned. This could potentially lead to increased seed production and decreased abundances of control agents. When the knapweed was mowed during the flowering stage, however, it was unable to re-form flowers that same year, and seed production was halted. This also led to a decrease in control agents, so this approach may be detrimental to the long-term control effort if mowing is not continued indefinitely.

Biological control and plant competition

Jacobs et al. (2006) studied the interation between plant competition and herbivory by *C. achates* on spotted knapweed. Seeds of either *Pseudoroegneria spicata* (Pursh), a native grass, or *Thinopyrum intermedium* (Host), a European grass were sown in plots established in a field dominated by spotted knapweed. Control plots were left unseeded. It was observed that *C. achates* significantly reduced the biomass and seed production in *C. stoebe* individuals that it infested, and the degree of fitness reduction was not significantly influenced by the presence of competition. The presence of *T. intermedium* strongly reduced the density of adult knapweed plants, presumably through competition with younger individuals which prevented them from reaching maturity. This effect was true of *P. spicata* to a lesser extent. This example of an exotic grass being a better competitor of knapweed than its native counterparts raises concerns about what will happen in areas once knapweeds are controlled.

The results obtained by Jacobs et al. (2006) do not support the “resource concentration” hypothesis, which predicts that the highest levels of herbivory will occur in resource-rich areas of large, dense stands of the host plant (Root 1973). Instead, their results show an inverse relationship between the density of knapweed and the proportion of plants attacked. This is characteristic of a “resource dilution” model (Otway et al., 2005), which predicts that more specialist herbivores will be present on a host population that is less densely distributed. This observation is important because it indicates that even if competing plants don’t directly inhibit knapweed growth, their presence can increase the efficiency of control insects. It also predicts that knapweed populations should decrease exponentially as they begin to be replaced by other species.

Knochel et al. (2010a) also investigated the effect reduced seed production has on spotted knapweed recruitment and survival in the presence of substantial native plant competition. This was done by sowing different densities of spotted knapweed seeds into plots established in an un-invaded meadow. The germination and persistence of spotted knapweed seedlings was then monitored for 2.5 growing seasons. There was a significant reduction in seedling recruitment with lower densities of seeds sown. Perhaps even more significant was that the recruitment of knapweed was very low (averaging 0.066%) across all plots, indicating that the presence of established vegetation can strongly limit knapweed invasion. Together, these results suggest that seedhead-feeding insects coupled with increased plant competition could be enough to control spotted knapweed, even without the presence of root-feeders such as *C. achates*.

One issue not addressed in Knochel et al. (2010) is whether the increase of plant competition could have as large of an effect if knapweed is established first; i.e. priority effects. Emery and Gross (2006) addressed part of this problem by experimentally invading plots dominated by either spotted knapweed, *Andropogon virginicus* (Linnaeus), *Bromus inermis* (Leyss), *or Solidago canadensis* (Linnaeus) with seeds of 19 different plant species. It was found that spotted knapweed is highly invasible, as was *B. inermis*. The authors speculate that this could be related to the relatively small amount of leaf litter beneath spotted knapweed as compared to *A. virginicus* and *S. canadensis.* While this study only monitored the establishment of seedlings, and not their competitive effect on the plants being invaded, it does provide useful insight into whether spotted knapweed is invasible.

Revegetation after reduction

Stephens et al. (2009) point out that despite the goal of restoration that most biological control projects state in their proposal, little research has been conducted on plant community responses to the successful removal of target plants. The successful restoration of a native flora is often dependent on site specific variables, such as the presence of a viable native remnant population, lack of other strongly competitive exotics, and the persistence of native seeds in the soil. For instance, it was shown by Blossey et al. (2001) that the successful control of purple loosestrife by *Galerucella* spp. could lead to either the re-establishment of diverse wetland plant assemblages, or to a dominance shift to other exotics like phragmites or reed canary grass.

While the successful reduction of knapweed populations is a relatively new phenomenon, the few studies investigating the plant community responses to these reductions have produced troubling results. In Western Montana, Story et al., (2006) observed that after spotted knapweed densities were reduced following the introduction of *C. achates*, the newly opened niches were quickly filled by two exotic plant species, *Bromus tectorum* and *Descurainia sophia*.

In a 9 yr study focusing on the community response to diffuse knapweed reduction following biological control in Colorado, Bush et al. (2007) came to a similar conclusion. The decrease in diffuse knapweed density left open an area of 25% relative cover available for colonization. Although there was a small increase in relative native forb cover (2.4 to 8.6%), native grasses were not able to shift into the open area. This was probably due to increased competition from the exotic grass species *Bromus arvensis* and *T. intermedium*, which were able to exploit much of the vacuum left by diffuse knapweed. Because of the response of other non-native plants, the overall ratio of native to exotic plant cover did not change following the reduction of diffuse knapweed.

After monitoring the plant community at six sites in White Lake, British Columbia, Stephens et al. (2009) found that exotics were quicker to capitalize on the available space left by declining diffuse knapweed, even though native plants were more species rich and abundant at the beginning of the study. There was a small increase in relative native plant cover, but exotic plants were able to colonize a larger proportion of the newly available space. There was no observed increase in species richness or diversity following knapweed reduction. The lack of native expansion is especially striking considering their abundance at these sites. However, at the end of the 4 yr study, native species still comprised about 80% of the relative plant cover, and the expansion of exotic species did not decrease the absolute cover of native plants (Stephens et al., 2009).

Together, these three studies from widely ranging geographic areas raise the concern that successful biological control may not always lead to successful restoration. If the reduction of the target weed leads to the dominance of several others for which control methods are not as well established, managers may be worse off than they were to start. It should also be noted that exotic grasses often fill in the available space when knapweed is reduced. In western rangeland systems, this grass has economic value as cattle forage (Giffith and Lacey 1991). In the Midwest, spotted knapweed is used as a nectar plant by a variety of pollinators, and reductions in its density that are not compensated with the addition of other nectar-producing species will translate to a net loss of floral resources.

Pollinators and invasive plants

Given the prevalence of introduced plants in modern ecosystems, interactions between invasive plant species and pollinator networks are common. Despite this, the impacts invasive plants have on pollinator communities are not yet well understood (Vanbergen, 2013). Bees (Hymenoptera: Anthophila) are responsible for the majority of plant pollination services in agriculture (Cane and Tepedino, 2001; Klein et al., 2007) and also contribute to the functioning of natural plant communities (Ollerton et al., 2011). Bee community structure is largely determined by the types and diversity of flowering plants available within a habitat, and increasing flowering plant species richness is linked to increasing bee species richness (Goulson 2004; Grundel et al., 2010; Potts et al., 2003; Wilson et al., 2009; Moron et al., 2009); thus, loss or alteration of native plant communities is a major concern for bee health.

Loss of floral resources, and in turn, loss of pollinator diversity in natural systems has been linked to invasions of non-native plant species. Because many exotic invasive plant species can decrease plant species richness and evenness of invaded communities, they can potentially have an adverse effect on native bees (Stout et al., 2009). However, when exotic plants become naturalized and reduce native floral resources, pollinator communities may come to rely on the nectar resources provided be the exotic species. Williams et al. (2011) found that many native bee species living in disturbed habitats primarily used exotic flowering plant species as nectar sources. The bees studied did not prefer exotic species, but rather used them at a rate proportional to their abundance in the flowering plant community.

Due to its tendency to form monotypic stands and its prevalence in sandy soils (McDonald et al., 2003), spotted knapweed is a super-abundant floral resource in parts of Michigan. While the negative impacts of this species on native plant communities are significant, spotted knapweed is likely to be providing significant floral resources to some native bees (pers. obs., author). The degree to which native bees use spotted knapweed is not yet known, though we do know that the plant is used by other pollinators, including the federally endangered Karner blue butterfly (*Lacaeides melissa samuelis*) (USFWS, 2003). Spotted knapweed also provides a nectar resource for the domesticated honeybee (*Apis mellifera).* After pollinating fruit crops such as apples and cherries in the early summer, many hives are transported to knapweed fields by beekeepers, and left by there through the summer. Beekeepers consider spotted knapweed, which they call star-thistle, to be an important nectar source in mid-summer months, when there is often a dirth of other floral resources (February 4, 2008 letter to United States Department of Agriculture Forest Service Huron-Manistee National Forests from the Michigan Beekeepers Association). Due to the floral resources spotted knapweed provide to a variety of pollinators and its prevalence in many landscapes, effort should be made to better understand the role spotted knapweed plays in supporting managed and wild pollinator communities. Furthermore, any attempt to control the plant should be coupled with the restoration of alternative floral resources.

APPENDIX

APPENDIX

Figure 1. Spotted knapweed flowerhead in bloom, being visited by *Halictus ligatus*, a common solitary bee in spotted knapweed-invaded fields (Photo by B. Carson). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Figure 2. Adult *Larinus minutus*. Body is densely pubescent, and the tarsi are red in color (Photo by WonGun Kim).

Figure 3. Adult *Larinus obtusus* on spotted knapweed flower (Photo by Eric Coombs, Oregon Department of Agriculture).

Figure 4. Adult *Cyphocleonus achates* captured during a sweep net survey at the 2010 Sharonville release site (Photo by B. Carson).

REFERENCES

REFERENCES

Albert, DA (1999) *Natural community abstract for open dunes* [Online]. Michigan State University, Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Open_dunes.pdf

Bais HP, Walker TS, Stermitz FR, Hufbauer RA, Vivanco JM (2002) Enantiomeric-dependent phytotoxic and antimicrobial activity of (+/-)-catechin. A rhizosecreted racemic mixture from spotted knapweed (Retracted article. See vol. 151, pg. 967, 2009). Plant Physiology 128**:**1173-1179

Bais HP (2010) Allelopathy and exotic plant invasion: From molecules and genes to species interactions (September, pg 1377, 2003). Science 327:781-781

Berube DE (1980) Interspecific competition between *Urophora affinis* and *U. quadrifasciata* (Diptera: Tephritidae) for ovipositional sites on diffuse knapweed (*Centaurea diffusa*: Compositae). Zeitschrift für Angewandte Entomologie 90:1 299-306

Blair AC, Hanson BD, Brunk GR, et al. (2005) New techniques and findings in the study of a candidate allelochemical implicated in invasion success. Ecology Letters 8:1039-1047

Blair AC, Schaffner U, Hafliger P, et al. (2008) How do biological control and hybridization affect enemy escape? Biological Control 46:358-370

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants - a hypothesis. Journal of Ecology 83:887-889

Blossey B, Skinner LC, Taylor J (2001) Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. Biodiversity and Conservation 10**:**1787-1807

Bourchier RS, Crowe ML (2011) Role of plant phenology in mediating interactions between two biological control agents for spotted knapweed. Biological Control 58:367-373

Bourchier RS, Mortensen K, Crowe M, (2002) *Centaurea diffusa* Lamarck, Diffuse Knapweed, and *Centaurea maculosa* Lamarck, Spotted Knapweed (Asteraceae). *Biological Control Programmes in Canada 1981–2000*. CABI, Wallingford, UK, pp. 302–313

Broz AK, Manter DK, Bowman G, et al. (2009) Plant origin and ploidy influence gene expression and life cycle characteristics in an invasive weed. BMC Plant Biology, 9:33

Broz AK, Manter DK, Vivanco JM (2007) Soil fungal abundance and diversity: another victim of the invasive plant *Centaurea maculosa*. Isme Journal 1:763-765

Bush RT, Seastedt TR, Buckner D (2007) Plant community response to the decline of diffuse knapweed in a Colorado grassland. Ecological Restoration 25:169-174

Caesar AJ, Campobasso G, Terragitti G (2002) Identification, pathogenicity and comparative virulence of *Fusarium* spp. associated with insect-damaged, diseased *Centaurea* spp. in Europe. Biological control 47**:**217-229

Callaway R, DeLuca T, Belliveau W (2000) Biological-control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. Ecology 81:3259-3259

Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290:2075-2075

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2:436-443

Callaway RM, Thelen GC, Barth S, et al. (2004) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. Ecology 85:1062-1071

Cane JH, Tepedino VJ (2001) Causes and extent of declines among native North American invertebrate pollinators: Detection, evidence, and consequences. Conservation Ecology 5**:**1

Cappuccino N, Carpenter D (2005) Invasive exotic plants suffer less herbivory than non-invasive exotic plants. Biology Letters 1**:**435-438

Carpenter D, Cappuccino N (2005) Herbivory, time since introduction and the invasiveness of exotic plants. Journal of Ecology 93**:**315-321

Clark SE, Van Driesche RG, Sturdevant N, et al. (2001) Effect of root feeding insects on spotted knapweed (*Centaurea maculosa*) stand density. Southwestern Entomologist 26:129-135

Cohen, JG (2000) *Natural community abstract for oakpine barrens* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Oak-pine_barrens.pdf

Cohen, JG (2001) *Natural community abstract for oak barrens* [Online]. Michigan State University, Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Oak_barrens.pdf

Corn JG, Story JM, White LJ (2006) Impacts of the biological control agent *Cyphocleonus achates* on spotted knapweed, *Centaurea maculosa*, in experimental plots. Biological Control 37:75-81

Corn JG, Story JM, White LJ (2007) Effect of summer drought relief on the impact of the root weevil *Cyphocleonus achates* on spotted knapweed. Environmental Entomology 36:858-863

Emery SM, Gross KL 2005. Effects of timing of prescribed fire on the demography of an invasive plant, spotted knapweed *Centaurea maculosa*. Journal of Applied Ecology 42**:**60-69

Emery SM, Gross KL (2006) Dominant species identity regulates invasibility of old-field plant communities. Oikos 115:549-558

Goulson D, Darvill B (2004) Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? Apidologie 35**:**55-63

Griffith D, Lacey JR (1991) Economic Evaluation of Spotted Knapweed (*Centaurea maculosa*) Control Using Picloram. Journal of Range Management 44:1 43-47

Groppe K, Sobhian R, Kashefi J (1990) A field experiment to determine host specificity of *Larinus curtus* Hochhut (Col., Curculionidae) and *Urophora sirunaseva* Hg. (Dipt., Tephritidae), candidates for the biological control of *Centaurea solstitialis* L. (Asteraceae), and *Larinus minutus* Gyllenhal, a candidate for biological control of *C. maculosa* Lam. and *C. diffusa* Lam. J. Appl. Ecol. 110:300-306

Grundel R, Jean RP, Frohnapple KJ, Glowacki GA, Scott PE, Pavlovic NB (2010) Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient. Ecological Applications 20:1678-1692

Hakim, SA (1979) Range condition on the Threemile Game Range in western Montana. Missoula, MT: University of Montana. 62 p. Thesis.

He WM, Feng YL, Ridenour WM, et al. (2009) Novel weapons and invasion: biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (+/-)-catechin. Oecologia 159:803-815

Hooper DU, Johnson L (1999) Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation. Biogeochemistry 46**:**247-293

Hufbauer RA, Sforza R (2008) Multiple introductions of two invasive *Centaurea* taxa inferred from cpDNA haplotypes. Diversity and Distributions 14:252-261

Jacobs JS, Sing SE, Martin JM (2006) Influence of herbivory and competition on invasive weed fitness: Observed effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and grass-seeding treatments on spotted knapweed performance. Environmental Entomology 35:1590-1596

Jakobs G, Weber E, Edwards PJ (2004) Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. Diversity and Distributions 10:11-19

Jogesh T, Carpenter D, Cappuccino N (2008) Herbivory on invasive exotic plants and their non-invasive relatives. Biological Invasions 10**:**797-804

Jordan K (1995) Host specificity of *Larinus minutus* Gyll (Coleoptera, Curculionidae), an agent introduced for the biological control of diffuse and spotted knapweed in North America. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 119:689-693

Kashefi JM, Sobhian R (1998) Notes on the biology of *Larinus minutus* Gyllenhal (Col., Curculionidae), an agent for biological control of diffuse and spotted knapweeds. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 122:547-549

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B-Biological Sciences 274**:**303-313

Knochel DG, Flagg C, Seastedt TR (2010a) Effects of plant competition, seed predation, and nutrient limitation on seedling survivorship of spotted knapweed (*Centaurea stoebe*). Biological Invasions 12:3771-3784

Knochel DG, Monson ND, Seastedt TR (2010b) Additive effects of aboveground and belowground herbivores on the dominance of spotted knapweed (*Centaurea stoebe*). Oecologia 164:701-712

Knochel DG, Seastedt TR (2010) Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. Ecological Applications 20:1903-1912

Kost MA (2004) *Natural community abstract for dry-mesic prairie.* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Dry-mesic_prairie.pdf

Kost MA & Slaughter BS (2009) *Natural community abstract for mesic sand prairie* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Mesic_sand_prairie.pdf

Lacey JR, Marlow CB, Lane JR (1989) Influence of spotted knapweed (*Centaurea maculosa*) on surface runoff and sediment yield. Weed Technology 3**:**627-631

Lang RF, Richard RD, Winkler J, et al. (2001) Distribution of *Urophora affinis* and *U. quadrifasciata* (Diptera : Tephritidae) for biological control of spotted knapweed (*Centaurea maculosa*) and diffuse knapweed (*Centaurea diffusa*) in Michigan. Great Lakes Entomologist 34:31-42

Lee, JG (2007) *Natural community abstract for dry-mesic southern forest.* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Dry-mesic_southern_forest.pdf

Lejeune KD, Suding KN, Sturgis S, et al. (2005) Biological control insect use of fertilized and unfertilized diffuse knapweed in a Colorado grassland. Environmental Entomology 34:225-234

Louda SM, Rand TA, Arnett AE, et al. (2005) Evaluation of ecological risk to populations of a threatened plant from an invasive biological control insect. Ecological Applications 15:234-249

Macdonald NW, Koetje MT & Perry BJ (2003) Native warm-season grass establishment on spotted knapweed-infested gravel mine spoils. Journal of Soil and Water Conservation 58**:**243-250

MacDonald NW, Scull BT, Abella SR (2007) Mid-spring burning reduces spotted knapweed and increases native grasses during a Michigan experimental grassland establishment. Restoration Ecology 15:118-128

Marshall JM, Storer AJ (2008) Comparative analysis of plant and ground dwelling arthropod communities in lacustrine dune areas with and without *Centaurea biebersteinii* (Asteraceae). American Midland Naturalist 159**:**261-274

Moron D, Lenda M, Skorka P, et al. (2009) Wild pollinator communities are negatively affected by invasion of alien goldenrods in grassland landscapes. Biological Conservation 142:1322-1332

Mullerscharer H, Schroeder D (1993) The biological-control of *Centaurea* spp. in North America - do insects solve the problem. Pesticide Science 37:343-353

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288**:**81-90

Myers JH, Risley C, Eng R (1990) The ability of plants to compensate for insect attack: why biological control of weeds with insects is so difficult. Proceedings of the VII International Symposium on Biological Control of Weeds. 1990 67-73

Myers JH, Risley C (2000) Why reduced seed production is not necessarily translated into successful biological weed control. Proceedings of the X International Symposium on Biological Control of Weeds, Bozeman, Montana, USA, 4-14 July. 1999:569-581

Myers JH (2007) Control of Weeds: a Case Study with Diffuse Knapweed. *Biological Control: A Global Perspective: Case Studies from Around the World*:70

Myers JH, Jackson C, Quinn H, et al. (2009) Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control 50:66-72

Notzold R, Blossey B, Newton E (1998) The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. Oecologia 113:82-93

Ochsmann, J (2001) On the taxonomy of spotted knapweed (*Centaurea stoebe* L.). Proceedings, 1st International Knapweed Symposium of the 21st century; 2001 March 15-16; Coeur d'Alene, ID. Albany, CA: U.S. Department of Agriculture, Agricultural Research Service: 33-41

Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120**:**321-326

Olliff T, Renkin R, McClure C, et al. (2001) Managing a complex exotic vegetation program in Yellowstone National Park. Western North American Naturalist 61:347-358

Ortega, YK, McKelvey KS, Six DL (2006) Invasion of an exotic forb impacts reproductive success and site fidelity of a migratory songbird. Oecologia 149**:**340-351

Ortega YK, Pearson DE (2005) Weak vs. strong invaders of natural plant communities: Assessing invasibility and impact. Ecological Applications 15**:**651-661

Otway SJ, Hector A, Lawton JH (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. Journal of Animal Ecology 74:2 234-240

Pearson DE, Callaway RM (2008) Weed-biocontrol insects reduce native-plant recruitment through second-order apparent competition. Ecological Applications 18:1489-1500

Pearson DE (2009) Invasive plant architecture alters trophic interactions by changing predator abundance and behavior. Oecologia 159**:**549-558

Perry LG, Thelen GC, Ridenour WM, Callaway RM, Paschke MW, Vivanco JM (2007) Concentrations of the allelochemical (+/-)-catechin in *Centaurea maculosa* soils. Journal of Chemical Ecology 33**:**2337-2344

Potts SG, Vulliamy B, Dafni A, Ne'eman G, Willmer P (2003) Linking bees and flowers: How do floral communities structure pollinator communities? Ecology 84:2628-2642

Reinhart KO, Rinella M (2011) Comparing susceptibility of eastern and western US grasslands to competition and allelopathy from spotted knapweed *Centaurea stoebe* L. subsp *micranthos* (Gugler) Hayek. Plant Ecology 212:821-828

Reznicek AA, Voss EG, Walters B S (2011) *MICHIGAN FLORA ONLINE* University of Michigan. Web. 11-14-2013. http://michiganflora.net/home.aspx. (Accessed 15 November 2013)

Ridenour WM, Callaway RM (2001) The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia 126:444-450

Ridenour WL, Callaway RM (2003) Root herbivores, pathogenic fungi, and competition between *Centaurea maculosa* and *Festuca idahoensis*. Plant Ecology 169:161-170

Ridenour WM, Vivanco JM, Feng YL, et al. (2008) No evidence for trade-offs: *Centaurea* plants from America are better competitors and defenders. Ecological Monographs 78:369-386

Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). Ecological Monographs 43:95–124

Rosburg TR (2001) Effects of late spring fires on the survival, growth, and reproduction of prairie forbs. Proceedings of the 17th N.A. Prairie Conference 48-58

Seastedt TR, Gregory N, Buckner D (2003) Effect of biocontrol insects on diffuse knapweed (*Centaurea diffusa*) in a Colorado grassland. Weed Science 51:237-245

Seastedt TR, Knochel DG, Garmoe M, et al. (2007) Interactions and effects of multiple biological control insects on diffuse and spotted knapweed in the Front Range of Colorado. Biological Control 42:345-354

Seastedt TR, Suding KN (2007) Biotic constraints on the invasion of diffuse knapweed (*Centaurea diffusa*) in North American grasslands. Oecologia 151:626-636

Seastedt TR (2007) Plant ecology - Resourceful invaders. Nature 446**:**985-986

Sheley RL, Larson LL (1996) Emergence date effects on resource partitioning between diffuse knapweed seedlings. Journal of Range Management 49:241-244

Sheley RL, Jacobs JS, Carpinelli MF (1998) Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technology 12:353-362

Smith L (2004) Impact of biological control agents on *Centaurea diffusa* (diffuse knapweed) in central Montana. Proceedings of the XI International Symposium on Biological Control of Weeds, Canberra, Australia, 27 April - 2 May, 2003:589-593

Stephens AEA, Krannitz PG, Myers JH (2009) Plant community changes after the reduction of an invasive rangeland weed, diffuse knapweed, *Centaurea diffusa*. Biological Control 51:140-146

Stinson CSA, Schroeder D, Marquardt K (1994) Investigations on *Cyphocleonus achates* (Coleoptera: Curculionidae), a potential biological-control agent of spotted knapweed (*Centaurea maculosa*) and diffuse knapweed (*Centaurea diffusa*) (Compositae) in North America. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 117:35-50

Story JM (1992) Biological control of weeds: selective, economical and safe. Western Wildlands 18:18-23

Story JM, Callan NW, Corn JG, et al. (2006) Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biological Control 38:227-232

Story JM, Corn JG, White LJ (2010) Compatibility of Seed Head Biological Control Agents and Mowing for Management of Spotted Knapweed. Environmental Entomology 39:164-168

Story JM, Good WR, White LJ (1997) First report of the establishment of *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) on spotted knapweed, *Centaurea maculosa* Lamarck, in the United States. Canadian Entomologist 129:373-374

Story JM, Smith L, Corn JG, et al. (2008) Influence of seed head-attacking biological control agents on spotted knapweed reproductive potential in western Montana over a 30-year period. Environmental Entomology 37:510-519

Story JM, Smith L, Good WR (2001) Relationship among growth attributes of spotted knapweed (*Centaurea maculosa*) in Western Montana. Weed Technology 15:750-761

Story JM, Stougaard RN (2006) Compatibility of two herbicides with *Cyphocleonus achates* (Coleoptera : Curculionidae) and *Agapeta zoegana* (Lepidoptera : Tortricidae), two root insects introduced for biological control of spotted knapweed. Environmental Entomology 35:373-378

Susanna A, Galbany-Casals M, Romaschenko K, Barres L, Martin J, Garcia-Jacas N (2011) Lessons from *Plectocephalus* (Compositae, Cardueae-Centaureinae): ITS disorientation in annuals and Beringian dispersal as revealed by molecular analyses. Annals of Botany 108**:**263-277

Thelen GC, Vivanco JM, Newingham B, et al. (2005) Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. Ecology Letters 8:209-217

Thorpe AS, Thelen GC, Diaconu A, et al. (2009) Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. Journal of Ecology 97:641-645

Treier UA, Broennimann O, Normand S, et al. (2009) Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. Ecology 90:1366-1377

USFWS (2003) Final recovery plan for the Karner Blue Butterfly (*Lycaeides melissa samuelis*). U.S. Fish and Wildlife Service, Fort Snelling, Minnesota.

Vanbergen AJ, Baude M, Biesmeijer et al. (2013) Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment 11**:**251-259

Watson AK, Renney AJ (1974) Biology of Canadian weeds *Centaurea diffusa* and *Centaurea maculosa*. Canadian Journal of Plant Science 54:687-701

Williams, NM, Cariveau D, Winfree R, Kremen C (2011) Bees in disturbed habitats use, but do not prefer, alien plants. Basic and Applied Ecology. 12: 332-341

Wilson JS, Messinger OJ, Griswold T (2009) Variation between bee communities on a sand dune complex in the Great Basin Desert, North America: Implications for sand dune conservation. Journal of Arid Environments 73:666-671

Wilson LM, Randall CB (2005) Biology and biological control of knapweeds. U.S. Forest Service Publication

CHAPTER 2

ESTABLISHMENT, IMPACTS, AND CURRENT RANGE OF SPOTTED KNAPWEED BIOLOGICAL CONTROL INSECTS IN MICHIGAN

Introduction

Spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos* (Gugler)) is a plant native to Southern and Eastern Europe. In its home range, the species occurs at relatively low densities, and is well integrated into grassland communities (Sheley et al., 1998). In contrast, in North America spotted knapweed is highly invasive and frequently becomes the dominant species in grassland, rangeland, and old field habitats. Spotted knapweed is now found throughout much of the lower 48 United States with the exceptions of Texas, Oklahoma, and Mississippi (USDA, 2013).

Considered a pest in western United States rangelands, it is estimated that spotted knapweed costs ranchers as much as 155 million dollars in annual gross revenue in Montana alone (Griffith and Lacey, 1991). Spotted knapweed has been shown to cause dramatic decreases in plant diversity, ecosystem functioning, and utilization of land by wild and domesticated foraging animals (Sheley et al., 1998; Mummey and Rillig, 2006; Lacey et al., 1989; Hakim, 1979; Watson and Renny, 1974).

In the Eastern and Midwestern United States, spotted knapweed has also become a serious invader, threatening the ecological integrity of natural areas in the region. In Michigan, spotted knapweed has been documented as an invader of rare native ecosystems including dry sand prairies, dry-mesic prairies (Kost, 2004), mesic sand prairies (Kost and Slaughter, 2009), dry-mesic Southern forests (Lee, 2007), oak barrens (Cohen, 2001), oak-pine barrens (Cohen, 2000), and open dunes, where it is often considered a primary threat to biodiversity (Albert, 1999). At the Pictured Rocks National Lakeshore in Northern Michigan, Marshall and Storer (2008) found that spotted knapweed invasion of open dune habitats had adverse effects on native plant communities and altered insect community composition. Native dune plants are adapted to the constantly changing structure of sand dunes. Spotted knapweed has been able to colonize the dunes and stabilize the sand, which facilitates invasion by other exotic plants. This change in the plant community and dune stability has altered the insect community, increasing the abundance of ants and weevils (Marshall and Storer 2008).

Despite the efforts of land managers, conventional control methods such as herbicide and mowing have been ineffective in providing adequate control of spotted knapweed. Western states, notably Montana, Colorado, and Washington have spearheaded the effort to use classical biological control to slow and reverse the invasion of spotted and diffuse (*Centaurea diffusa*) knapweeds, (Watson and Renney, 1974; Story and Anderson 1978; Sheley and Jacobs, 1997; Corn et al., 2009). More recently, agencies in the eastern United States have begun making their own biological control releases targeting spotted knapweed (http://www.biocontrol.entomology.cornell.edu/weedfeed/Larinus).

Biological control of spotted knapweed in Michigan began with the release of two species of seedhead flies, *Urophora affinis* and *U. quadrifasciata* (Diptera: Tephritidae). The two flies were released in Isabella County in 1994 by USDA APHIS PPQ. Subsequent surveys in 1998-2000 detected *U. quadrifasciata* in all 83 Michigan counties and *U. affinis* in 24 counties (Lang et al., 2001). A 2009 survey by Landis and Sebolt (unpub. data) show that both seedhead flies remain established in both peninsulas in Michigan. The high rates of infestation (*U. quadrifasciata* averaged 78.1% and *U. affinis* averaged 52.5%) by the seedhead flies combined with the overall abundance of knapweed at these sites confirm the reported inefficiency of these two agents (Myers et al., 2009).

In 2007, USDA-APHIS issued permits for the interstate transport of the root-boring weevil *Cyphocleonus achates* (Coleoptera: Curculionidae) and the two seedhead-feeding weevils *Larinus minutus* and *Larinus obtusus* (Coleoptera: Curculionidae) into Michigan. Releases of these insects were subsequently made at two sites in Southern Michigan (**Table 1**). In 2009, the USDA-Forest Service received a permit to conduct releases of *C. achates* and *L. minutus* in the western part of Michigan's upper peninsula. Due to the concerns of commercial beekeepers in Michigan, who value spotted knapweed as a midsummer nectar source, further issuance of biological control release permits was suspended in 2008.

While performing preliminary searches for knapweed biological control insects in 2011, we unexpectedly found *L. minutus* in four counties in the south-central part of the state, far from any known Michigan release sites. With additional background research, we discovered that the Indiana Department of Natural Resources had made releases of *L. minutus* and *C. achates* in Bristol, Indiana, at a site 2.5 km from the Michigan border in 1996 (**Table 1**), and we currently believe this to be the most likely source of the *L. minutus* population occurring in south-central Michigan.

Following the 2007 and 2009 releases, increased interest in spotted knapweed biological control among land managers prompted the Michigan Department of Natural Resources (MDNR) to move forward with controlled experimental releases on MDNR property. They approached

Michigan State University with a request to set up several experimental biological control release sites. The goal of these releases is to test the efficacy of spotted knapweed biological control in Michigan while simultaneously investigating methods for establishing native flowering plant species that may supplement the floral resources the plant provides to native and managed pollinators. Here we report the initial establishment and dispersal of these recently introduced biological control insects, their impacts on knapweed populations, the current range of each species in Michigan and native plant establishment.

Methods

Site selection and control agent releases

Sites: In collaboration with MDNR staff, we identified 6 sites in Michigan during the summer of 2009 in which the impacts of knapweed biological control release and re-vegetation with native nectar plants could contrasted (2010 release sites, **Table 1).** These sites were all located on state-owned lands and formed a north-south transect allowing for the exploration of latitudinal differences in establishment and effectiveness of biological control agents. At each site we selected potential biological control release and control (no-release) spotted knapweed-dominated fields on similar soils and located ≥ 800 m apart (**Figure 5**).

Sites were monitored in fall 2009 to ensure the absence of biological control agents other than *Urophora* spp. In late-July through mid-August, 2010 we visited each site to conduct initial monitoring. In release fields, we established a grid of 3 x 3 m plots with 1 m buffers (n=16) to allow for knapweed monitoring and replicated trials of native plant overseeding (**Figure 5**). In control fields we established four 5 x 5 m plots to monitor the plant community and potential dispersal of biological control agents. We collected baseline plant community and knapweed demographic data using the methods detailed below. Permanent photo points were established by taking photos of each set of plots from a distance of 15 m, facing the plots in each of the four cardinal directions. At the Seney location we established two release fields (North and South) and a single control field. The North release site was set up as the site for native plant restoration research and the South site as a biological control release evaluation site only. Thus, the South site is similar in design to a control field in that it only has four plots to monitor changes in plant communities.

Agent collection: In collaboration with Monika Chandler of the Minnesota Department of Agriculture (MNDA), adult *L. minutus* and *C. achates* were field collected from well-established sites near Bemidji, Minnesota on August 9-10, 2010. Personnel slowly walked through infested sites and hand collected adults into small cups. At the end of each day, all *Larinus* were carefully examined and any suspected *L. obtusus* were removed with the intent that only *L. minutus* were included in Michigan releases. Insects were provided fresh knapweed foliage and held at 4-6 C. Representative specimens of each species were retained to be deposited as vouchers in the A.J. Cook Arthropod Research Collection at Michigan State University.

Agent release: On August 11-13, releases of both biological control agents were made at all 6 release sites (**Table 1**). Insects were pre-sorted into plastic vials, four with *C. achates* and four with *L. minutus*. The total number available for release at each site (368 *L. minutus*, 41 *C. achates*) were divided evenly among the four containers and kept in a cooler at approximately 6

C. At the time of release, containers were opened at the base of knapweed plants at four equidistant locations within each release site **(Figure 5).**

Native plant seed additions at biological control release sites: We developed “low” and “high” diversity mixes of native plants for re-vegetation of knapweed sites undergoing biological control. Both contained a mix of native grasses and forbs that were comparatively low-cost, readily available, provided a range of flowering times, and were deemed appropriate for sites typical of knapweed infestations. (i.e. dry to dry mesic soils, full to partial sun, generally low fertility). To choose plants for each mix we started from Michigan Natural Features Inventory lists of plants common to such sites historically (oak barrens, pine barrens, dry sand, and dry-mesic prairies) and use additional resources to identify: plants native throughout Michigan (both upper and lower peninsulas) from Voss’ Michigan Flora, and that were currently available as seed from Michigan native plant producers (Michigan Native Plant producers website and associated catalogs). Our low diversity mix contained eight forb species and four grass species, and our high diversity mix contained 16 forb species and four grass species. The total weight of grasses and forbs was kept equal in both treatments, allowing us to investigate the effect seed treatment diversity has in re-establishment success.

Native plant seed mixes were overseeded into 3.0 m x 3.0 m experimental plots (**Figure 5**) in the fall of 2011, with four plots receiving the high diversity mix, four plots receiving the low diversity mix, four designated as no-seed treatments, and four plots left without treatment. We used a completely randomized design, and assigned treatments at random. In 2012, plots that had received no treatment in 2011 were seeded with the high diversity mix, using fresh seeds purchased from Michigan Wildflower Farm. Prior to the 2012 seeding, the western half of each plot to be seeded was disturbed with a rake, creating “safe sites” for seeds to germinate in. Seed mixes for each plot were prepared in the lab and stored in airtight plastic cups until seeding. In the field, the perimeter of each plot was delineated with a 1.0 m tall corrugated plastic frame to confine the seed to the exact site. The seed mix for each plot was evenly mixed with sand and 100 ml of water, and this mixture hand-sown evenly within the plots receiving seed treatments. All seeding was conducted after the first frost and in the period between October 15 and November 15. Research plots at control sites received no seeding treatment.

Weevil establishment at 2010 release sites

To monitor the establishment and growth of *L. minutus* and *C. achates* populations, each release site was surveyed in 2011, 2012 and 2013. Surveys occurred during mid-July and mid-August, when the greatest number of adults were expected to be present. This expectation was verified by the results of our phenological study (Carson Chapter 3). When possible, insect populations were sampled during warm days, when insects are most active.

At each site, 200 sweeps with a standard sweep net were made within 10 m of the perimeter of the research plots. These were made within 10 m of each side (North, South, East, West) of the plots, with 50 sweeps taken from each side. The sweeps were focused on dense knapweed patches where the insects were likely to be located. The contents of each set of 50 sweeps were examined, and *C. achates* and *L. minutus* weevils were counted. A subsample of 20 *Larinus* spp. individuals were kept for identification. When fewer than 20 individuals were captured, we collected as many as possible. These were brought back to the lab and frozen, and then identified to species level.

Sweeps were also conducted at each control site to determine whether biological control insects had spread to the control site from the release point. These were made within 10 m of each side (North, South, East, West) of the plots, with 25 sweeps taken from each side (100 total). Fewer sweeps were taken at the control sites because the research plot area was smaller than that at the release sites.

In May 2012, a minimum of 40 randomly selected knapweed roots were collected from each release site and inspected for *C. achates* larvae. The roots were collected from outside of the research plot area, with 10 roots taken from each side. Roots were placed in a cooler and dissected in the lab for *C. achates* larvae and evidence of feeding damage. Larvae were identified, counted, and preserved in ethanol as voucher specimens.

In addition to conducting biological control weevil population monitoring at the release sites, we sampled the surrounding landscape to detect outward dispersal from the release sites. See Carson Thesis Chapter 3 for a complete description of dispersal sampling methodology. In all dispersal sampling we used detectability as a proxy for measuring dispersal, and recognize that the insects likely dispersed outward from release sites in stages, and not nesessarily in single dispersal events. We evaluated dispersal rates using several metrics. The “maximum dispersal detected” was defined as the furthest dispersal observed at a site. The “average maximum dispersal detected” was defined as the mean of the maximum dispersal observed for each cardinal direction (n=4). To calculate the “average rate of spread between 2012 and 2013” we took the difference between the maximum detected dispersal in each cardinal direction from 2012 and 2013, and averaged these four values (n=4).

Native plant establishment and biological control impacts on the plant community

In the early, mid, and late summer in 2012, and the early and late summer in 2013, we searched each 3 x 3 m research plot for signs of establishment of the native plant mixes. Close to the end of knapweed’s peak bloom period in early-mid August, we took measurements of plant community diversity and knapweed population demographics at each site in 2011-2013. In each year we began sampling at southern sites, working our way northward.

Within each 3 x 3 m research plot, we established a permanent 1 x 1 m sampling quadrat (**Figure 5**). Within this quadrat, two researchers independently evaluated the percent cover of each plant species. The average of the two estimates was recorded. Within one 20 x 50 cm microplot located in the southwestern corner of each 1 x 1 m quadrat, we counted the number of knapweed rosettes, stems, adult plants, and seedheads, and measured the height of the five tallest knapweed plants. To investigate biological control impacts on spotted knapweed populations, we compared the percent cover, rosette density, and mean plant height of *C. stoebe* across years at the release and control sites using Repeated Measures ANOVA. For all statistical tests alpha = 0.05.

State-wide distribution of knapweed biological control insects

To evaluate the statewide distribution, density, and rate of spread of spotted knapweed biological control insects in Michigan, we conducted sweep net surveys along roadsides in 2011-2013.

Sampling was conducted within the peak of *Larinus* spp. activity, between June 20 and July 25 (See *Larinus* spp. phenology, Carson thesis chapter 3). Once a location was chosen for sampling and its longitude and latitude were recorded (Garmin GPS 2 Plus), we took 60 sweeps in the knapweed patch with a standard 37 cm diameter sweepnet. The sweeps were aimed at the top half of the plants, where adult *Larinus* spp. and *C .achates* are reported to reside (Wilson and Randall, 2005; Stinson et al., 1994). After 60 sweeps were taken, the contents of the sweepnet were examined for *Larinus* spp. and *C. achates*. Any biological control weevils were counted, and where possible, 20 *Larinus* spp. were collected for identification at the lab. If fewer than 20 individuals were found, all of them were collected.

Surveys for knapweed biological control insects were conducted along arterial highways and connecting main roads throughout much of Michigan's lower peninsula (except Northeast) at intervals of approximately 20 km. Starting in Southern Michigan and proceeding northward, a circular driving route was planned that encompassed a quadrant of the state that had not yet been sampled that season. While driving, we located patches of spotted knapweed along the roadside or at highway on-ramps. To qualify for sampling, patches had to be a minimum of 50 m^2 in area and contain at least 70% cover of spotted knapweed. After sampling we returned to driving along the designated route. After traveling 15 km, we began searching for another patch of spotted knapweed to sample. This process was repeated until we completed the route.

Due to limited resources, we could not intensively survey spotted knapweed throughout the state. Therefore, we concentrated our sampling efforts in the southern part of the state, where we knew the biological control insects *L. minutus*, *L. obtusus*, and *C. achates* had been released in 2007.

During this sampling in 2011-2013, it became apparent that there was a large unexplained and nearly contiguous population of *L. minutus* in Southwestern Michigan that may have originated from a release made in Bristol, Indiana (**Table 1**). To delineate the edges of this population, we narrowed the distance between samplings to 1.5 km as we approached the suspected edge of the population (i.e. as detections decreased). Sampling was continued at this distance until two samples in a row were negative. Sampling then resumed along the designated route at intervals of approximately 20 km.

In 2011, we sampled 29 different sites in 24 counties. In 2012, we sampled 66 sites in 28 counties, and in 2013 we sampled 74 sites in 28 counties. We also obtained knapweed biological control monitoring data from the USFS that was taken from three counties in the Western Upper Peninsula of Michigan.

Results

Release observations

As they warmed to ambient temperatures, both species of biological control agents became active, leaving the release containers and climbing nearby plants. At the Sharonville site, observations were made for the initial hour after release. Adult *C. achates* generally climbed and remained motionless near the top of knapweed stems. In contrast, *L. minutus* were more active and began to seek out spotted knapweed flowers. One adult was observed to fly from the release area and orient towards and land on a flower about 1.5 m away. The individual flew towards the flower and while in the air, circled it at a distance of a few centimeters before landing directly on the flower. Once on the flower, the adults would typically crawl head first into the flower where they remained with abdomen visible.

Weevil establishment at 2010 release sites

In 2011, adult *L. minutus* were recovered at Grayling and Houghton Lake, and adult *C. achates* at the two Seney sites (**Table 3**). In 2012, adult *L. minutus* were recovered at all six release sites and adult *C. achates* were recovered at two additional sites, Grayling and Houghton. Finally, in 2013, adult *L. minutus* were recovered at all six sites and adult *C. achates* were recovered at Sharonville and again at Seney Release 1. In all, *C. achates* was recovered from every site except Flat River over the 3 yr sampling period. Additionally, with the exception of Grayling in 2013, the observed density of *L. minutus* increased each year at every site.

Root sampling did not prove to be an effective means of detecting *C. achates*. Houghton Lake was the only site that we found any larvae in spotted knapweed roots, despite having recovered adult weevils at five of the six release sites using sweep nets. This monitoring technique may be more effective once populations have reached higher densities (Clark et al. 2001a).

Individual beetles appearing to be *L. obtusus* were found at Sharonville and Grayling in 2012, and Flat River, Houghton Lake, and Sharonville in 2013, indicating that there were probably low numbers of *L. obtusus* mixed in with the stock of *L. minutus* that were released.

Of the five 2010 release sites, we detected biological control insect dispersal beyond the immediate release zone at two sites in 2012, and at four sites in 2013 (**Table 4**). In 2012, we collected *L. minutus* weevils 100 m East of the release point at Flat River, and 100 m North of the release point at Sharonville. In 2013, we detected dispersal 100 m to the North of the release point at Camp Grayling, 100 m East and 1800 m South of the release at Houghton Lake, and 100 m in all four cardinal directions at both Sharonville and Flat River. Additionally, at Flat River we detected weevil dispersal 500 m to the South and 600 m to the West. No weevil dispersal was detected from Seney release site. Between 2012 and 2013, the average rate of spread was 175 m (+/- 92 m). All *Larinus* spp. recovered beyond the release points occurred in low densities, usually less than 0.05 weevils per sweep.

Native plant establishment and biological control impacts on the plant community

The yearly plant community and knapweed demographic sampling from 2010-2013 did not provide clear evidence of biological control agent impact. While we detected statistically significant differences in the mean number of *C. stoebe* rosettes, mean *C. stoebe* plant height, and mean *C. stoebe* percent cover between many release and control sites **(Figures 6-10)**, these differences do not follow a consistent pattern, and the release sites never showed a decrease in these metrics over time that was not also matched by the control site. For example, consistent differences in mean knapweed cover at Seney (**Figure 6**) and in mean rosette number and plant height at Flat River probably reflect preexisting field-level conditions. While rosette number consistently declined at the Flat River release site, similar changes occurred at the control site. Thus there was no indication that these differences were caused by the effect of newly introduced biological control weevils.

We detected no germination of native plants introduced to the release sites, with the exception of trace amounts of *Monarda punctata* found in one 2011 seeded plot in 2013. This species was not found elsewhere at the site and it is likely these seedlings originated from our seed mix, though this is not definitive. Apart from this single occurrence, no introduced species were found growing in plots seeded in 2011 with high diversity and low diversity treatments, or in plots seeded in 2012 with high diversity treatments.

State-wide distribution of knapweed biological control insects

In 2011, we found *C. achates* at two sites in Schoolcraft County in the Upper Peninsula. In 2012 we found *C. achates* in two additional counties, and by 2013, C. *achates* had been recovered in five counties. All of the sites of *C. achates* recovery were known to be release sites for the species (**Figure 11A**). *C. achates* was never recovered from the 2009 USFS release sites in the western upper penninsula. *C. achates* was never observed more than 10 m from a known release.

In 2011, we found *L. minutus* in eight counties in the lower penninsula, and it was also recovered by the USFS in three additional counties in the Upper Peninsula (**Figure 11B**). Seven of the counties it was found in were known to be release sites of the species, but four counties in the south-central part of the state did not contain any known release sites. During 2012, we found *L. minutus* in 17 counties. Again, the species was found in eight south-central counties that had made no known releases. In these counties *L. minutus* occurred in high abundances, with the density upon sampling often exceeding one weevil per sweep. It seemed unlikely that these *L. minutus* populations had spread and grown from any known release site in Michigan, because weevils at the known release sites of *L. minutus* appeared to be locally contained (Carson

Chapter 3). After additional background research, we discovered that the Indiana Department of Natural Resources had made releases of *L. minutus* and *C. achates* in Bristol, Indiana, at a site 2.5 km from the Michigan border. These releases were made in 1996, and we concluded that they are the most likely source of the *L. minutus* population occurring in south-central Michigan. As part of our work documenting the extent of knapweed biological control insects in Michigan, we located the northernmost point of this population of *L. minutus* so we can monitor its future rate of spread.

In 2013 we observed an apparent continued expansion of Indiana population of *L. minutus*. They were found in six additional counties, and the northern-most point that they existed in detectable levels shifted 45 km northward. *L. minutus* continued to be recovered at all of the release sites in Michigan, but no additional populations were found outside of those derived from either the Indiana release or known Michigan releases.

In 2011 the only observed population of *L. obtusus* occurred in Kalamazoo County (**Figure 11C**), at the site of the 2007 release of this species. In 2012 we observed a dispersal of this population to two additional counties directly west of the release site. We also recovered *L. obtusus* at two sites (Grayling and Sharonville) where we had made releases of *L. minutus*. These recoveries indicate that there were probably small numbers of *L. obtusus* mixed in with the *L. minutus* released at those sites in 2010. By 2013 we had found *L. obtusus* in eight additional counties. Four of the additional counties are adjacent to the population established in Kalamazoo, and presumably a nearly continuous population of *L. obtusus* now occupies that region. Two of the additional counties were also 2010 release sites (Flat River and Houghton Lake), pointing again to the likelihood of small numbers of *L. obtusus* mixed in with *L. minutus* from the original collections in Minnesota. However, *L. obtusus* was also found in Lenawee and Monroe Counties, the two south-easternmost counties in the state. The nearest known *Larinus* spp. release to these two recovery sites was in Sharonville in neighboring Jackson County. However, at Sharonville we have only recovered two individual *L. obtusus* weevils and they were confined to the immediate release site, and thus it is unlikely that such a small population would have spread to Lenawee and Monroe counties.

Discussion

Of the six 2010 MSU knapweed biological control release sites, *L. minutus* has strongly established at the three southernmost sites, and seems likely to persist at all six sites. *C. achates* has been recovered in low numbers at five of the six sites, and its future persistence is somewhat less certain. However, *C. achates* has been reported to have a slow initial population growth rate (Story et al., 1997; Story and Stougaard, 2006), and it is entirely possible that all release sites will have robust populations of the root boring weevil in the future.

While we did observe establishment of *L. minutus* and *C. achates* at our release sites, our plant community data shows that there has not yet been a quantifiable impact on spotted knapweed. This is likely because biological control insects have not been present at release sites for long enough to reach the densities necessary to impact knapweed recruitment. It is uncertain whether seedhead predation by *Larinus* weevils alone will be enough to reduce spotted knapweed populations (Story et al. 2008).

Anecdotally, sites that are near to the 1996 Bristol, Indiana release still have knapweed populations that appear to be robust, despite the presence of *L. minutus* for over a decade. While *C. achates* was reported to be released at this site, it has not been recovered (pers. comm. Richard Dunbar, Indiana Department of Natural Resources). Studies conducted in the western United States and Canada suggest that while *Larinus* spp. are able to reduce diffuse knapweed, spotted knapweed is able to persist in the presence of substantial seedhead predation (Story et al. 2008).

Spotted knapweed's ability to maintain high densities in the early years of seedhead predation may be a reflection of the biology of both species. Large, dense populations of spotted knapweed usually occur on sites characterized by well-drained, coarse textured soils that have been relatively recently disturbed. Spotted knapweed is very competitive in these locations, and often is one of the only forb species present. Each knapweed plant is capable of producing up to 600 seeds, and production per square meter has been documented to range from 5,000 to 40,000 seeds (Sheley et al., 1998). Spotted knapweed's perennial nature allows each plant to contribute to the seedbank several times during its lifespan Story et al. (2008). These seeds persist in the seedbank for up to 9 yr (Sheley and Larson, 1996). Even if seedhead predators are able to infest most of the seedheads at a site, the seedbank still contains viable seeds, and it is likely that a significant number of new seeds are still added each year. Thus, it will take some time before spotted knapweed populations at these sites become seed-limited. However, several studies have observed sharp declines in the number of viable knapweed seeds in the seedbank less than 20 yr after *L. minutus* establishment (Story et al. 2008; Knochel et al. 2010a).

Western studies indicate that *C. achates* is the key to reducing spotted knapweed densities (Jacobs et al., 2006; Seastedt et al., 2003; Clark et al., 2001b; Story and Stougaard, 2006), and the combination of *L. minutus* and *C. achates* has an even stronger impact on knapweed populations (Knochel et al. 2010b). Because *C. achates* has been slow to establish at release sites in Michigan, it will be some time before we can confirm if a similar trend will occur in this region.

It is conceivable that seedhead predators that are able to disperse to newly colonizing knapweed populations could prevent them from establishing dominance. By reducing knapweed's seed production from the offset, they could allow other plant species to maintain a competetive balance with the newly-invading knapweed. We found that *L. minutus* was a relatively rapid disperser (see Carson thesis chapter 3), and thus it may be able to prevent newly established knapweed populations from spreading via the mechanism described above.

During the two years we monitored the plant communities at our biological control release sites, we did not find any evidence that the native plants seeded into experimental plots established. Other research has shown that is possible to germinate (Emery and Gross, 2006) and establish (McDonald et al., 2013) native plant species into spotted knapweed stands without additional control or soil preparation. Notably, the summer of 2012 was a drought year characterized by high temperatures, which may have decreased the survival potential of seedlings that did germinate. However, even after additional seeding in the fall of 2012, we saw no germination in 2013. It may be that 2 yr was simply not enough time for the seeds to germinate, though we had expected had expected to see at least a few species establish within the 2 yr study. We also consider it possible that the allelopathic effects of spotted knapweed (Callaway and Ridenour, 2004) may have prevented the survival of native plant seedlings. Another alternative hypothesis is that, as described above, the density of viable knapweed seeds in the soil was much greater than the density of introduced native seeds. This could have allowed knapweed seedlings to outcompete the native species by sheer seedling quantity.

In all three years of surveying for spotted knapweed biological control insects, *C. achates* was never observed outside of its original points of release. The reported mean daily travel rate of *C. achates* is 0.27 m, which would add up to less than 10 m over the adult insects lifespan (Rondeau, 2007). The reportedly slow dispersal rate of *C. achates* is consistent with the lack of dispersal that we saw in both our roadside and transect-based dispersal studies. *C. achates* has been shown to be a more effective biological control of spotted knapweed than *Larinus minutus* (Jacobs, 2006; Knochel et al., 2010), and this effectiveness coupled with its poor ability to self-disperse once it is released makes *C. achates* a good candidate for targeted biological control of spotted knapweed.

Throughout the three years of sampling, we demonstrated that Michigan contains multiple expanding populations of *L. minutus*. The origin of each population is believed to be known, and no unexplained populations were found in our roadside surveys. The six populations derived from the 2010 MSU release sites are growing, but all of them can only be detected within 2 km of their origin. These sites are located along a latitudinal gradient in the central part of the state, and *L. minutus* from the sites could eventually expand to form one continuous population. The population of *L. minutus* derived from the 2007 release at Lake Orion is still contained within Oakland County, though it is beginning to increase its rate of expansion. By far the largest of these populations is that which we believe descended from the 1996 release made in Bristol Indiana. This population was shown to be moving rapidly northward, covering a distance of 45 km between 2012 and 2013. Moving at this rate, it could reach the northernmost extent of Michigan's Lower Peninsula within 7 yr.

In 2011, *L. obtusus* was only known to occur at a single release site in Kalamazoo County. By the next year, weevils from that 2007 release had spread to occupy two adjacent counties. It also became evident that there were small populations of *L. obtusus* at some of the 2010 MSU release sites. By 2013, the population of *L. obtusus* derived from the 2007 Kalamazoo release had expanded rapidly, and the species could be found in a total of 13 counties. Most of the occurrences of *L. obtusus* can be explained by either dispersal from the Kalamazoo release, or by the undetected presence of low numbers of *L. obtusus* in 2010 releases of *L. minutus*. However, there seems to be an unexplained population in the Southeast corner of the state. It is possible that these weevils came from an unreported release in Michigan, or a release in nearby Ohio. It is also possible that some dispersal of weevils in the state occurs inadvertently via human activity. For example, we have observed *C. stoebe* seedheads caught in the bumper of research vehicles after driving on two-track roads with knapweed infestations. Alternatively, roadside mowing equipment such as large "brush hog" mowers frequently accumulate plant matter on their decks, which, if not cleaned off, could result in the long-distance transport of *C. stoebe* and associated biological control insects. Finally, due to individual citizens' enthusiasm for biological control it is possible that unauthorized intentional spread of the biological control agents may have occurred.

While *L. obtusus* seems to be gaining a strong foothold in Michigan, it is uncertain what will happen when *L. minutus* growth approaches levels of saturation. Right now both species have almost unlimited resources to expand into, as the spotted knapweed population is still very large across the state. Even where *Larinus* spp. populations are dense, there are still many seedheads that are not preyed upon (personal observation). But both *Larinus* species share a nearly identical niche, and there does not appear to be any temporal partitioning that we could observe from our phenological studies (Carson Chapter 1). It is not yet known whether the species hybridize. It is possible that they could, and in the lab we have observed the two species attempting to copulate. However, we have found very few weevils with intermediate morphologies, even after looking at more than 1500 *Larinus* individuals from the Kalamazoo release site where both species occur in relatively high densities. This suggests that the two species are probably biologically isolated as the result of allopatric speciation in their native range.

If the two species are capable of hybridization, it is possible that the less-abundant *L. obtusus* genotype will be diluted, and eventually disappear. If they are not capable of hybridization and they do indeed share the same niche, according to ecological theory one species should eventually competitively exclude the other (Bess et al., 1961). Because *L. minutus* has a substantial head-start in the region, it seems a likely candidate for success.

APPENDIX

APPENDIX

Table 1.
Sites and species of spotted knapweed biological control weevils released in Michigan and bordering states.

Site Name	County	Year of Release	*Larinus minutus*	*Larinus obtusus*	*Cyphocleonus achates*	Longitude	Latitude
Bristol	Elkhart (Indiana)	1996	+	-	+	W85.77141	N41.73578
Lake Orion	Oakland	2007	+	-	+	W83.29482	N42.75865
Kalamazoo	Kalamazoo	2007	-	+	+	W85.76245	N42.28951
Ottawa National Forest	Iron, Gogebic, Houghton	2009	+	-	+	Not available	Not available
Seney North	Schoolcraft	2010	+	-	+	W86.04817	N46.34626
Seney South	Schoolcraft	2010	+	-	+	W86.05508	N46.34072
Camp Grayling	Crawford	2010	+	-	+	W84.62825	N44.78453
Houghton Lake	Missaukee	2010	+	-	+	W84.89275	N44.31051
Flat River	Ionia	2010	+	-	+	W85.21220	N43.12400
Sharonville	Jackson	2010	+	-	+	W84.14593	N42.19637

Table 2.
Species and seeding rate/cost information for low and moderate diversity seed mixes for re-vegetation of 2010 spotted knapweed biological control sites with native nectar plants in Michigan.

Species	g/A	estimate seed/g	seed/A	seed/square ft	Total Cost/Acre
Low Diversity Mix					
FORBS					
Anemone cylindrica	56.6	737100	52000	1.19	96
Asclepias syriaca	396.8	113400	56000	1.29	70
Coreopsis lanceolata	170.1	567000	120000	2.75	75
Euphorbia corollata	170.1	226800	48000	1.10	330
Liatris aspera	226.8	453600	128000	2.94	304
Monarda fistulosa	56.7	2551500	180000	4.13	40
Rudbeckia hirta	56.7	2608200	184000	4.22	16
Solidago nemoralis	14.2	8505000	150000	3.44	22.5
Total Forbs	1148.2	15762600	918000	21.07	953.5
GRASSES					
Andropogon gerardii	340.2	283500	120000	2.75	42
Koeleria macrantha	113.4	5670000	800000	18.37	100
Schizachyrium scoparium	453.6	340200	192000	4.41	64
Total grasses	907.2	6293700	1112000	25.53	206
Total seed	2055.3	22056300	2030000	46.60	1159.5
High Diversity Mix					
FORBS					
Anemone cylindrica	28.3	737100	26000	0.60	48
Asclepias syriaca	198.4	113400	28000	0.64	35
Aster laevis	56.7	1559250	110000	2.53	90
Coreopsis lanceolata	85.0	567000	60000	1.38	37.5
Desmodium canadense	226.8	155925	44000	1.01	200
Euphorbia corollata	85.0	226800	24000	0.55	165
Gnaphalium obtusifolium	2.8	14175000	50000	1.15	4.5
Helianthus divaricatus	141.7	136080	24000	0.55	250
Liatris aspera	113.4	453600	64000	1.47	152
Monarda fistulosa	28.3	2551500	90000	2.07	20
Monarda punctata	56.7	2551500	180000	4.13	60
Penstemon digitalis	56.7	3685500	260000	5.97	30
Rudbeckia hirta	28.3	2608200	92000	2.11	8
Solidago juncea	7.1	8221500	72500	1.66	11.25
Solidago nemoralis	7.1	8505000	75000	1.72	11.25

Table 2. (cont'd)

Solidago speciosa	28.3	2693250	95000	2.18	20
Total forbs	1150.9	48940605	1294500	29.72	1142.5
<u>GRASSES</u>					
Andropogon gerardii	340.2	283500	120000	2.75	42
Koeleria macrantha	113.4	5670000	800000	18.37	100
Schizachyrium scoparium	453.6	340200	192000	4.41	64
Total grasses	907.2	6293700	1112000	25.53	206
Total seed	2058.2	55234305	2406500	55.25	1348.5

Table 3.

The number of *Larinus* spp. and *C. achates* recovered at 2010 release sites, Michigan 2011-2013.

Site	**Sample Year**	**Total *Larinus* spp. recovered**	***L. minutus* in subsample**	***L. obtusus* in subsample**	***Larinus* sp. per sweep avg**	**Total *Cyphocleonus* recovered**
Seney Release 1	2011	0	na	na	0.00	1
Seney Release 1	2012	1	1	0	0.01	0
Seney Release 1	2013	5	5	0	0.03	2
Seney Release 2	2011	0	na	na	0.00	1
Seney Release 2	2012	3	3	0	0.02	0
Seney Release 2	2013	9	9	0	0.05	0
Grayling	2011	1	na	na	0.01	0
Grayling	2012	7	5	1	0.04	1
Grayling	2013	2	2	0	0.01	0
Houghton Lake	2011	1	na	na	0.01	0
Houghton Lake	2012	28	10	0	0.07	2
Houghton Lake	2013	56	18	1	0.28	0
Flat River	2011	0	na	na	0.00	0
Flat River	2012	85	11	0	0.21	0
Flat River	2013	226	17	1	1.13	0
Sharonville	2011	0	na	na	0.00	0
Sharonville	2012	23	10	1	0.04	0
Sharonville	2013	91	22	1	0.46	1

Table 4.
The maximum dispersal, average maximum dispersal (± SE), average annual rate of spread since release, and average rate of spread from 2012-2013 (± SE) for recovered *Larinus* spp. Includes data from 2010 spotted knapweed biological control release sites in Michigan.

Site	**Year of Release**	**Sampling Year**	**Maximum Dispersal Detected (m)**	**Average Maximum (m)**	**Average annual rate of spread since release (m/year)**	**Average rate of spread 2012-2013 (m)**
Seney Release 1	2010	2012	0	0 ± 0	0	0±0
Seney Release 1	2010	2013	0	0 ± 0	0	na
Camp Grayling	2010	2012	0	0 ± 0	0	25 ± 25
Camp Grayling	2010	2013	100	25 ± 25	8.3 ± 8.3	na
Houghton Lake	2010	2012	0	0 ± 0	0	475 ± 442
Houghton Lake	2010	2013	1800	475 ± 442	158.3 ± 147.3	na
Flat River	2010	2012	100	25 ± 25	12.5 ± 12.5	300 ± 147
Flat River	2010	2013	600	325 ± 131	108.3 ± 43.70	na
Sharonville	2010	2012	100	25 ± 25	12.5 ± 12.5	75 ± 25
Sharonville	2010	2013	100	100 ± 000	33.3 ± 0.00	na

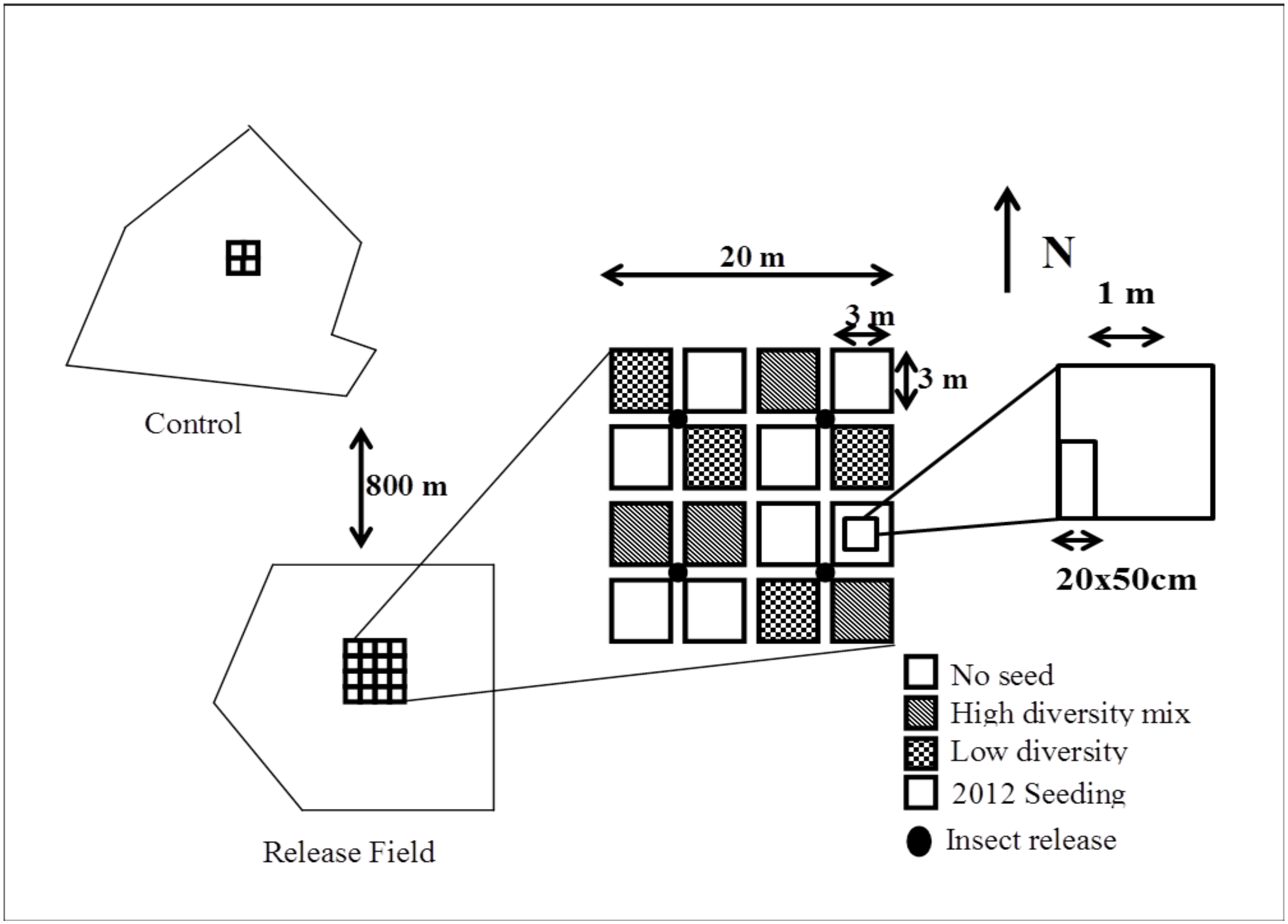


Figure 5. The experimental design of 2010 spotted knapweed biological control release and control sites in Michigan. Each release site had subplots which either received no seed additions, high diversity 2011, low diversity 2011, or high diversity 2012 seed addition treatments.

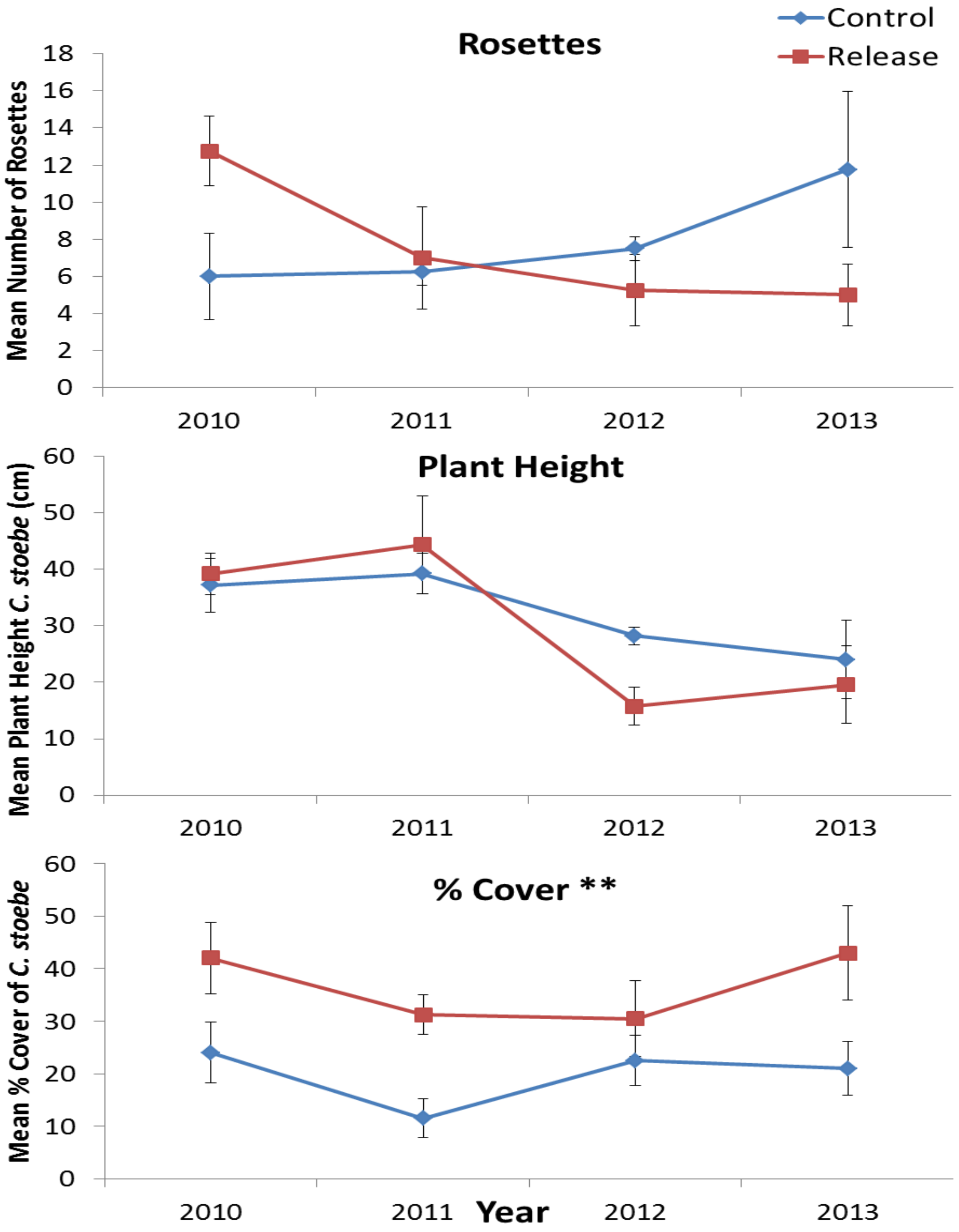


Figure 6. Mean (±SEM) of *Centaurea stoebe* plant response variables at **Seney** in year of biological control agent release (2010) and three subsequent years. * indicates significant difference within year and ** indicates a consistent overall significant difference between release and control treatments.

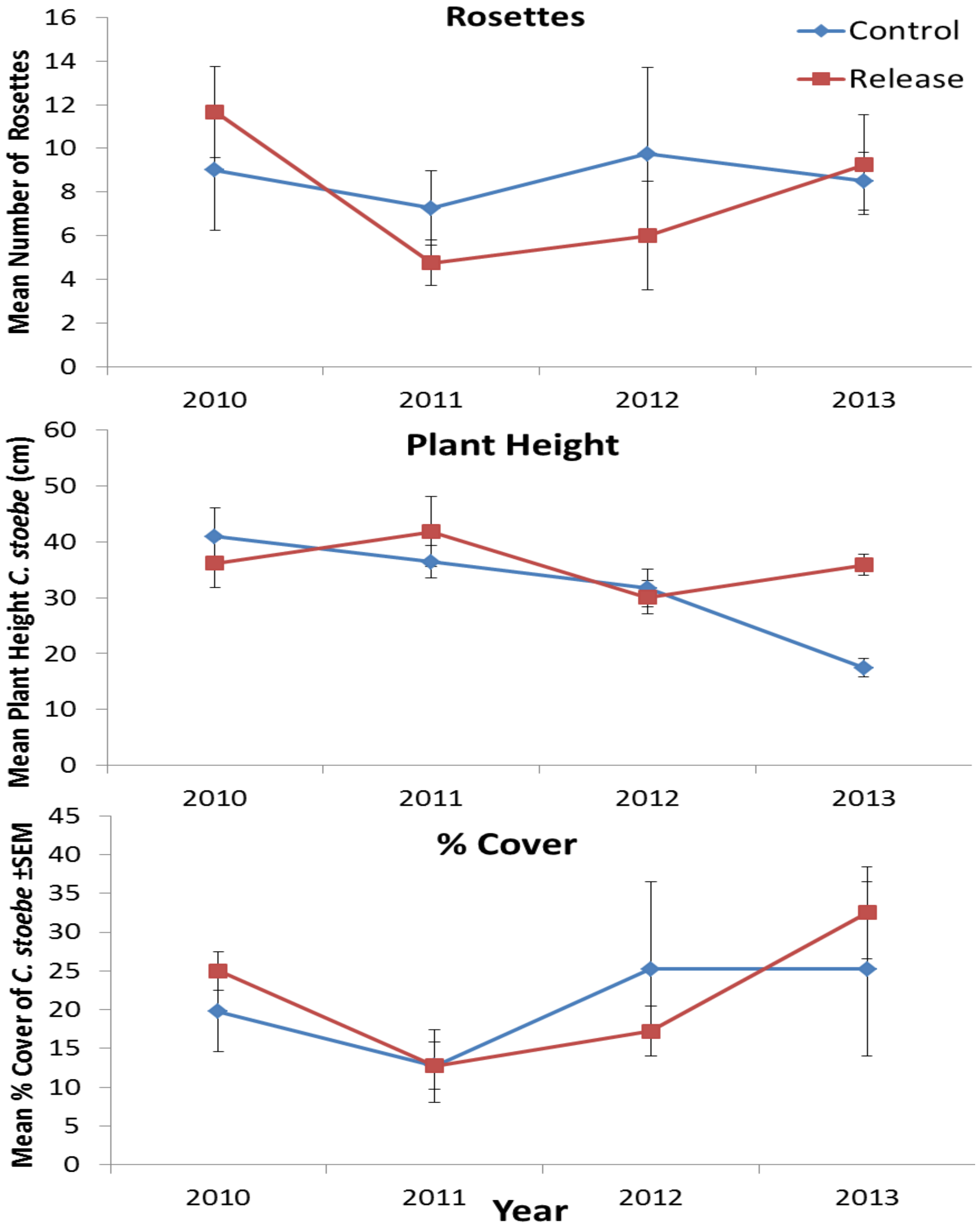


Figure 7. Mean (±SEM) of *Centaurea stoebe* plant response variables at **Grayling** in year of biological control agent release (2010) and three subsequent years. * indicates significant difference within year and ** indicates a consistent overall significant difference between release and control treatments.

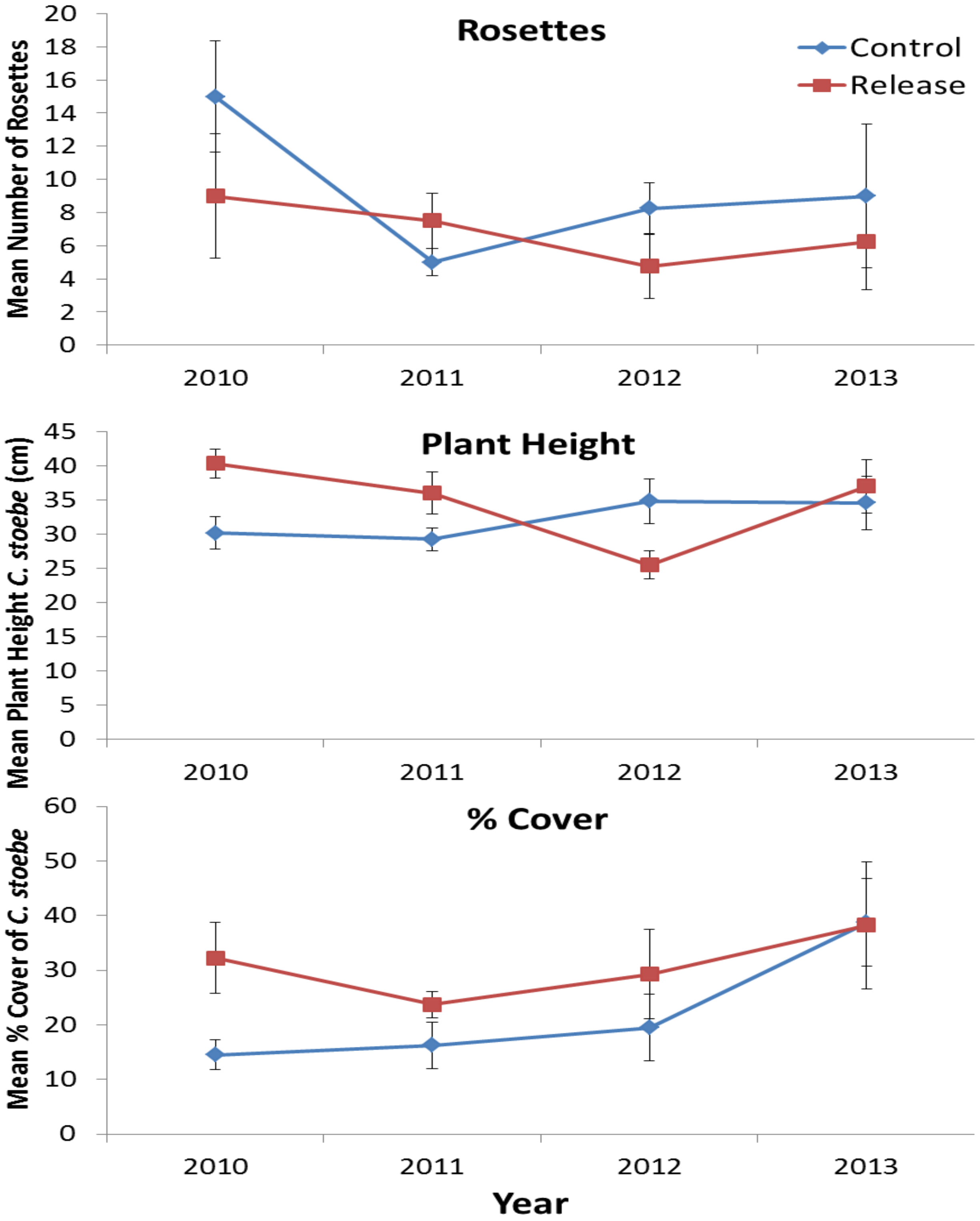


Figure 8. Mean (±SEM) of *Centaurea stoebe* plant response variables at **Houghton Lake** in year of biological control agent release (2010) and three subsequent years. * indicates significant difference within year and ** indicates a consistent overall significant difference between release and control treatments.

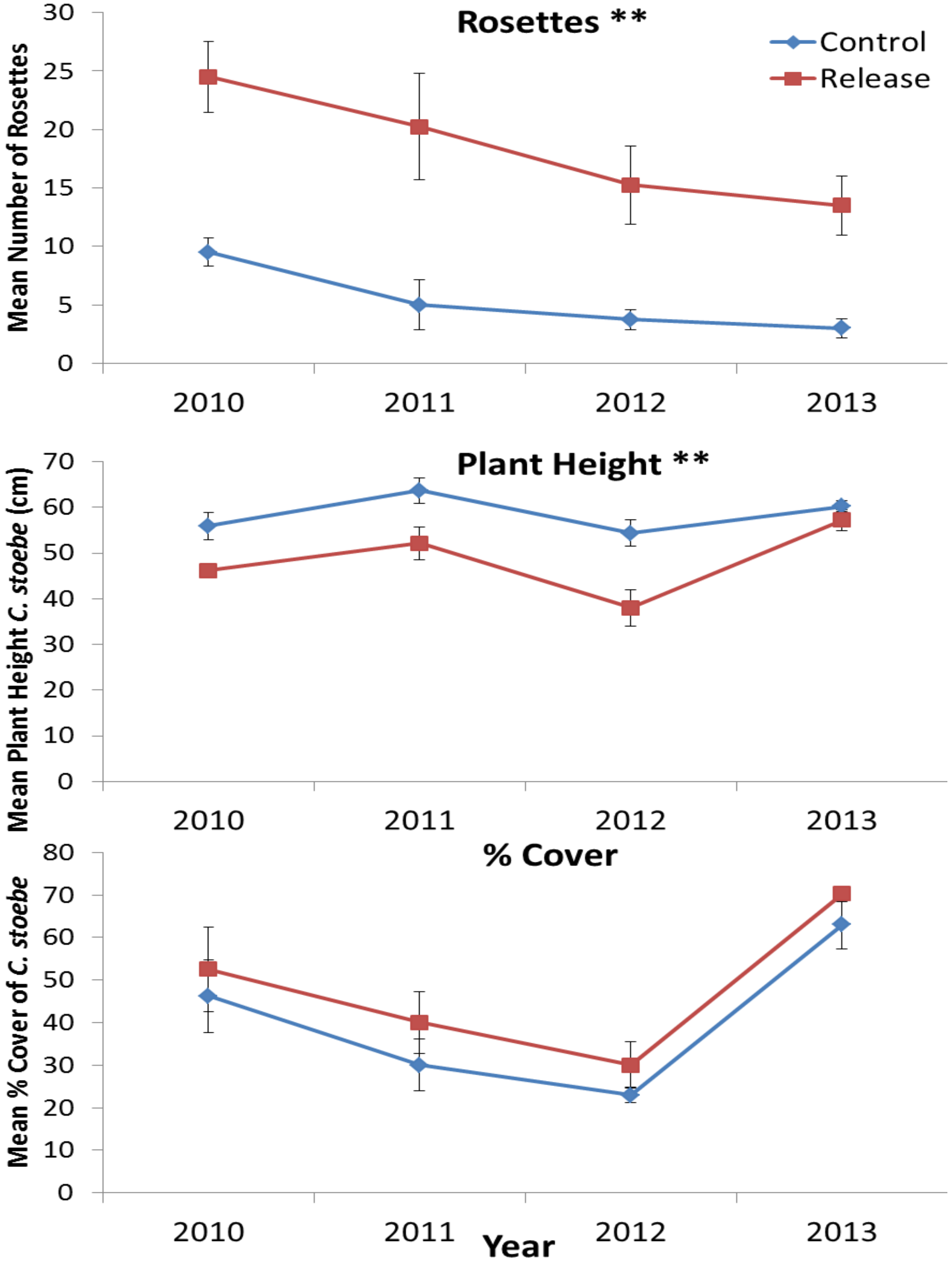


Figure 9. Mean (±SEM) of *Centaurea stoebe* plant response variables at **Flat River** in year of biological control agent release (2010) and three subsequent years. * indicates significant difference within year and ** indicates a consistent overall significant difference between release and control treatments.

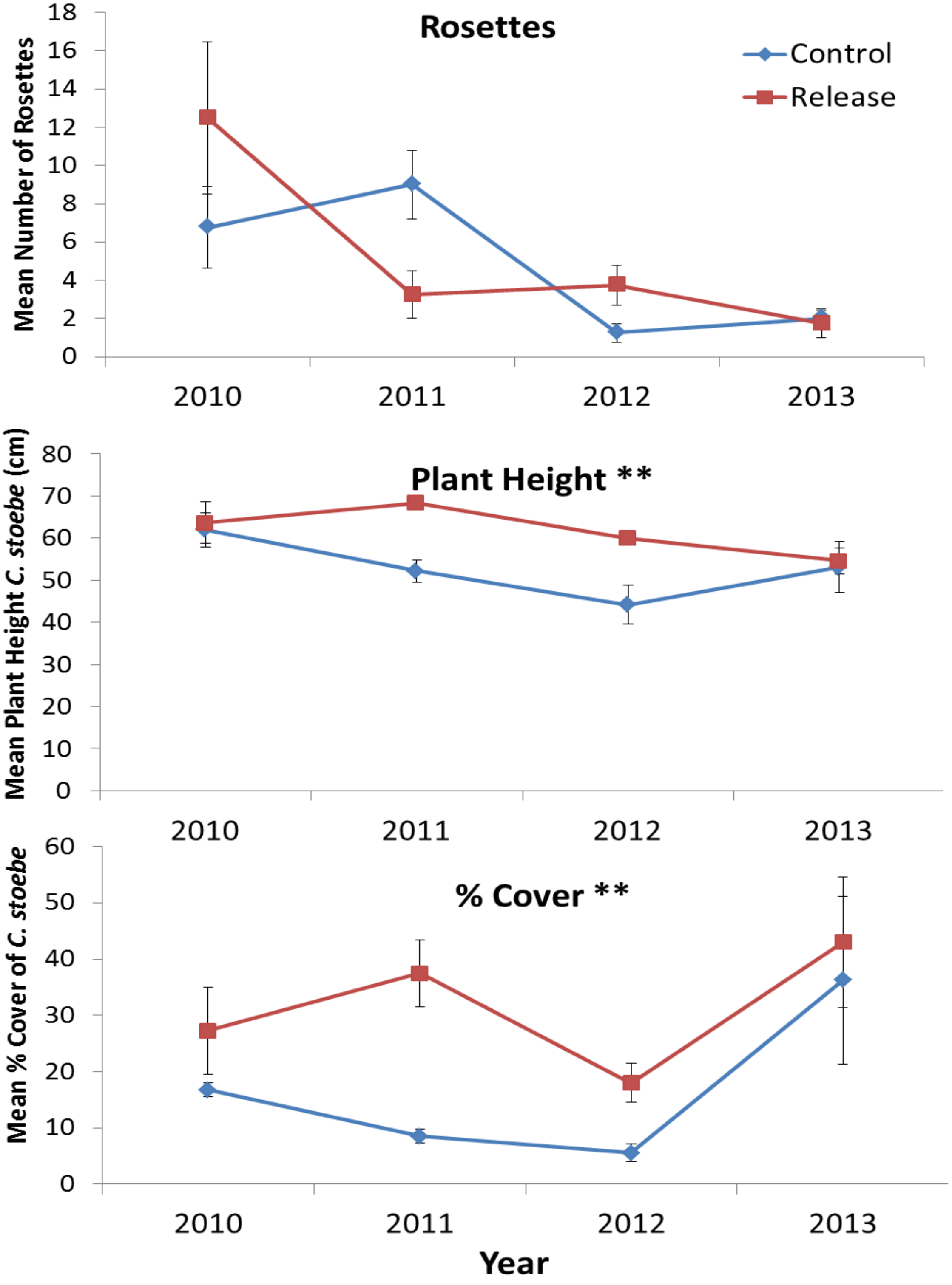


Figure 10. Mean (±SEM) of *Centaurea stoebe* plant response variables at **Sharonville** in year of biological control agent release (2010) and three subsequent years. * indicates significant difference within year and ** indicates a consistent overall significant difference between release and control treatments.

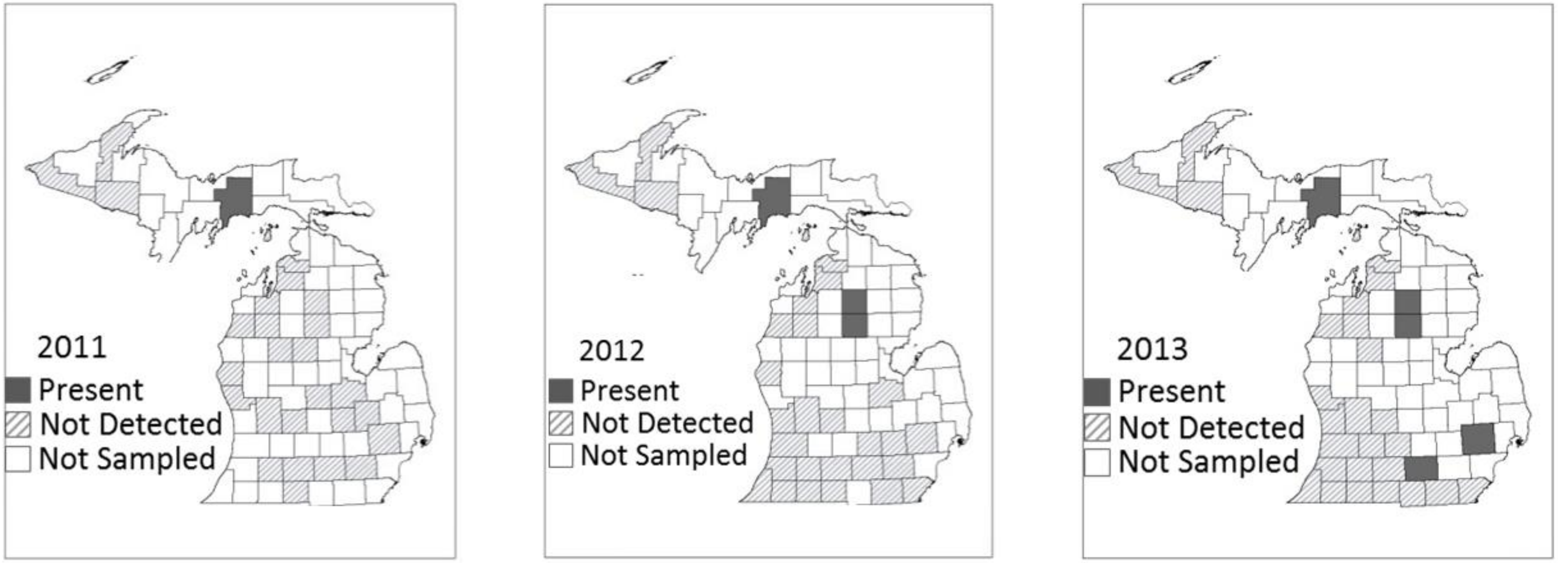


Figure 11A. The known range of the spotted knapweed biological control weevil *C. achates* in Michigan in 2011, 2012, and 2013.

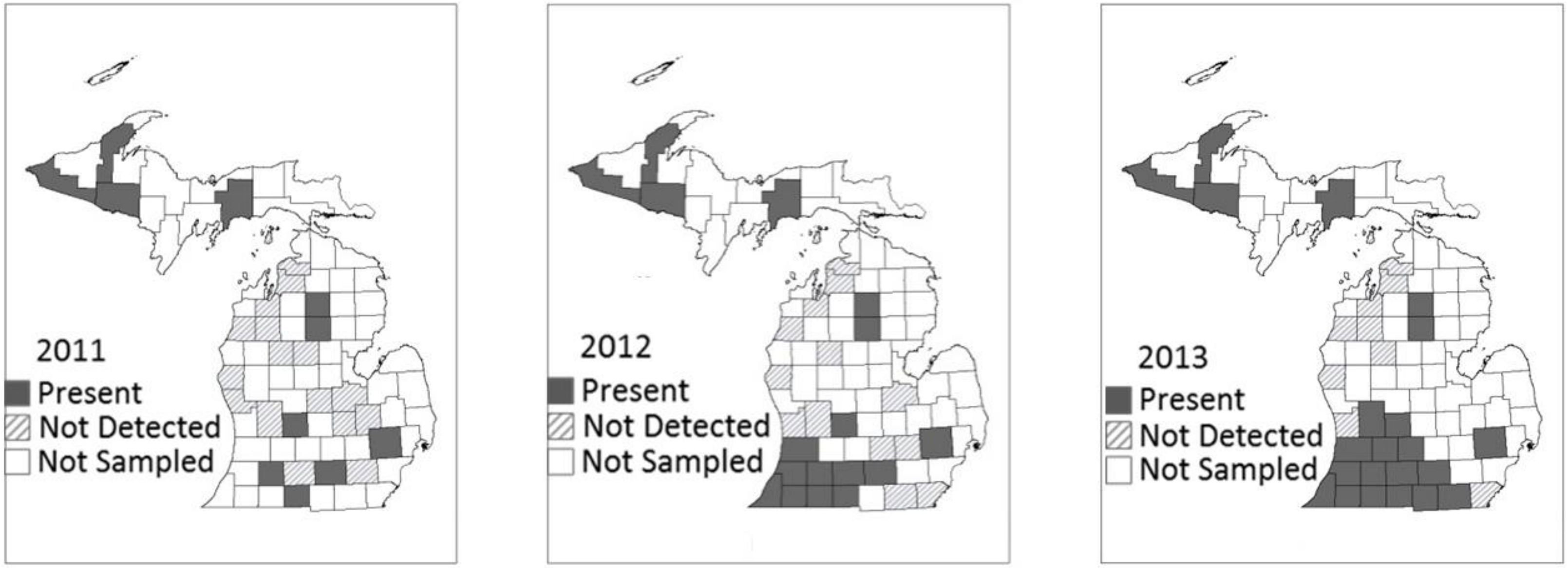


Figure 11B. The known range of the spotted knapweed biological control weevil *L. minutus* in Michigan in 2011, 2012, and 2013.

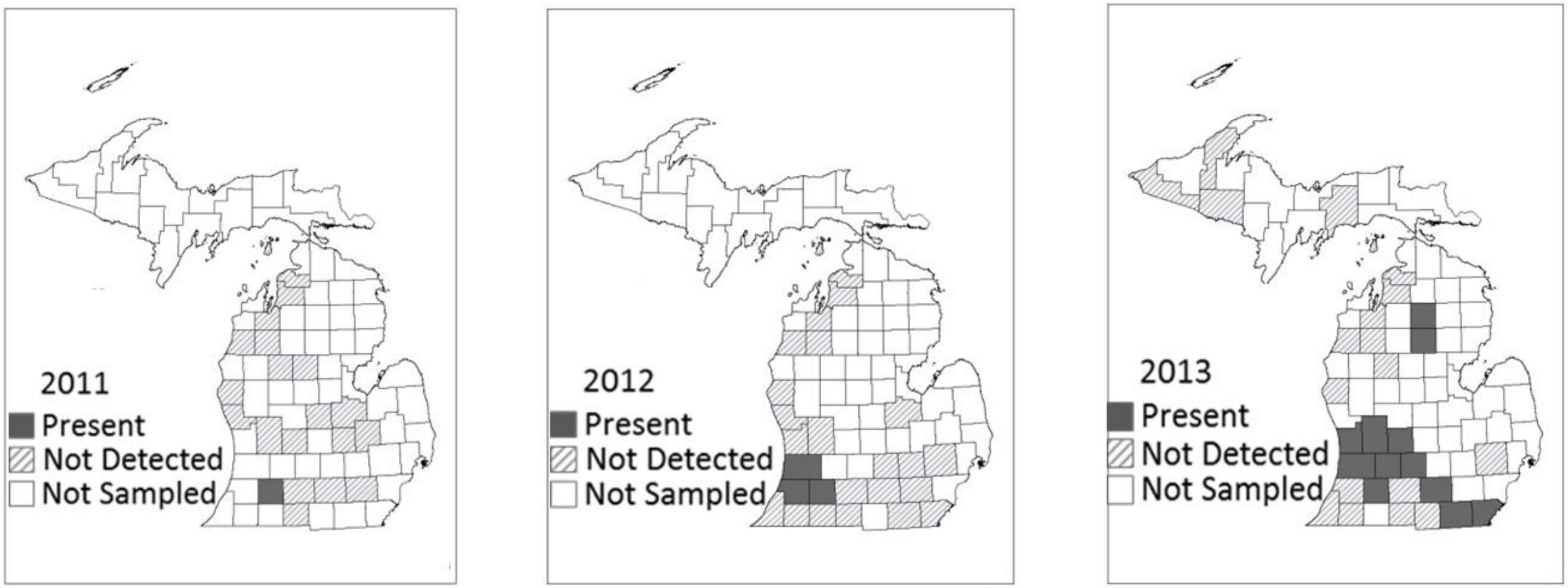


Figure 11C. The known range of the spotted knapweed biological control weevil *L. obtusus* in Michigan in 2011, 2012, and 2013.

REFERENCES

REFERENCES

Albert DA (1999) *Natural community abstract for open dunes* [Online]. Michigan State University, Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Open_dunes.pdf

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2:436-443

Clark SE, Driesche RGV, Sturdevant NJ, Elkinton JP (2001a) Effects of site characteristics and release history on establishment of *Agapeta zoegana* (Lepidoptera: Cochylidae) and *Cyphocleonus achates* (Coleoptera: Curculionidae), root-feeding herbivores of spotted knapweed, *Centaurea maculosa*. Biological Control 22:122

Clark SE, Van Driesche RG, Sturdevant N, et al. (2001b) Effect of root feeding insects on spotted knapweed (*Centaurea maculosa*) stand density. Southwestern Entomologist 26:129-135

Cohen JG (2000) *Natural community abstract for oakpine barrens* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Oak-pine_barrens.pdf

Cohen, JG (2001) *Natural community abstract for oak barrens* [Online]. Michigan State University, Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Oak_barrens.pdf

Corn JG, Story JM, White LJ (2009) Comparison of Larval Development and Overwintering Stages of the Spotted Knapweed Biological Control Agents *Agapeta zoegana* (Lepidoptera: Tortricidae) and *Cyphocleonus achates* (Coleoptera: Curculionidae) in Montana Versus Eastern Europe. Environmental Entomology 38:971-976

Emery SM, Gross KL (2006) Dominant species identity regulates invasibilityof old-field plant communities. Oikos 115**:**549-558

Griffith D, Lacey JR (1991) Economic Evaluation of Spotted Knapweed (*Centaurea maculosa*) Control Using Picloram. Journal of Range Management 44:1 43-37

Hakim SA (1979) Range condition on the Threemile Game Range in western Montana. Missoula, MT. Masters Thesis, University of Montana.

Jacobs JS, Sing SE, Martin JM (2006) Influence of herbivory and competition on invasive weed fitness: Observed effects of *Cyphocleonus achates* (Coleoptera:

Curculionidae) and grass-seeding treatments on spotted knapweed performance. Environmental Entomology 35**:**1590-1596

Knochel DG, Seastedt TR (2010) Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. Ecological Applications 20:1903-1912

Knochel DG, Monson ND, Seastedt TR (2010) Additive effects of aboveground and belowground herbivores on the dominance of spotted knapweed (*Centaurea stoebe*). Oecologia 164:701-712

Kost, MA (2004) *Natural community abstract for dry-mesic prairie*. [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Dry-mesic_prairie.pdf

Kost MA, Slaughter BS (2009) *Natural community abstract for mesic sand prairie* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Mesic_sand_prairie.pdf

Lacey JR, Marlow CB, Lane JR (1989) Influence of spotted knapweed (*Centaurea maculosa*) on surface runoff and sediment yield. Weed Technology 3**:**627-631

Lang RF, Richard RD, Winkler J, Wheeler G (2001) Distribution of *Urophora affinis* and *U. quadrifasciata* (Diptera : Tephritidae) for biological control of spotted knapweed (*Centaurea maculosa*) and diffuse knapweed (*Centaurea diffusa*) in Michigan. Great Lakes Entomologist 34**:**31-42

Lee JG (2007) *Natural community abstract for dry-mesic southern forest.* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Dry-mesic_southern_forest.pdf

Marshall JM, Storer AJ (2008) Comparative analysis of plant and ground dwelling arthropod communities in lacustrine dune areas with and without *Centaurea biebersteinii* (Asteraceae). American Midland Naturalist 159**:**261-274

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288**:**81-90

Myers JH, Jackson C, Quinn H, White SR, Cory JS (2009) Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control 50**:**66-72

Rondeau KJ (2007) Dispersal of the Biocontrol Agent, *Cyphocleonus achates* (Coleoptera: Curculionidae), on the Invasive Plant, Diffuse Knapweed (*Centaurea diffusa*) (Asteraceae). Master's Thesis, University of Alberta.

Seastedt TR, Gregory N, Buckner D (2003) Effect of biocontrol insects on diffuse knapweed (*Centaurea diffusa*) in a Colorado grassland. Weed Science 51:237-245

Sheley RL, Larson LL (1996) Emergence date effects on resource partitioning between diffuse knapweed seedlings. Journal of Range Management 49:241-244

Sheley RL, Jacobs JS (1997) "Acceptable" levels of spotted knapweed (*Centaurea maculosa*) control. Weed Technology 11:363-368

Sheley RL, Jacobs JS, Carpinelli MF (1998) Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technology 12:353-362

Stinson CSA, Schroeder D, Marquardt K (1994) Investigations on *Cyphocleonus achates* (Coleoptera: Curculionidae), a potential biological-control agent of spotted knapweed (*Centaurea maculosa*) and diffuse knapweed (*Centaurea diffusa*) (Compositae) in North America. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 117:35-50

Story JM, Anderson NL (1978) Release and establishment of *Urophora affinis* (Diptera Tephritidae) on spotted knapweed in western Montana. Environmental Entomology 7:445-448

Story JM, Good WR, White LJ (1997) First report of the establishment of *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) on spotted knapweed, *Centaurea maculosa* Lamarck, in the United States. Canadian Entomologist 129:373-374

Story JM, Stougaard RN (2006) Compatibility of two herbicides with *Cyphocleonus achates* (Coleoptera: Curculionidae) and *Agapeta zoegana* (Lepidoptera: Tortricidae), two root insects introduced for biological control of spotted knapweed. Environmental Entomology 35:373-378

Story JM, Smith L, Corn JG, et al. (2008) Influence of seed head-attacking biological control agents on spotted knapweed reproductive potential in western Montana over a 30-year period. Environmental Entomology 37:510-519

Watson AK, Renney AJ (1974) Biology of Canadian weeds *Centaurea diffusa* and *Centaurea maculosa*. Canadian Journal of Plant Science 54:687-701

Wilson LM, Randall CB (2005) Biology and biological control of knapweeds. U.S. Forest Service Publication

CHAPTER 3

PHENOLOGY AND DISPERSAL OF *LARINUS MINUTUS* AND *LARINUS OBTUSUS*, TWO BIOLOGICAL CONTROL AGENTS OF SPOTTED KNAPWEED IN MICHIGAN

Introduction

Spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos* (Gugler)) is an invasive plant in North America, found in every U.S. state in lower the 48 with the exceptions of Texas, Oklahoma, and Mississippi (USDA, 2013). It has been shown to cause dramatic decreases in plant diversity, ecosystem functioning, and utilization of land by wild and domesticated foraging animals (Mummey and Rillig, 2006; Lacey et al., 1989; Hakim, 1979; Watson and Renny, 1974), and has a substantial economic cost (Griffith and Lacey, 1991). In the Eastern and Midwestern United States, spotted knapweed has become a serious invader, threatening the ecological integrity of natural areas in the region. Despite the efforts of land managers, conventional control methods such as herbicide and mowing have been ineffective at slowing the spread of spotted knapweed (Wilson and Randall, 2005). Over the last four decades, Western states, notably Montana, Colorado, and Washington, have spearheaded the effort to use classical biological control to slow and reverse the invasion of spotted and diffuse knapweed (eg. Watson and Renney, 1974; Story and Anderson 1978; Sheley and Jacobs, 1997; Corn et al., 2009). More recently, states in the Eastern United States have begun making their own biological control releases targeting spotted knapweed. The most common insect species released in the eastern region have been the root-boring weevil *Cyphocleonus achates* (Coleoptera: Curculionidae), *Larinus minutus* (Coleoptera: Curculionidae), and, to a lesser extent, *L. obtusus* (Coleoptera: Curculionidae) (http://www.biocontrol.entomology.cornell. edu/weedfeed/Larinus).

L. minutus and *L. obtusus* adults are brown to black beetles, 4 to 5 mm long, and their larvae are white, C-shaped grubs with brown head capsules (Wilson and Randall, 2005). Each species shares a similar life history, overwintering as adults in the leaf litter beneath knapweed plants, and emerging in the late spring. The adults feed on knapweed stems and foliage when they emerge. Once spotted knapweed flowers appear, the weevils mate and lay their eggs throughout the summer. The female lays from one to five eggs at a time in the flowerhead, and up to 130 eggs in a season. The larvae hatch three days after oviposition, and immediately begin feeding on the pappus hairs. They then migrate downward and begin feeding on the seeds. The larvae are aggressive and will often eat each other and other insects in the same seedhead (Wilson and Randall, 2005). These weevils impact knapweed populations by reducing the amount of seeds each plant produces, potentially limiting knapweed recruitment. Releases of *L. minutus* in the Western United States and Canada have effectively reduced diffuse knapweed (*Centaurea diffusa*) (Myers et al., 2009), though *Larinus* spp.'s ability to reduce spotted knapweed has yet to be demonstrated (Story et al., 2008).

Despite widespread releases of *Larinus* spp. that have been made throughout the United States and Canada since 1991 (Müller-Schärer and Schroeder 1993), there is still a dirth of information regarding several aspects of their biology that are relevant to biological control planning and efficacy. An ISI Web of Science search using the keywords "*Larinus*" and "phenology" shows that there are no reported studies documenting the season-long phenology of *L. minutus* or *L. obtusus* anywhere in North America. In a U.S. Forest Service publication, Wilson and Randall (2005) report that adults of both species are generally active from May or June until August, but they do not report what regions this information applies to. Knowledge of biological control agent phenology is important because it informs decisions of when to collect weevils for redistribution, and when to conduct population monitoring.

It is also important to know how quickly biological control agents disperse through the landscape, as this can help determine where releases sites should be located (Paynter and Bellgard, 2011). There have been no reported studies of the dispersal of *L. obtusus*, and *L. minutus* dispersal has only been documented in two studies. One of these took place in a landscape dominated by diffuse knapweed in Alberta, Canada, and reported an average rate of spread of 1.9 km/year five years after the initial release. However, this study was not replicated, and it only documented the spread of weevils in one direction (VanHezewijk and Bourchier 2011). Furthermore, because diffuse knapweed is *L. minutus'* preferred host plant and may facilitate greater rates of reproduction (Groppe et al., 1990), it is uncertain whether data obtained from a diffuse knapweed system can be extrapolated to spotted knapweed-dominated landscapes. A second study has recently reported that *L. minutus* could be expected to spread "at least 225 m from the release point two years post release," or 112.5 m/yr (Alford 2013).

These studies show two very different dispersal rates for *L. minutus*, 1.9 km/yr vs. 112.5 m/yr. We suspect that the difference in dispersal rates observed at the two sites is the result of a dispersal rate that increases throughout time, though there are no published studies on how *Larinus* spp. dispersal rates change with time since release. After monitoring the dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* from 5 release sites in Florida, Porter et al. (2004) report that they exhibited dispersal rates that increased dramatically over time, spreading up to 6 km from the release point two years post-release, and then increasing their range to 36 km from the release point three years post-release.

In 2007, the USDA issued permits for the release of *C. achates*, L. *minutus*, and *L. obtusus* in Michigan. Releases of these insects were subsequently made at two sites in Southern Michigan (Chapter 2 **Table 1**). In 2012, we became aware of a biological control release that was made in Bristol, Indiana in 1994 (Chapter 2 **Table 1**) from which *L. mintutus* weevils appear to have emigrated into southern Michigan, and have formed a substantial population in the region (Carson Chapter 2).

Here we examined well-established *Larinus* spp. populations descended from the 2007 releases in Michigan to collect data on the phenology and dispersal rates of *Larinus* spp. in the Midwestern U.S. The objective of the phenology study were to establish when *L. minutus* and *L. obtusus* adults are active throughout the growing season. This will inform future collection efforts of these species by allowing researchers to sample during to the period of their peak activity. The objective of our dispersal study was to establish the dispersal rates of *Larinus* spp. in the Midwestern region. In addition to collecting data from 2007 *Larinus* releases, we used data from releases made in 2010 and 1996 to infer how dispersal rates change as time since release increases. This will help us understand the geographical distribution of these species, and will allow land managers to better plan future biological control releases.

Methods

Phenology

Studies were conducted in 2012 and 2013 at the Lake Orion and Kalamazoo sites (Chapter 2 **Table 1**), as these represented well-established populations of *L. minutus* and *L. obtusus*. The

Kalamazoo site experienced an influx of *L. minutus*, presumably from the 1996 release made in Bristol, Indiana. The presence of both *L. minutus* and *L. obtusus* gave us the opportunity to compare their phenology in the same location.

We conducted abundance sampling at each 2007 release site in 2012 and 2013, between May 1 and September 25. The abundance of active adult *Larinus* weevils was monitored weekly at Kalamazoo, and bi-weekly at Lake Orion. Sampling was conducted between 10:00 and 17:00, and when possible sampling was done during warm days, when insects are more active. We used 37 cm diameter sweepnets to collect adult weevils, taking sweeps in sets of 20 at one time. Sweeps were taken in knapweed vegetation and targeted the upper portion of each plant. After one set of 20 sweeps, the net’s contents were examined and *Larinus* weevils were counted and recorded. A subset of five weevils were collected from each round of 20 sweeps. This procedure was repeated 10 times, with each set of sweeps taken from a different patch of vegetation. All sampling was performed within 100 m of the 2007 biological control release points. At the lab, the weevils were frozen for later identification. At Lake Orion, sampling was conducted by members of the Oakland County Parks Natural Resources staff. To ensure consistency, staff were trained at the beginning of each field season by MSU researchers. Sampling at Lake Orion took place bi-weekly due to scheduling limitations. In all other respects, sampling at Lake Orion was identical to sampling at Kalamazoo.

In the lab, subsamples of 50 weevils collected on each sampling date were identified to species using a dissecting microscope (Leica M125, Leica Camera AG). The characters used in separating the species were as follows: *L. minutus*: longer, more dense hair patterns on integument, bifurcated hairs behind the ocular region, red tinted tarsi. *L. obtusus*: sparser, shorter hairs on integument, hairs behind ocular region not bifurcated, black tarsi (Draney and Oehmichen 2007). The first occurrence of teneral adults appeared in the subsamples, as indicated by a red tint throughout the integument, was also recorded.

Data analysis

Time series abundance data are displayed by sample date and by degree day (DD_{10C}) for each sampling year. Because no physiological data is available for *Larinus* spp., it was assumed that DD_{10C} is an appropriate measure because that value has commonly been used to measure arthropod emergence in the Midwest region (http://www.entomology.umn.edu/cues/Web/049DegreeDays.pdf). Abundance data were not normally distributed. To compare the changes in *Larinus* spp. abundance at each site between 2012 and 2013 we used a non-parametric Kruskal Wallis test on the total abundances all *Larinus* weevils, and *L. obtusus* and *L. minutus* independently, using year and date as fixed factors. No assumptions of the Kruskal Wallis test were violated.

Dispersal

To determine the rate of spread of *C. achates* and *Larinus* spp. populations, we conducted surveys of knapweed-infested fields in the landscape surrounding 2007 biological control release sites (**Table 1**). In all dispersal sampling we used detectability as a proxy for measuring dispersal, and recognize that the insects likely dispersed outward from release sites in stages, and not nesessarily in single dispersal events. Sampling took place during the summers of 2012 and 2013, between June 15 and August 15. Starting from the release point at each site, we located patches of knapweed in each cardinal direction and conducted 60 sweeps at each site, counting the number of biological control weevils from each group of sweeps. Our goal was to find sampling sites in each direction at a distance of 100 m, 500 m, and 1000 m from each release point. If no biological control weevils were found at the 500 m and 1000 m points, sampling in that direction was discontinued. If we continued to find weevils at 1000 m, we sampled at 2000 m and 3000 m from the release point. It was not always possible to find knapweed at the exact distances described in our sampling protocol. As a result, the sampling sites varied somewhat in their absolute distance and direction from the release point.

The species of weevils released at the Kalamazoo site was *L. obtusus*. However, by 2012 the surrounding landscape contained *L. minutus* weevils as well. It is very difficult to tell *L. obtusus* and *L. minutus* apart in the field, so we had no way to know when we were finding *L. obtusus* that had spread from the 2007 release site, and when we were finding *L. minutus* that had spread from the 1996 release in Indiana. As a solution we conducted sweep surveys at 100 m, 500 m, 1000 m, 2000 m, and 3000 m in each direction from the central release point. We then continued to sample at 8 and then 16 km intervals from the release point to try to detect long-distance dispersal. The decision to sample further from the release point was informed by the previous detection of *L. obtusus* as far as 45 km from the original 2007 release site in 2012 (Carson Chapter 2). A subsample of 20 weevils was collected at each sampling site. These were later identified, and it was assumed that any *L. obtusus* had dispersed from the Kalamazoo release.

We evaluated dispersal rates using several metrics. The “maximum dispersal detected” was calculated as the furthest dispersal observed at a site. The “average maximum dispersal detected” was calculated as the mean of the maximum dispersal observed for each cardinal direction (n=4). To calculate the "average rate of spread between 2012 and 2013" we took the difference between the maximum detected dispersal in each cardinal direction from 2012 and 2013, and averaged these four values (n=4). The "average maximum dispersal rate" was calculated as the "average maximum dispersal detected" divided by the years since release.

Results

Phenology

In 2012 at Kalamazoo, *Larinus* weevils were first observed on May 10 (242 DD_{10C}) (**Figure 12**). There was a period of increase in both species' populations beginning on June 1 (404 DD_{10C}). This increase continued until its peak on June 15 (523 DD_{10C}). The population then decreased slightly, and there was a second, lesser peak on July 13 (911 DD_{10C}). After this point, the populations underwent a steady decrease until the end of sampling on September 6. Teneral adults first appeared on August 3, and we continued to find them in our subsamples through our last observation on September 13.

In 2013 at Kalamazoo, we observed the first weevils emerging on May 14 (125 DD_{10C}) (**Figure 13**). Both *L. minutus* and *L. obutsus* showed rapid increases in population between the June 6 and June 14 (298 to 369 DD_{10C}). June 14 was the first peak in population. There was then a decrease in weevil abundance, followed by a second peak lasting from July 3 through July 12 (567 and

673 DD_{10C}, respectively). Populations of both weevil species showed a steady decline after this second peak, and by Aug. 9 (958 DD_{10C}) they had reached relatively low population levels that were maintained until our last sampling day on September 17. Teneral adults first appeared on August 9, and continued to occur through September 17.

In 2012, mean abundance of *L. obtusus* was 9.97 ± 2.31 weevils/20 sweeps, and *L. minutus* was 6.29 ± 0.99 weevils/20 sweeps. *L. obtusus* populations were greater until August 3, 2012, when *L. minutus* population levels rose to surpass those of *L. obtusus.* In contrast, *L. minutus* was more abundant than *L. obtusus* throughout 2013, with a mean of 17.45 ± 4.48 individuals/20 sweeps vs. 7.61 ± 1.97 weevils/20 sweeps, respectively. There was significantly higher total *Larinus* spp. abundance in 2013 than in 2012 (chi-squared = 11.971, df = 1, p-value <.0001). There was not a significant difference in *L. obtusus* abundance between the two years (chi-squared = 0.615, df = 1, p-value = 0.433), and there were significantly more *L. minutus* in 2013 than in 2012 (chi-squared = 44.424, df = 1, p-value<.0001). Over the three years of study, *L. minutus* steadily increased in percent of total *Larinus* spp. abundance (**Table 5**).

At Lake Orion, *L. minutus* was the only *Larinus* species observed in both years of the study. In 2012, *L. minutus* was first observed on May 11 (210 DD_{10C}) and its population increased between May 30 and its first peak on June 28 (329 and 600 DD_{10C}, respectively) (**Figure 14**). The population then underwent a decline before peaking again on September 7 (1473 DD_{10C}). After the second peak, there was again a steep decline and then maintenance of a low population level until the end of sampling on October 3. Due to inconsistent collection of subsamples during the period of teneral appearance, we are unable to accurately report when teneral adults first emerged. However, they were present in subsamples from the September 7 sampling date, and we continued to find them until the final sampling date on September 27.

In 2013, the first weevils were observed on May 2 (104 DD_{10C}) (**Figure 15**). Weevil populations maintained low levels until a sharp increase beginning on July 3 and peaking on July 15 (945 and 1222 DD_{10C}, respectively). Following this sharp peak, the observed *L. minutus* population declined abruptly and maintained low levels for the remainder of the season. Teneral adults first appeared on August 29, and we continued to find them in our subsamples through the last sampling date on September 24.

Dispersal

We detected *Larinus spp.* dispersal from both 2007 release sites on both a local and landscape scale. In 2012 at Lake Orion, we found local *L. minutus* dispersal 500 m from the release point in each cardinal direction. At this distance, weevil densities were substantially less than densities at the release point (**Figure 16**). In 2013, the densities of *L. minutus* 500 m from the release site were roughly equivalent to densities at the release, indicating that the population had grown to be evenly distributed up to this distance (**Table 6, Figure 16**). In 2013 *L. minutus* could consistently be found up to 1 km from the release in each direction.

In the landscape surrounding the Lake Orion release (**Figure 17**), we detected maximum dispersal of *L. minutus* up to 1 km to the North, 1.35 km to the East, 500 m to the South, and 1. 4

km to the West of the release point in 2012. In 2013 we collected *L. minutus* up to 10.5 km to the North, 8.1 km to the East, 1.0 km to the South, and 4.6 km to the West of the release point.

In 2012 at Kalamazoo, weevil densities at the release point and up to 800 m from the release point were equivalent, indicating that *L. obtusus* was evenly dispersed up to 800m from the release site five years post-release (**Table 6, Figure 18**). *L. obtusus* could be found up to 1.8 km of the release point in every direction. In 2013, we continued to find *L. obtusus* in densities equivalent to the release point up to 800 m in each direction. Beyond this, *L. obtusus* densities declined with distance from release in every direction but South. We found *L. obtusus* in similar density to the release point up to 3.4 km to the South. Sampling to the South was conducted along a power line, which may have served as a corridor for dispersal. *L. obtusus* could be found up to 1.8 km from the release in each cardinal direction.

On a landscape scale at Kalamazoo in 2012, we detected maximum dispersal of *L. obtusus* 3 km to the North, 3 km to the East, 1.8 km to the South, and 3.3 km to the West of the release point in 2012 (**Figure 19**). In 2013 we detected *L. obtusus* dispersal up to 12.9 km to the North, 24.0 km to the East, 3.3 km to the South, and 43.3 km to the West of the release point.

Between 2012 and 2013, the average rate of spread of *L. obtusus* from Orion Oaks was 4.9 km, and the average rate of spread from the Kalamazoo release was 18.1 km (**Table 7**). The rate of dispersal increased with time since release at both sites (**Table 8**), and this pattern of increasing dispersal rates is further supported from *L. minutus* releases made in 2010 (Carson Chapter 2).

While we did not perform a dispersal transect originating from the 1996 release made in Bristol, Indiana, we were able to infer the rate that this population of *L. minutus* have spread across the landscape based on data collected during roadside biological control insect surveys performed in 2012 and 2013 (Carson Chapter 2). Since being released in 1996, this population of *L. minutus* has spread 145 km over the course of 17 years. Based on this distance, we calculated that the population has spread at an overall average rate of 9.1 km per year. Between 2012 and 2013, this population was observed to move 45 km North.

Discussion

Phenology

Observations at the Kalamazoo site allow us to compare the phenology of *L. minutus* and *L. obtusus.* In both 2012 and 2013, the trend for each species followed a very similar pattern, indicating that there is no substantial difference in the phenology of these two species.

Weevils first appeared in mid-May, and the first peak in *Larinus* populations occurred near the middle of June. The second peak occurred 28 days later, in mid-July. It has recently been reported that the period of time of development from egg to adult in *Larinus minutus* ranges from 25.6 to 26.9 days in Arkansas (Alford 2013). The 28 days that we observed between population peaks corresponds to this timeframe, and we initially hypothesized that the first observed population peak is made up of adults that had overwintered from the previous year emerging, feeding, and mating, and the second peak consists the emergence of the next generation of weevils. However, after searching for freshly emerged teneral weevils in our collected samples, we discovered that this new generation of weevils did not appear until the beginning of August in either study year, well after we observed a second peak. This finding led us to conclude that the second peak in weevil abundance in July is most likely caused by variation in the activity of the first generation of adult weevils, rather than a mass emergence of that generation's offspring.

The pattern that emerges when comparing *Larinus* spp. abundances to the accumulated degree days shows similar trends. The range of degree day accumulation the corresponds to the initial population increase was between ~300 and ~500 C, with initial peaks occurring at 523 DD_{10C} in 2012 and 369 DD_{10C} in 2013. The variation in *Larinus* abundances with regard to degree days may be explained by the highly divergent weather patterns of the two study years. The early spring of 2012 was much warmer than average, with the mean temperature of March the highest on record (http://enviroweather.msu.edu/ run.php?stn=htc&mod=w_ddy). During this early warm period, degree days accumulated quickly, though apparently not enough to trigger weevil emergence. Then temperatures cooled for several weeks, with little degree day accumulation. In 2013, the weather trended in the opposite direction, with a relatively cool spring. Despite this, *Larinus* spp. weevils emerged in mid-June again, this time when the degree day accumulation was ~200 DD_{10C} less than it had been the previous year. Because 2013 was a cool spring with a more steady accumulation of degree days, we believe that the first peak abundance observed that year, at ~370 DD_{10C} may correspond to the minimum degree day requirements for peak *Larinus* spp. emergence.

The results of our phenology study at Lake Orion differ somewhat from those of Kalamazoo. Some of these differences may be the result of data collection, which occurred at half the frequency of sampling at Kalamazoo (bi-weekly instead of weekly). Similarly to Kalamazoo, in 2012 the *L. minutus* population achieved its first peak when the degree day accumulation was ~600 DD_{10C}. However, the time between the first observed peak on June 28 and the second observed peak on September 7 was more than twice the time between the two peaks in both study years at Kalamazoo. The greatly increased time between these two population peaks is not explainable by the reported larval development period (Alford 2013), which again suggests that there is variability in the activity of adult weevils throughout the season. Days with high levels of *Larinus* spp. weevils did not correspond to trends in temperature or cloud cover, and thus there seems to be a large degree of natural variability in *Larinus* spp. weevil activity that is not explainable by parameters we measured.

Data from Lake Orion in 2013 further support the hypothesis of high variability in adult weevil activity throughout the season. In 2013 there was only one population peak observed at Lake Orion, and it occurred on July 15, when the degree day accumulation had reached 1222 DD_{10C}. This single peak differs from with the pattern of emergence we saw at Lake Orion in 2012 and at Kalamazoo in 2012 and 2013.

Even with the high level of variation in adult *Larinus* spp. activity we observed at each site in each year, each sampling effort between mid-June and the end of August recovered relatively high abundances of weevils. In each case, after 700 DD_{10C} accumulation weevils remained at easily detectable levels until the end of August, when their numbers were in steady decline. Land managers or researchers who wish to survey for the presence of *L. minutus* or *L. obtusus* should do so within this time period. Due to the observed variation in abundance within the time period of adult activity, researchers seeking to establish accurate abundance estimates for a site should sample on several dates during this period of time.

At Kalamazoo, *L. mintus* and *L. obtusus* followed similar trends in both 2012 and 2013. However, the relative abundance of each species reversed toward the end of the 2012 season. In 2013, there were significantly more *L. minutus* at the site than in 2012, while the population of *L. obtusus* showed no change between 2012 and 2013. Because only *L. obtusus* weevils were released at Kalamazoo, we believe that all *L. minutus* at the site immigrated from the population that was established in Bristol, Indiana in 1996. The fact that there was a sharp increase in *L. minutus* at the Kalamazoo site in 2013 suggests that the population of *L. minutus* in the surrounding landscape is still growing and expanding rapidly. This hypothesis is further supported when we calculate the percent of the total Kalamazoo *Larinus* spp. population that were comprised of *L. minutus* and *L. obtusus* on July 11 2011, when we made a preliminary observation (**Table 5).** In 2011, 6% of *Larinus* spp. at the site were *L. minutus*. In 2012 this rose to 39%, and in 2013 70% of the *Larinus* spp. population was made up of *L. minutus*.

In the future, it is possible that *L. minutus* will displace *L. obtusus* in this region. Even though there was no observed decrease in *L. obtusus* in 2013, this lack of change could be accounted for by the success of the previous year's population of *L. obtusus*, which was able to reproduce before the site was inundated with *L. mintutus*. Future studies at this site could show whether these congeners will competitively exclude each other, hybridize, or co-exist as separate species.

Dispersal

In general, we saw a pattern of increasing dispersal rate as the time since release increased. The average rate of dispersal since release (**Table 8**) increased at Kalamazoo and Lake Orion between 2012 and 2013 sampling. An increasing dispersal rate is also evident when comparing the average annual dispersal rates (the average distance that the population's edge moved since the year before) from 2007 releases and 2010 releases (Carson Chapter 2), and considering the limited data available from the 1996 Indiana release furthers the support for dispersal rates increasing as the time since release increases. The distance each population expanded between 2012 and 2013 increased substantially with time since initial release in every case.

This observation is not surprising, as a newly founded population needs time to grow before it is capable of expanding. Once a new population grows to a certain density, individuals would likely experience more intra-specific competition for host-plant resources, triggering dispersal. Even if these density dependent mechanisms are not at play, and the insects naturally disperse upon emergence, it would take some time for their population to reach detectable densities at any distance from the release site. In this alternative scenario, detectable dispersal would still be dependent on the population's growth rate. Because the newly released *Larinus* weevils have nearly unlimited resource availability, their population growth rate should be exponential. Thus, it follows that their rate of dispersal would also increase with time, though we do not have sufficient data to speculate on the growth function that the *Larinus* dispersal rate follows.

The overall pattern of increasing dispersal rate with time since release in *L. minutus* and *L. obtusus* is similar to that reported for *Pseudacteon tricuspis* by Porter et al. (2004). However, while we found that it took 5-6 yr for *Larinus* spp. populations to begin substantial dispersal, *P. tricuspis* was dispersing on a landscape scale 3 yr post-release. This difference could be due to differing rates of reproduction, the mobile nature of Dipterans, or a longer period of insect activity in Florida due to mild climate.

Our average rates of dispersal since release are somewhat less than those calculated in other published studies of *Larinus* dispersal rates. While Alford (2013) reported that *L. minutus* had an average dispersal of 112 m/yr 2 yr following release, our mean annual dispersal rate 2 yr post release was only 5.0 ± 3.1 m.

VanHezewijk and Bourchier (2011) reported an average annual dispersal rate for *L. minutus* of 1900 m/yr 5 yr after initial release, while we calculated an average annual dispersal rate five yr post release of 381 ± 169 m/yr. Our average was calculated from two 2007 *Larinus* releases, only one of which was of *L. minutus*. If we only consider the average annual dispersal rate of the Lake Orion population of *L. minutus*, the average then becomes 212.4 ± 41.6 m/yr. Comparing just this 2007 release of *L. minutus* to the *L. minutus* dispersal that VanHezewijk and Bourchier observed in Alberta, the mean annual dispersal rate of the Lake Orion weevil population is much less. However, by the following year *L. minutus* at Lake Orion had undergone further dispersal, with their average annual dispersal rate increasing to 1008 ± 345 m/yr. This is closer to the 5 yr post-release rate calculated by VanHezewijk and Bourchier (2011), and shows that while *L. minutus* dispersal rates at Lake Orion were initially slow, weevils at the site are beginning to disperse at a rapid pace approaching that of the Alberta study.

There are many possible explanations for the differences in observed dispersal rates between our study and those of VanHezewijk and Bourchier (2011) and (Alford 2013). The fact that the study in Alberta was conducted in diffuse and not spotted knapweed may be important. The climatic conditions at a site could also influence the rate of population growth and subsequent expansion. At our northernmost site in Seney (see Carson thesis Chapter 2), we did not see any dispersal of weevils into the surrounding landscape, while at our southernmost sites we saw the beginnings of substantial dispersal 3 yr post release. The initial number of weevils released will clearly have an influence on dispersal rates, though this effect should only be evident during the first few years of establishment. The size and host plant density at the release site, as well as the resource availability in the surrounding landscape are also likely to be important parameters affecting *Larinus* spp. dispersal rates.

Because of the myriad of factors affecting biological control dispersal rates, it is likely to prove difficult to precisely predict how fast any one release will expand into the surrounding landscape. However, several relevant patterns have emerged from our work. First, rates of dispersal increase with time since release, and, considering the rapidly expanding population of *L. minutus* released in Bristol, Indiana 17 yr ago, it appears that this pattern is maintained for some time. Second, drawing from our observations of the 2007 releases in Kalamazoo and Lake Orion, it is clear that there can be a large degree of variation in dispersal distances that is independent of time since release. While both populations are expanding at a fast pace six yr after their respective releases, *L. obtusus* weevils descended from the Kalamazoo release have dispersed an average of four times as far as the *L. minutus* weevils dispersing from Lake Orion. It is uncertain whether these differences are driven by differential dispersal abilities of the two species, landscape composition, anthropogenic assistance, or other unknown factors.

Finally, it is evident from our work that *Larinus* weevils are very mobile biological control agents, capable of distributing themselves on a landscape scale in less than a decade. This is in stark contrast to *C. achates*, which has a very slow dispersal rate (Rondeau 2007) and in our studies was never once found outside of a known release site (Carson Chapter 2). Despite the reported effectiveness *C. achates* as a biological control of spotted knapweed, it seems that *Larinus* weevils will spread to occupy knapweed-dominated habitats across the region long before *C. achates* arrives in most places. It remains to be seen how significant of an effect these seedhead predators will have on knapweed populations in the Midwest.

APPENDIX

APPENDIX

Table 5.
The percent of the total *Larinus* spp. comprised of *L. minutus* and *L. obtusus* in 2011, 2012, and 2013 at Kalamazoo Co release site. 2011 numbers are based on a single preliminary observation date.

Site	Year	*L. minutus* %	*L. obtusus* %
Kalamazoo	2011	6%	94%
Kalamazoo	2012	39%	61%
Kalamazoo	2013	70%	30%

Table 6.
Average *Larinus* spp. per sweep recovered at release point and <800m, 601-3000m, and >3000m from release point. Data includes 2007 spotted knapweed biological control releases made in Oakland Co and Kalamazoo Co, Michigan.

Site	Species	Sampling Year	*Larinus* spp. /sweep, at release	Avg. *Larinus* spp. /sweep, <800m from release	Avg. *Larinus* spp. /sweep 800m- 3000m from release	Avg. *Larinus* spp. /sweep >3000m from release
Lake Orion	*L. minutus*	2012	2.15	0.37 ± .16	0.02 ± 0.01	0.00 ± 0.00
Lake Orion	*L. minutus*	2013	5.42	4.28 ± .35	0.21 ± 0.06	0.14 ± 0.13
Kalamazoo	*L. obtusus*	2012	0.74	0.76 ± .22	0.49 ± 0.13	0.02 ± 0.02
Kalamazoo	*L. obtusus*	2013	1.36	1.48 ± .55	0.89 ± 0.27	0.25 ± 0.15

Table 7.
The maximum dispersal, average maximum dispersal (calculated using the mean of the maximum dispersal seen in each cardinal direction (± SE)), and average rate of spread from 2012-2013 (±SE) for *Larinus minutus* in Oakland Co and *Larinus obtusus* in Kalamazoo Co.

Site	Year of Release	Sampling Year	Maximum Dispersal Detected (km)	Average Maximum Dispersal Detected (km)	Average rate of spread 2012-2013 (km)
Lake Orion	2007	2012	1.4 (West)	1.06 ± 0.21	4.99 ± 1.98
Lake Orion	2007	2013	10.5 (North)	6.05 ± 2.07	na
Kalamazoo	2007	2012	3.27 (West)	2.75 ± 0.34	18.11 ± 8.32
Kalamazoo	2007	2013	43.3 (West)	20.87 ± 8.50	na

Table 8.
The average dispersal rate based on the years since release. Two and three-year post-release data is from five 2010 MSU release sites (See Carson Thesis Chapter 2). Five and six-year post-release data is from 2007 releases in Kalamazoo and Oakland Counties.

Years Post Release	Average Maximum Dispersal Rate (m/yr since release)
2 (2010 Releases)	5 ± 3
3 (2010 Releases)	61 ± 30
5 (2007 Releases)	381 ± 169
6 (2007 Releases)	2243 ± 1235

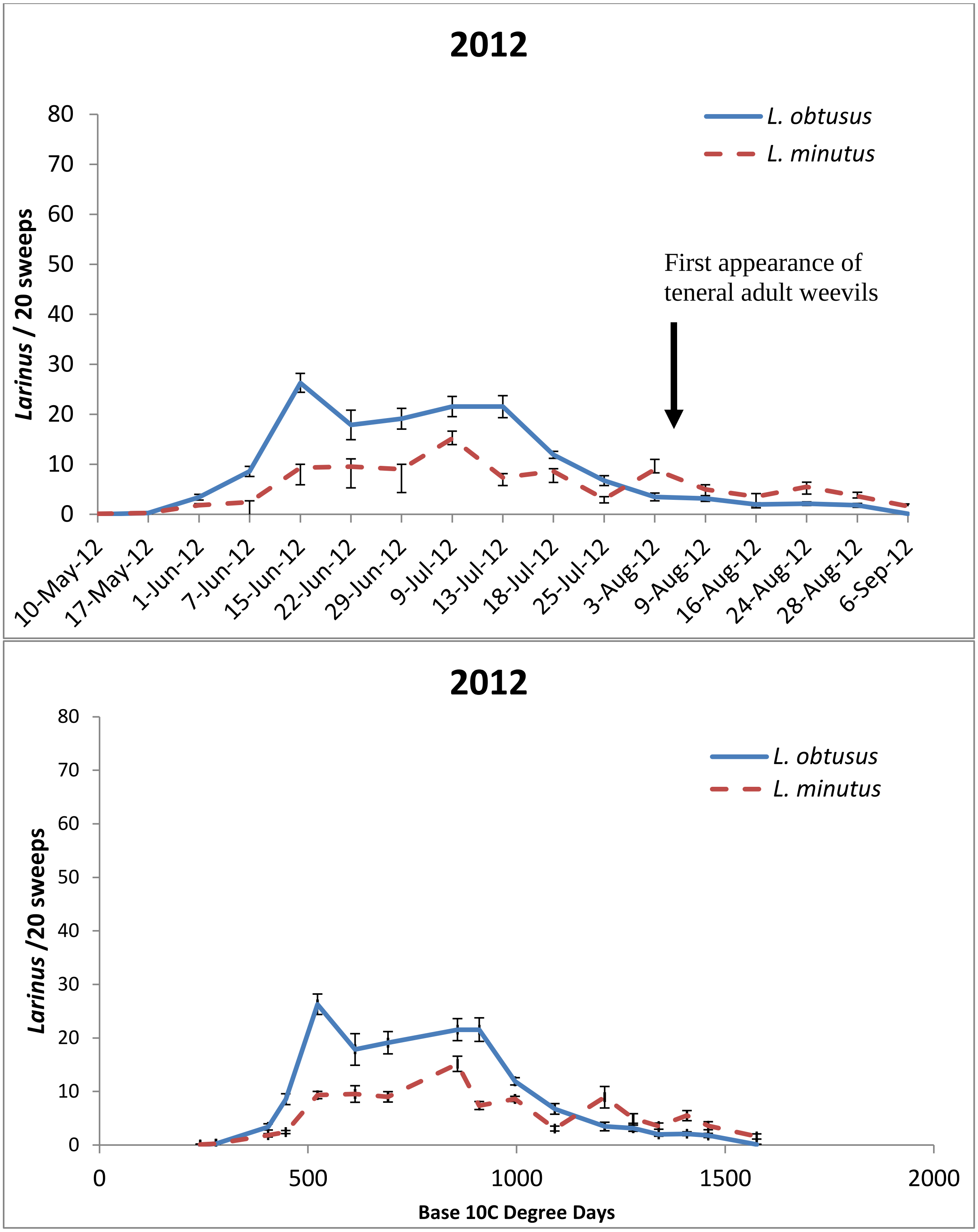


Figure 12. The average abundance of *L. minutus* and *L. obtusus* sampling date and degree day accumulation. Averages are based on the number of weevils found in each set of 20 sweeps (n=10). Error bars indicate SE. Data is from 2012 sampling at a 2007 spotted knapweed biological control release site in Kalamazoo Co, Michigan.

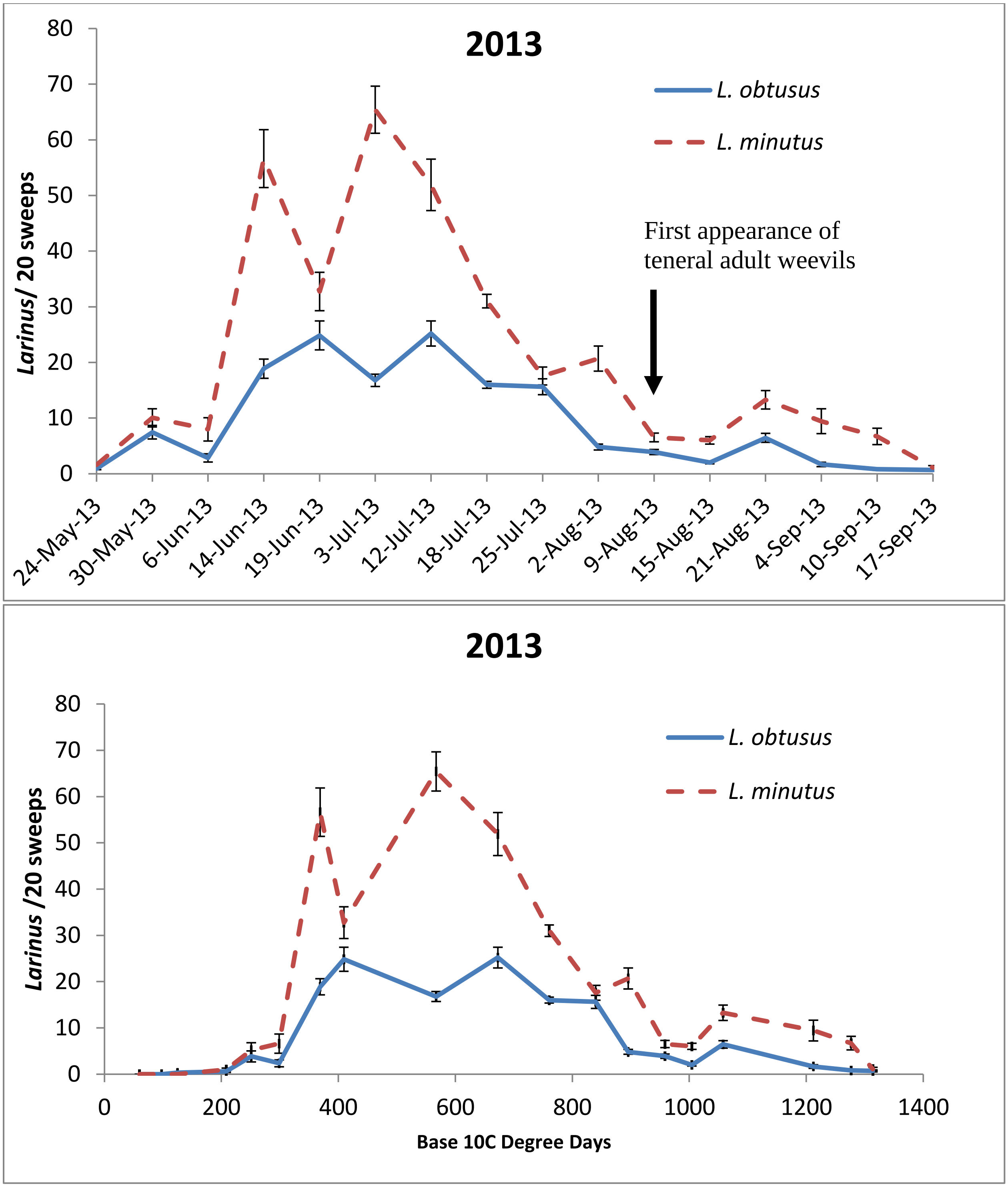


Figure 13. The average abundance of *L. minutus* and *L. obtusus* by sampling date and degree day accumulation. Averages are based on the number of weevils found in each set of 20 sweeps (n=10). Error bars indicate SE. Data is from 2012 sampling at a 2007 spotted knapweed biological control release site in Kalamazoo Co, Michigan.

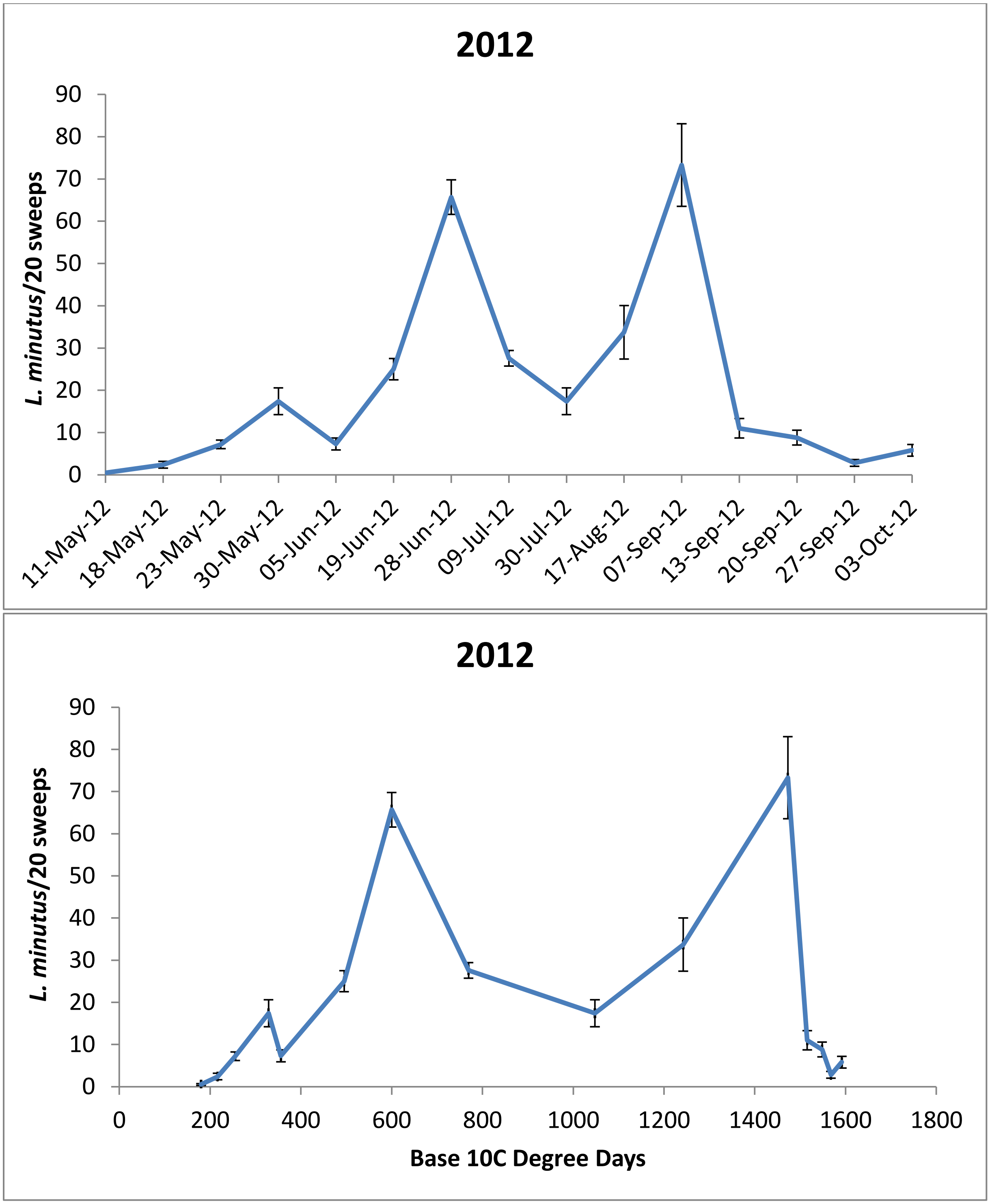


Figure 14. The average abundance of *L. minutus* by sampling date and degree day accumulation. Averages are based on the number of weevils found in each set of 20 sweeps (n=10). Error bars indicate SE. Data is from 2012 sampling at a 2007 spotted knapweed biological control release site in Oakland Co, Michigan.

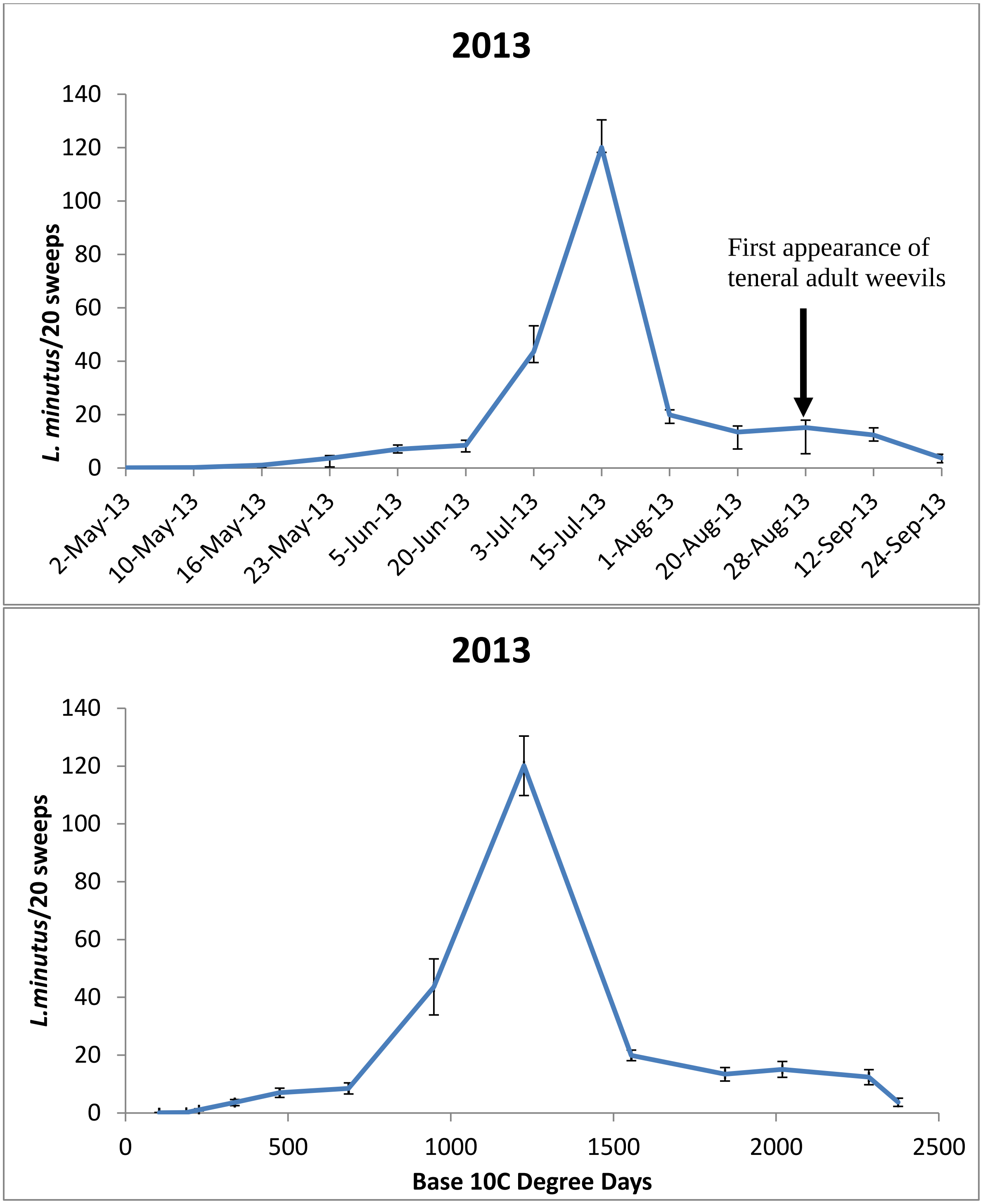


Figure 15. The average abundance of *L. minutus* by sampling date and degree day accumulation. Averages are based on the number of weevils found in each set of 20 sweeps (n=10). Error bars indicate SE. Data is from 2012 sampling at a 2007 spotted knapweed biological control release site in Oakland Co, Michigan.

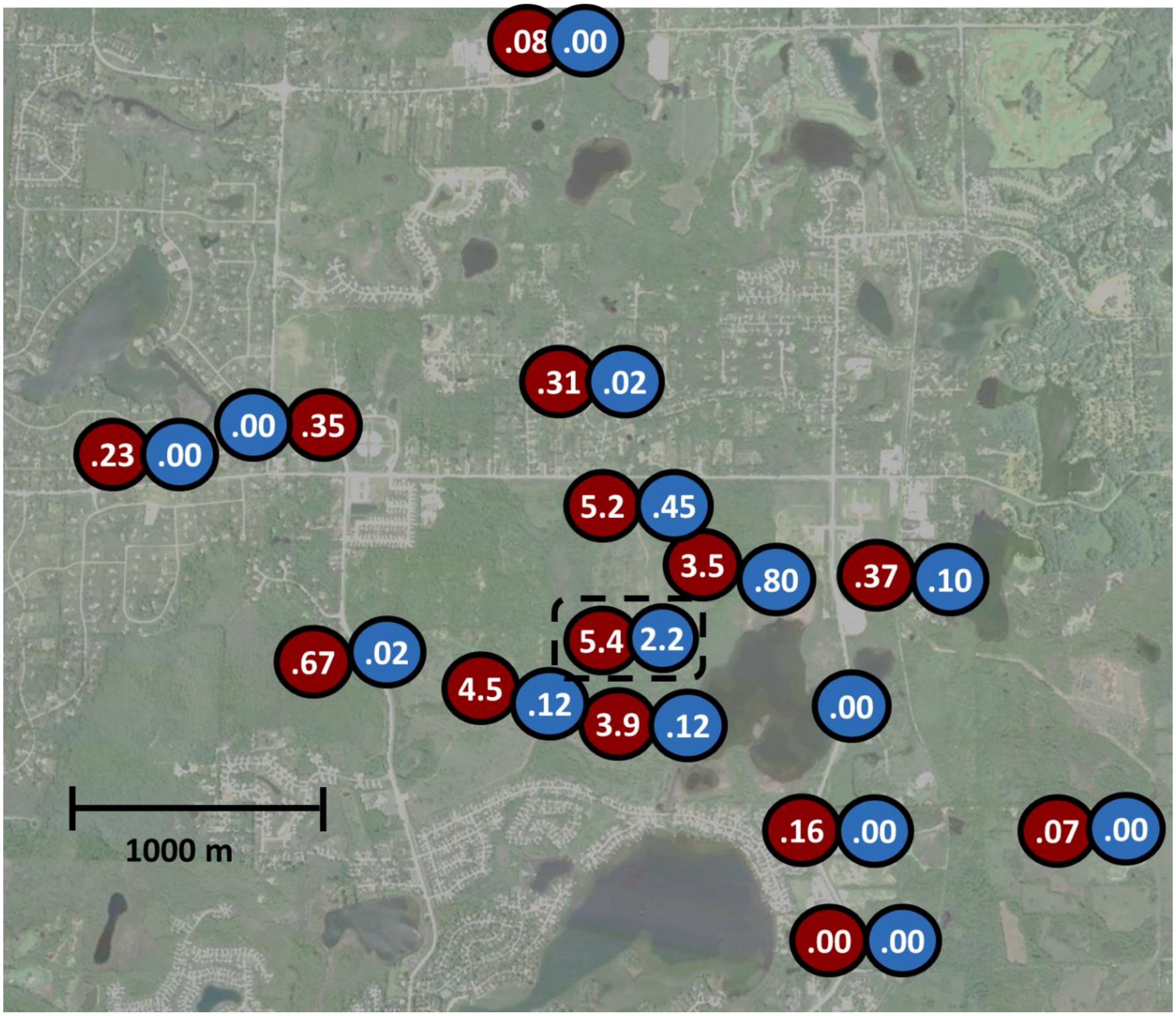


Figure 16. The number of *L. minutus*/sweep at 2012 (blue) and 2013 (red) Lake Orion dispersal sampling points shown on a local scale. Release site is circled with a dashed line. Paired 2012 and 2013 sampling points are in the same location. Oakland Co, Michigan

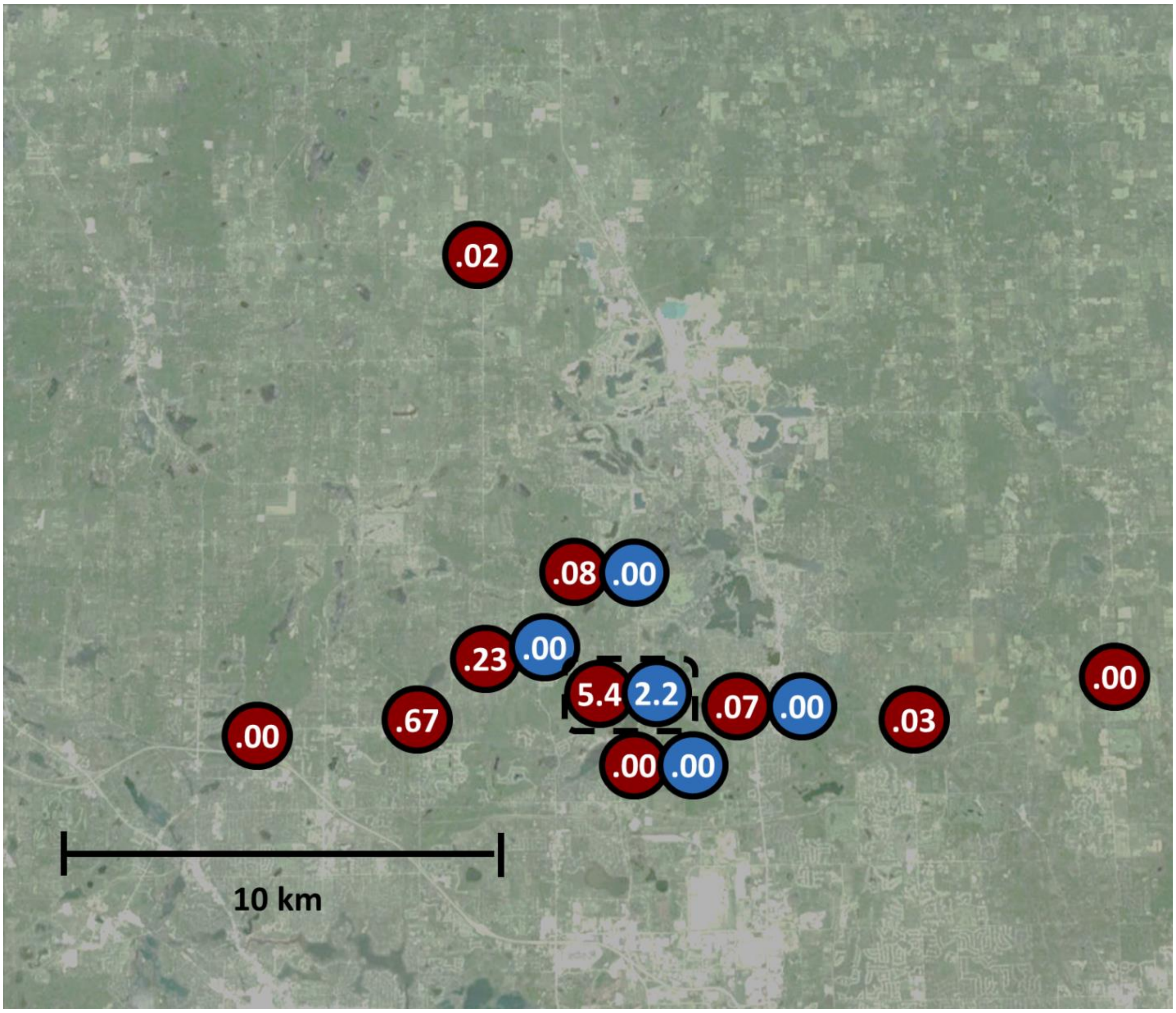


Figure 17. The number of *L. minutus*/sweep at 2012 (blue) and 2013 (red) Lake Orion dispersal sampling points shown on a landscape scale. Release site is circled with a dashed line. Paired 2012 and 2013 sampling points are in the same location. Oakland Co, Michigan

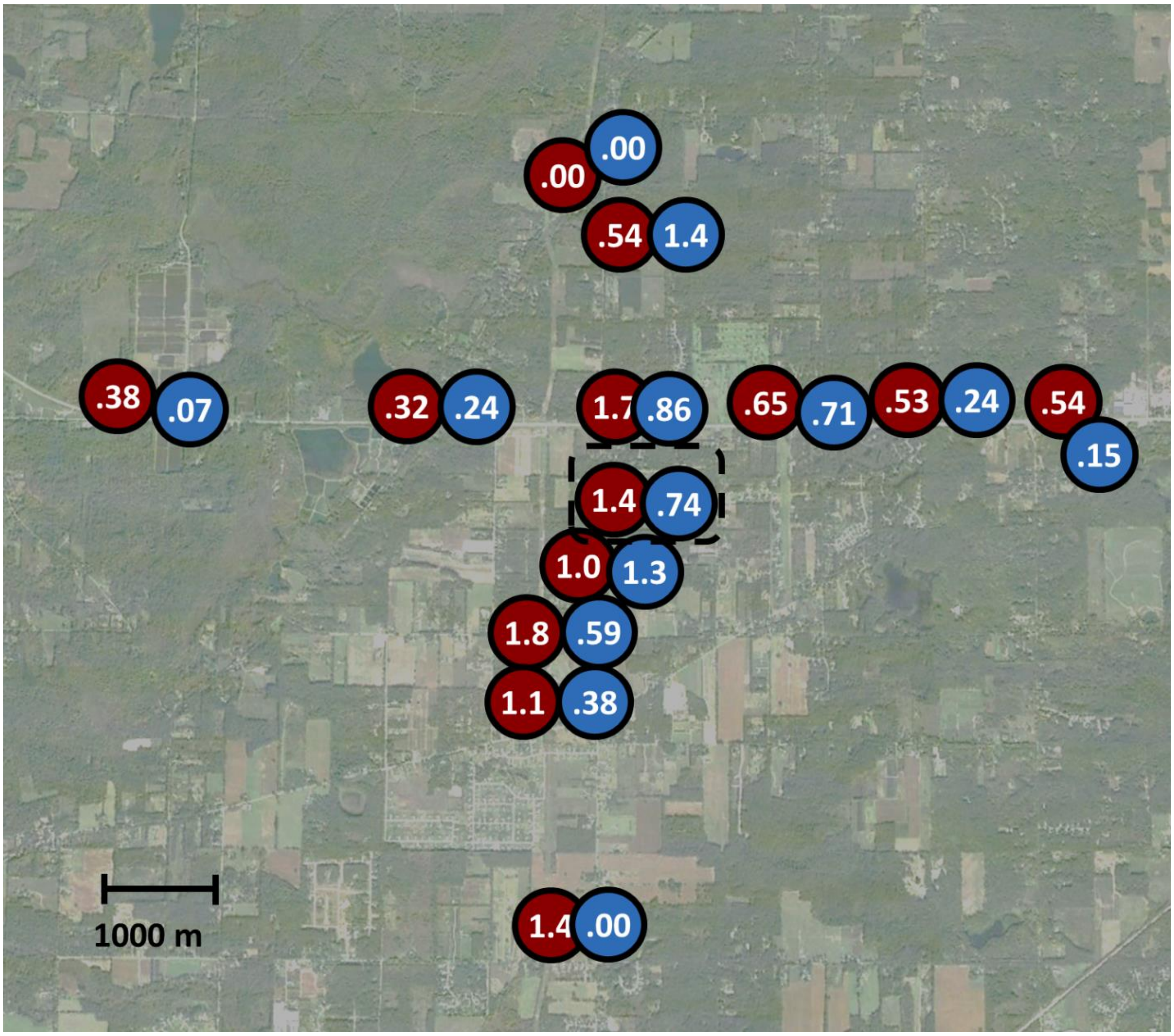


Figure 18. The number of *L. minutus*/sweep at 2012 (blue) and 2013 (red) Kalamazoo dispersal sampling points shown on a local scale. Release site is circled with a dashed line. Paired 2012 and 2013 sampling points are in the same location. Kalamazoo Co, Michigan

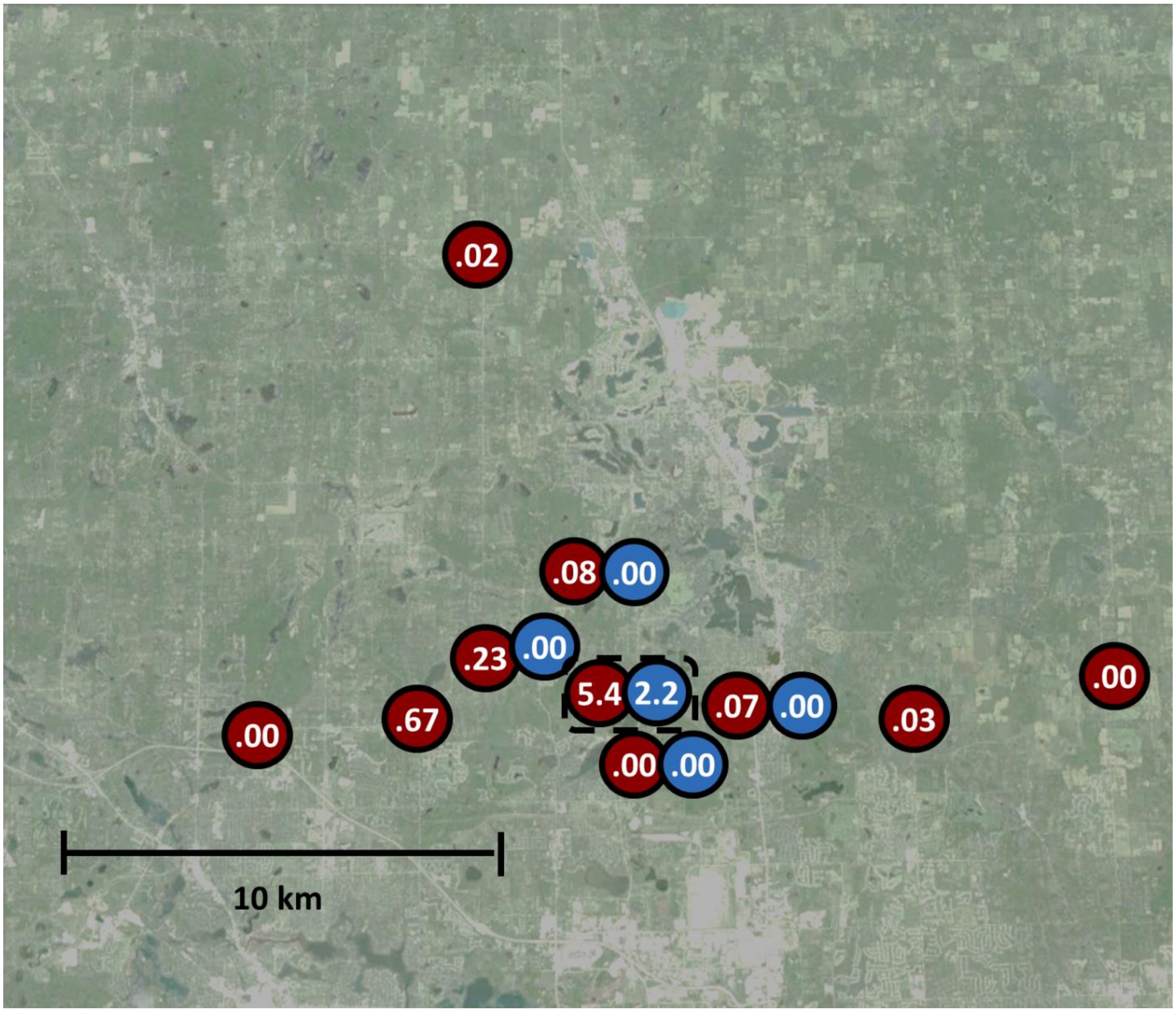


Figure 19. The number of *L. minutus*/sweep at 2012 (blue) and 2013 (red) Kalamazoo dispersal sampling points shown on a landscape scale. Release site is circled with a dashed line. Paired 2012 and 2013 sampling points are in the same location. Kalamazoo Co, Michigan

REFERENCES

REFERENCES

Alford AM (2013) The life history of *Larinus minutus*, a biological control agent of invasive knapweeds, and its dispersal from release sites in Arkansas. Master's Thesis University of Arkansas.

Clark SE, Driesche RGV, Sturdevant N, Elkinton J, and Buonaccorsi JP (2001) Effects of site characteristics and release history on establishment of *Agapeta zoegana* (Lepidoptera: Cochylidae) and *Cyphocleonus achates* (Coleoptera: Curculionidae), root-feeding herbivores of spotted knapweed, *Centaurea maculosa*. Biological Control 22:122

Corn JG, Story JM, White LJ (2009) Comparison of Larval Development and Overwintering Stages of the Spotted Knapweed Biological Control Agents *Agapeta zoegana* (Lepidoptera: Tortricidae) and *Cyphocleonus achates* (Coleoptera: Curculionidae) in Montana Versus Eastern Europe. Environmental Entomology 38:971-976

Draney M L, Oehmichen WT (2007) Spotted Knapweed Bioagents Reaction to Fire (WI) *National Fish and Wildlife Foundation Final Programmatic Report*, 2007-0079-003

Griffith D, Lacey JR (1991) Economic Evaluation of Spotted Knapweed (*Centaurea maculosa*) Control Using Picloram. Journal of Range Management 44:1 43-37

Groppe K, Sobhian R, Kashefi J (1990) A field experiment to determine host specificity of *Larinus curtus* Hochhut (Col., Curculionidae) and *Urophora sirunaseva* Hg. (Dipt., Tephritidae), candidates for the biological control of *Centaurea solstitialis* L. (Asteraceae), and *Larinus minutus* Gyllenhal, a candidate for biological control of *C. maculosa* Lam. and *C. diffusa* Lam. J. Appl. Ecol. 110:300-306

Hakim SA (1979) Range condition on the Threemile Game Range in western Montana. Missoula, MT. Masters Thesis, University of Montana.

Lacey JR, Marlow CB, Lane JR (1989) Influence of spotted knapweed (*Centaurea maculosa*) on surface runoff and sediment yield. Weed Technology 3**:**627-631

Müller-Schärer H, Schroeder D (1993) The biological control of *Centaurea* spp. in North America: do insects solve the problem? Pestic. Sci. 37:343-353

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288**:**81-90

Myers JH, Jackson C, Quinn H, White SR, Cory JS (2009) Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control 50**:**66-72

Paynter Q, Bellgard S (2011) Understanding dispersal rates of invading weed biocontrol agents. Journal of Applied Ecology 48:2 407-414

Porter SD, Nogueira LA, et al. (2004) Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biological Control 29:2 179-188

Rondeau, KJ (2007) Dispersal of the Biocontrol Agent, *Cyphocleonus achates* (Coleoptera: Curculionidae), on the Invasive Plant, Diffuse Knapweed (*Centaurea diffusa*) (Asteraceae). Master's Thesis, University of Alberta

Sheley RL, Jacobs JS (1997) "Acceptable" levels of spotted knapweed (*Centaurea maculosa*) control. Weed Technology 11:363-368

Stinson CSA, Schroeder D, Marquardt K (1994) Investigations on *Cyphocleonus achates* (Coleoptera: Curculionidae), a potential biological-control agent of spotted knapweed (*Centaurea maculosa*) and diffuse knapweed (*Centaurea diffusa*) (Compositae) in North America. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 117:35-50

Story JM, Anderson NL (1978) Release and establishment of *Urophora affinis* (Diptera Tephritidae) on spotted knapweed in western Montana. Environmental Entomology 7:445-448

Story JM, Smith L, Corn JG, et al. (2008) Influence of seed head-attacking biological control agents on spotted knapweed reproductive potential in western Montana over a 30-year period. Environmental Entomology 37:510-519

Van Hezewijk BH, Bourchier RS (2011) Dispersal and Impact of *Larinus minutus* among *Centaurea diffusa* Patches in Alberta, Canada. XIII International Symposium on Biological Control of Weeds pp. 328

Watson AK, Renney AJ (1974) Biology of Canadian weeds *Centaurea diffusa* and *Centaurea maculosa*. Canadian Journal of Plant Science 54:687-701

Wilson LM, Randall CB (2005) Biology and biological control of knapweeds. U.S. Forest Service Publication

CHAPTER 4

IMPACT OF THE INVASIVE PLANT *CENTAUREA STOEBE* ON BEE COMMUNITY ABUNDANCE, RICHNESS, AND DIVERSITY

Introduction

Bees (Hymenoptera: Anthophila) are responsible for the majority of plant pollination services in agriculture (Cane and Tepedino, 2001; Klein et al., 2007) and also contribute to the functioning of natural plant communities (Ollerton et al., 2011). The decline of native pollinator populations coupled with agricultural intensification has led farmers to depend more heavily on the domesticated honey bee (*Apis mellifera* L.) for the pollination of fruit and vegetable crops (Cane and Tepedino, 2001). Commercial pollination services are more expensive than using wild pollinators, and, given recent declining trends in honey bee health, their future reliability is uncertain (Steffen-Dewenter 2005; Cane and Tepedino, 2001). Therefore, practices to ensure the conservation of native bees while also providing resources to support honey bee populations are essential for maintaining robust pollination services within agricultural landscapes.

The decline of wild bee populations is linked to habitat loss and fragmentation (Potts et al., 2010; Goribaldin and Steffen-Dewenter, 2011), a reduction in the diversity of available floral resources (Kleijn and Raemakers, 2008), and climate-change induced shifts in host-plant phenology (Memmott et al., 2010). Because bees often use many habitats within a landscape (Williams and Kremen 2007), increasing the concentration and quality of resource-rich habitats (i.e., habitats containing abundant floral resources) should enhance wild bee abundance, species richness, and species evenness within a given landscape as well as support populations of honey bees when flowering crop resources are lacking. Wild bee community structure is largely determined by the types and diversity of flowering plants available within a habitat, and increasing flowering plant species richness is linked to increasing bee species richness (Goulson and Darvill, 2004; Grundel et al., 2010; Potts et al., 2003; Wilson 2009; Moron 2009), thus, loss or alteration of native plant communities is a major concern for bee health.

Loss of floral resources, and in turn, loss of bee diversity in natural systems has been linked to invasions of non-native plant species. Because many exotic invasive plant species can decrease plant species richness and evenness of invaded communities, they can potentially have an adverse effect on native bees (Stout and Morales 2009). For example, Moron et al. (2009) found that the invasion of the exotic goldenrods *Solidago canadensis* and *Solidago gigantea* in Poland caused a significant decrease in the abundance, species richness, and evenness of bees in wet meadows. A similar effect was measured for hoverflies (Syrphidae) and butterflies (Lepidoptera). The authors had initially predicted that because these goldenrods provide an abundant nectar resource, only specialist foragers would be affected; however, the abundance of generalist nectar-feeding species also decreased significantly (Moron et al., 2009). While this study showed a strong negative influence on native bee diversity, community-level impacts invasive plants have on bees are relatively unknown (Vanbergen et al., 2013).

Much of modern natural areas management involves the removal of exotic plant species and the restoration of native plant communities (Stephens et al., 2009). However, when exotic plants become naturalized and reduce native floral resources, pollinator communities may come to rely on the nectar resources provided be the exotic species. Williams et al. (2011) found that many native bee species living in disturbed habitats primarily used exotic flowering plant species as nectar sources. The bees studied did not prefer exotic species, but rather used them at a rate proportional to their abundance in the flowering plant community. Thus, to maintain bees and pollination services, the removal of any exotic flowering plant species should coincide with the introduction of flowering plant species that provide a similar or greater level of resource provisioning.

Spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos* (Gugler)) is an asteraceous, perennial forb native to Eastern Europe. The plant was introduced to North America in the 1890s, and has since become a widespread invasive species (Sheley et al., 1998). In the Western U.S., it displaces rangeland plants, reducing feed available for livestock and wildlife (Sheley et al., 1998). Spotted knapweed has allelopathic properties that allow it to outcompete native plant species in many situations (Calloway and Ridenour, 2004), and it often forms large, dense, monotypic stands (Sheley et al., 1998). In Michigan, spotted knapweed dominates many disturbed sites, and threatens intact natural areas with invasion (Kost 2004; Kost and Slaughter 2009; Lee, 2007; Cohen 2000 and 2001; Albert, 1999).

While the negative impacts of spotted knapweed on native plant communities are significant, this species provides a valued nectar resource to domestic honeybees and beekeepers in the state. The relative longevity of its bloom period, attractiveness, nectar quality, and abundance in the landscape all contribute to its importance as a floral resource (Watson and Renny, 1974). It is also likely to be providing floral resources to some native bee species, especially those that are generalist foragers (pers. obs., author).

Due to the threat spotted knapweed presents to native plant communities, five arthropod biological control agents have been released in Michigan over the past two decades to control this plant (Carson, Chapter 2). Since their introduction in Michigan, these agents have become established and are slowly spreading from release sites. These same natural enemies have shown some success in reducing spotted knapweed populations in Western systems, though spotted knapweed has never been completely eliminated (Jacobs et al., 2006; Seastedt, 2003; Clark et al., 2001; Storyet al., 2006). Following successful reductions in knapweed densities, several western sites have had exotic grasses move into the space left open by knapweed (Stevens et al., 2009; Story et al., 2006; Bush et al., 2007). In rangelands, these exotic grasses are preferred to knapweed because of the forage they provide (Griffith and Lacey, 1991). In the Midwest, however, replacement of spotted knapweed with exotic grasses would decrease the amount of floral resources available in the landscape, with potential detrimental effects on managed and wild pollinators alike.

Any biological control-facilitated reduction in spotted knapweed densities is expected to happen over decades; however, understanding how these changes will affect pollinator communities is essential to direct management and restoration efforts. The goal of our work is to understand the role spotted knapweed currently plays in supporting bee abundance and diversity, and what plant species might be used to support bee communities if spotted knapweed declines. To address this goal, we conducted a common garden study that contrasted bee visitation to *C. stoebe* and 12 native plant species in a field setting. The goal of this experiment was to gain an understanding of which plant species, alone or in combination, may provide equivalent or better floral resources under similar conditions to spotted knapweed. We coupled this study with a natural experiment pairing knapweed-dominated fields to fields that contained diverse flowering plant communities, including spotted knapweed, within a larger landscape context. The goal of this experiment was to gain understanding how bee communities differed in composition, diversity, abundance and seasonality between the two plant communities.

Methods

Common garden experiment

Native species selection: Native plant species were selected based on two criteria: that they would be well-suited to habitat conditions similar to spotted knapweed, and that they are considered good pollen and nectar sources. To choose suitable plant species, we started from Michigan Natural Features Inventory (http://mnfi.anr.msu.edu/) lists of plants common to such sites historically (oak barrens, pine barrens, dry sand, and dry-mesic prairies) and use additional resources to identify plants native throughout Michigan (both upper and lower peninsulas) from Reznicek et al. (2011) that were currently available as seed from members of the Michigan Native Plant Producers Association (http://www.mnppa.org/) and associated catalogs. **Table 9** shows the plant species in the common garden experiment.

Planting design: Four replications of 13 forb species (12 native forbs, plus spotted knapweed, **Table 9**) were grown in a randomized complete block design. The blocks were laid out on a rectangular grid, with each block consisting of two rows of five plant species, and one row of six plant species (**Figure 20**). Each species by rep unit consisted of three plants of a single species, planted 0.5 m apart in a triangle, and each unit was spaced 2 m from the next.

Rooted plant material was obtained from Wildtype Nursery, Mason, MI. The garden was planted on May 15, 2012, and watered up to three times a week for the first month of establishment. Plants that died in the first four weeks were replaced. The first blooms were observed in experimental plots at the beginning of July, 2012, at which time bee observations were initiated.

Bee observations: Each plant species was sampled once per week beginning with its first bloom event and continuing until the last bloom of the season. Only plant species with actively blooming flowers were observed. When a plot had at least one individual in bloom, that species was observed in all four blocks. This led us to observe many clusters of each species that were not in bloom, and allowed us confirm that it was the floral display that bees were attracted to and not extrafloral or Homopteran honeydew sources.

All of the blooming plant species in a block were observed before moving on to the next block. The order that blocks were sampled was rotated each week so that no one block received all of its observations at the same time of day. Bee observation occurred between 10:00 and 16:00 on sunny days when the temperature was a minimum of 21 C, except one date when the temperature reached 15.5° C during observations (September 14, 2013).

Each plant cluster was observed for 6 min during each data collection period. During this time, we attempted to capture every bee that landed on a flower. Bees were collected using an insect-sampling vacuum (Bioquip model 2820GA). All bee visits were recorded as either “honey bees” or other bees,” regardless of whether each bee was successfully collected. After capture, we killed the bees using ethyl acetate kill jars and returned to the lab for pinning and identification.

All identifications were made to the species level using an online key (Discoverlife.org) and verified by Dr. Jason Gibbs, Michigan State University.

To estimate the attractiveness of the basic unit of floral display, we divided the total bee visitation each plant species received by the total area (cm^2) of floral display which that species presented throughout the season. To estimate the attractiveness of each plant as a whole, we divided the number of bee visits to a plant species by the number of individual plants in bloom at the time of observation. By averaging this metric for each observation within each month of the summer, we were able to compare the relative attractiveness of each plant species that was in bloom for June, July, August, and September.

Field-level floral diversity experiment

This experiment was conducted at Orion Oaks County Park in Oakland County, Michigan (42.75°N, -85.29°E). The park contains remnant oak barrens, mesic hardwood forest, and lower elevation wetlands. The uplands primarily consist of well-drained sandy soil, and the majority of the non-forested upland habitat has been invaded by spotted knapweed which has become the dominant plant cover in some fields. We selected four fields within the park that were dominated by spotted knapweed (**Figure 21**) and four that contained a diversity of flowering plant species, including spotted knapweed. This allowed us to compare the use of different plant species by bees in both knapweed near-monocultures and diverse plant communities. To control for spatial variation in the bee communities, each field with a knapweed dominated plant community was paired with a nearby (within 250 m) field with a diverse plant community.

We conducted sampling on August 2, August 21, and September 17 in 2012, and July 10, July 30, August 19, and September 11 in 2013. We used criteria identical to that of the common garden to choose sampling days. Air temperature and percent cloud cover were recorded at the beginning of each sampling period.

Floral community sampling: Immediately following bee observations (see below), the flowering plant community at each transect was characterized. In 2012, observers placed a 2 x 2 m quadrat at the 0 and 10 m marks on each transect and counted the number of blooms of each plant species present in each quadrat. In 2013, sampling efforts were doubled, with quadrats placed at the 0, 5, 10, and 15 m marks on each transect.

Bee community sampling: Two permanent 20 m sampling transects were established in each field (**Figure 21**). The order in which field pairs were sampled in was rotated to minimize bias caused by sampling time. Pollinator observations were conducted by two observers walking each 20 m transect for 10 min. Observers walked parallel to each other on opposite sides of the transect line, and all bees visiting flowers within 1.5 m of the transect were documented. The flower species being visited was recorded, as well as the type of bee (honey bee, bumble bee, or small solitary bee). Bees were collected using a bee-vacuum (Bioquip model 2820GA) and a butterfly net, with each device capturing and holding the bees from one plant species. Prior to sampling, the plant species present were recorded and split into two groups. During sampling, each observer collected off half of the plant species. After 5 min, observers exchanged nets and vacuums and collected off the other half of plant species. Thus, up to four species of plant could be observed at a time. If more than four plants were being visited by bees, observers paused the timer to process captured bees, and then resumed the transect. After each 20 m transect, the bees were killed using ethyl acetate. Bees were pinned and labeled soon after returning to the lab. All identifications were made to the species level using an online key (Discoverlife.org) and verified by Dr. Jason Gibbs, Michigan State University.

Data analysis

To standardize the floral display of different species, we measured the diameter of all of the individuals of each species present in several quadrats. For blooms that were not radially symmetrical (ex. *Solidago* sp.) we estimated the mean floral area in square centimeters. From this data we calculated the average floral abundance (cm^2) of each plant species in each field type, as well as the floral diversity present in each field type. The floral diversity was estimated using Shannon's diversity index (Shannon 1948).

For all model selection experiments, Akaike's Information Criterion (AIC) was used to rank competing models. Models that had lower relative AIC values were considered to better explain variation in the data, and models producing AIC values within two units of each other were considered to have equivalent performance. When two models were ranked equivalently, the model with fewer parameters was selected for further investigation.

To test for differences in floral and bee diversities and abundances between field types, we included the factors of sampling year, sampling date, field pair, and field type (knapweed-dominated or diverse) in a linear model. We used Two-way ANOVAs to analyze floral data and bee diversity data. Because many observations had no bees in a particular category, zero bias caused bee abundance data to follow a non-normal distribution, and thus violated the normality assumption of ANOVA. To account for this, we used a non-parametric Kruskal-Wallis test to examine trends in bee abundance data.

We used a model selection approach employing generalized linear mixed models (Burnham and Anderson, 1998) (R 2.15.3 glmmADMB) to investigate what factors influenced bee abundance and diversity. We only used 2013 data for model selection, because floral abundance data from that year provides a closer approximation of the plant community at each transect due to increased sampling effort. A negative binomial error structure was used in all models, and field pair was included as a random factor to account for potential spatial variation of the bee community within the park.

To explore factors influencing total bee abundance, we first used the floral abundance of each plant species as independent factors to determine which plant species had the greatest impact on bee abundance. Before beginning the model selection process, we used a decision rule to drop plant species that had fewer than four observations throughout the season. We then performed model selection using total floral abundance, floral species richness, floral diversity, temperature, and percent cloud cover as independent variables, and total bee abundance, solitary bee, honey bee, and bumblebee abundances as dependent variables. Finally, to investigate variables linked to bee diversity, we used stepwise selection in a linear model with the independent factors of floral abundance, floral species richness, floral diversity, temperature, and percent cloud cover as independent factors.

To test for overall differences in the bee communities of diverse and knapweed-dominated fields, we used the Bray-Curtis index to generate a matrix of similarities based on bee species and identities found in each field type. We used combined bee species data from 2012 and 2013. Before generating this matrix we removed bee species that only appeared once in the dataset. We then plotted each value of this similarity matrix using non-metric dimensional scaling (NMDS) (Primer 6, Version 6.1.13, Primer-E ltd.). Plotted this way, each point represents a bee community from a given observation and the distance between each point shows how much similarity there is between communities, with closer points showing more community similarity. Finally we used an analysis of similarity (ANOSIM) to test whether between-treatment dissimilarities were greater than within-treatment dissimilarities, which would indicate that there are different bee communities in diverse and knapweed-dominated fields. All statistical tests used an alpha of 0.05 to determine significance.

RESULTS

Common garden experiment

Twenty five hours of bee observations were completed in 2012, and 23.5 hr were completed in 2013. **Figure 22** shows the observed bloom period of each plant species in the common garden. With the exception of *Anenome canadensis*, every plant species was observed receiving at least one bee visitor. *C. stoebe* was visited by more total bees than any other plant over the course of the study (**Figure 23**). Very few honey bees were observed at the common garden experiment in 2012 (n=25), while more were observed in 2013 (n=117). Unlike the total bee community, honey bees were more frequently observed visiting several native plant species than *C. stoebe*. (**Figure 24**).

The following results report combined bee visitation values for 2012 and 2013. In June, *Penstemon hirsutus* and *Coreopsis lanceolata* were the only species in bloom. *P. hirsutus* was more attractive, with a mean of 1.85 bees per plant in bloom/6 minute observation. In July, 12 plant species were in bloom (**Figure 25**). Of these, *C. stoebe* had the highest visitation rate (1.86 bees per plant in bloom/6 min observation), closely followed by *P. hirsutus, C. lanceolata*, and *Monarda fistulosa*. Eight species were in bloom in August, of which *C. stoebe* had the highest visitation rate, with a mean of 1.55 bees per plant in bloom/6 min observation. Ten plant species were in bloom in September, with *S. speciosa* having the highest visitation rate (2.08 bee visits per plant in bloom/6 minute observation).

In 2012, *C. stoebe* had the greatest visitation to floral area ratio (0.019 bees/cm^2), followed by *Gnaphalium obtusifolium* (0.005 bees/cm^2). While *C. stoebe's* high visitation ratio was probably due to the attractiveness of its bloom to a wide variety of bee species. *G. obtusifolium*'s high ratio was more than likely the result of the tiny area (0.5 cm diameter) represented by its floral display. In 2013, *P. hirsutus* had the highest ratio of visits to floral area (0.012 bees/cm^2). It was closely followed by *C. stoebe* (.012 bees/cm^2) and then *C. lanceolata* (0.005 bees/cm^2) and *A. laevis* (0.005 bees/cm^2).

In total, we collected 37 bee species during our common garden experiment, with 18 bee species in 2012, and 31 bee species in 2013. *C. stoebe* was visited by 23 bee species, more than any other plant in our experiment. *Rudbeckia hirta* was visited by 10 bee species, *H. occidentalis* was visited by eight species, and *P. hirsutus* was visited by six species. **Table 10** shows the bee species captured in the common garden experiment in 2012-2013, and the percentage of visits that were to *C. stoebe*. 23/37 bee species (62%) visited *C. stoebe* at least once, while 14/37 bee species (38%) never visited *C. stoebe* during observations. **Figure 26** shows the bee species richness for each plant species.

Field-level floral diversity experiment

Floral community sampling: Table 11 shows the total season-long floral abundance by plant species for 2013 transects. In diverse fields, the plant species with the greatest floral abundance were *Solidago speciosa, Monarda fistulosa, C. stoebe, Solidago juncea, Solidago nemoralis, Achillea millefolium,* and *Asclepias tuberosa*. In fields dominated by *C. stoebe,* the vast majority of floral units were represented by that species. The season-long total floral abundance in diverse field transects was 2,721cm^2/m^2, and in knapweed-dominated fields it was 1,646 cm^2/m^2. The mean season-long floral resource diversity was significantly higher in the diverse versus knapweed-dominated fields ($F_{(1,46)}$ =23.56, p < .001) (**Figure 27**) and within each individual field pair ($F_{(1,46)}$=21.44, p < .001).

The mean season-long floral abundance was also higher in diverse versus knapweed-dominated fields. This finding was statistically significant across both sampling years (**Figure 28**) ($F_{(6,36)}$=6.374, p=0.015). Date was found to significantly influence floral abundance ($F_{(6,36)}$=6.788, p < .001), and there was a significant interaction between sampling date and field type ($F_{(6,36)}$ =3.043, p=0.016), indicating that floral abundance varied across dates in different ways in diverse and knapweed-dominated fields. This finding is explained by the high number of knapweed blooms present in both field types during knapweed's peak bloom period (**Figure 29**).

Bee community sampling: A total of 50 species of bees were collected across both years of the study; 19 in 2012, and 43 in 2013 (**Table 12**). A total of 36 bee species were found in diverse fields and 22 in knapweed-dominated fields. In diverse fields, 38% of bee species (14 total) collected were observed using *C. stoebe*, while in knapweed-dominated fields, 86% (19 total) of bee species collected were observed using spotted knapweed. In 2013, a total of 20 bee species were observed using *C. stoebe*. Nine bee species were solely collected from knapweed, while 23 species were never observed using spotted knapweed. Four species were only collected on July 10 (i.e. before knapweed began blooming), and nine species were only collected on September 11, after knapweed had stopped blooming.

Table 11 shows the number of bee visitors each plant species received during 2013 transect sampling. In knapweed-dominated fields, *C. stoebe* received 235 bee visits, far more than the second most visited plant, *Bertoa incana*, which received nine bee visits throughout the season. In diverse fields, *C. stoebe* still had more bee visitors than any other plant species, with 125 total observed visits. However, in diverse fields other plant species were also heavily used. *M.fistulosa*, *S. speciosa*, and *S. nemoralis* received 106, 96, and 48 bee visits. Notably, *M. fistulosa* blooms before and during the beginning of *C. stoebe's* peak bloom period, while *S. speciosa* and *S. nemoralis* both bloom after *C. stoebe* has ceased blooming.

There was no significant difference in total bee visits between field types in 2012 (Kruskal-Wallis χ^2= 0.236, df = 1, p-value = 0.627). In 2013, diverse fields had significantly higher total bee abundance (Kruskal-Wallis chi-squared = 4.798, df = 1, p-value = 0.029) (**Figure 30**). Solitary bees (Kruskal-Wallis chi-squared = 5.111, df = 1, p-value = 0.024) and honey bees (Kruskal-Wallis chi-squared = 6.141, df = 1, p-value = 0.013) were significantly more abundant in diverse fields across sampling years. Bumblebees showed no significant trends across years, but were numerically more abundant in knapweed-dominated fields in 2012, and in diverse fields in 2013.

The abundance of each bee group varied between sampling dates, and each group was found in numerically higher abundance in knapweed-dominated fields on at least one sampling date. However, in every case diverse fields contained significantly higher bee abundance on the final sampling date: (Solitary bees in 2012: Kruskal-Wallis chi-squared = 4.091, df = 1, p-value = 0.043; 2013: Kruskal-Wallis chi-squared = 5.600, df = 1, p-value = 0.018; honeybees in 2012 Kruskal-Wallis chi-squared = 3.857, df = 1, p-value = 0.049, 2013: Kruskal-Wallis chi-squared = 6.137, df = 1, p-value = 0.013; bumblebees in 2012: Kruskal-Wallis chi-squared = 3.857, df = 1, p-value = 0.049, 2013: Kruskal-Wallis chi-squared = 6.054, df = 1, p-value = 0.014).

The mean bee richness of diverse fields was greater than that of knapweed-dominated fields ($F_{(1,47)}$=16.900, p < .001) (**Figure 31**). Sampling date was a significant factor ($F_{(6,42)}$=3.131, p=.013), indicating that the total bee species richness varied with sampling date. There was also an interaction between sampling date and field type ($F_{(1,42)}$=21.504, p < .001). This finding was correlated with a seasonal waxing and waning of *C. stoebe* floral resources in knapweed-dominated fields (**Figure 29**).

The season-long mean bee diversity as measured by Shannon's H was statistically higher in diverse fields than in knapweed-dominated fields across both sampling years ($F_{(1,47)}$ =31.590, p <.001) (**Figure 32**). Sampling date was not a significant factor, but there was an interaction between field type and sampling date ($F_{(6,36)}$=2.808, p=0.024), again linked to waxing and waning of *C. stoebe* floral resources (**Figure 29**).

Overall, *S. ericoides*, *C. stoebe,* and *M. fistulosa* abundance had more influence on total bee abundance than any other plant species. Total floral abundance was the best predictor of total bee abundance, as well as solitary bee, bumble bee, and honey bee abundance. Variance in bee diversity was significantly explained by floral abundance and floral diversity. The floral abundance of *C. stoebe* (slope=2.370e-4 ± 6.720e-5, p < 0.001), *S. ericoides* (slope=4.350e-3 ± 1.530e-3, p=.004) and *M. fistulosa* (2.340e-4 ± 1.190e-4, p=0.049) positively influenced total bee abundance. The floral abundance of *B. incana* (-1.750e-3 ± 7.140e-4, p=0.014) had a negative relationship with total bee abundance.

Total floral abundance (slope=0.035 ± 0.009, p<0.001) and temperature (slope=0.090 ± 0.034, p=0.017) both had a positive influence on total bee abundance, and cloud cover (slope=-0.018 ± 0.006, p=0.001) had a negative effect on total bee abundance. Floral abundance had a significant positive effect on solitary bee abundance (slope=0.019 ± 0.007, p=0.009) and temperature had a non-significant positive relationship with solitary bee abundance (slope= 0.042 ± 0.024± 0.081).

Floral abundance had a significant positive effect on honeybee abundance (slope=0.083 ± 0.028, p=0.003) and temperature (slope= 0.028 ± 0.087, p=0.001) had a significant positive relationship with honeybee abundance. Cloud cover (slope=-0.046 ± 0.019, p=0.013) had a significant negative relationship with honeybee abundance. Floral abundance had a significant positive effect on bumblebee abundance (slope=0.039 ± 0.059, p=0.153) and temperature (slope=0.085 ± 0.059, p=0.152) had a non-significant positive relationship with bumblebee abundance. Cloud cover (slope=-0.022 ± 0.008, p= 0.009) had a significant negative relationship with bumblebee abundance. Bee diversity was positively related to floral abundance (slope=.003 ± .001, p=.001) and floral diversity (slope=.436 ± .161, p=.014).

The two-dimensional representation of bee communities found in diverse and knapweed-dominated fields produced using NMDS had a relatively high stress level (0.21), indicating that there was a large degree of bee community variability between observations, regardless of whether observations were from diverse or knapweed-dominated fields. ANOSIM analysis showed that there was no statistical difference in bee communities between field types (Global R=-0.025, p=0.620), indicating that diverse and knapweed-dominated fields had similar bee communities throughout the study.

Discussion

The results of our common garden study and our field observations both demonstrate that *C. stoebe* is an attractive plant that is visited by a large quantity and diversity of bees throughout its bloom period. However, both studies also demonstrate the important role that plant diversity plays in increasing the amount of time floral resources are available throughout the season. By extending the length of time floral resources are available, increases in plant diversity led to greater overall bee visitation at sites containing more plant diversity. Greater plant diversity favored bee species richness. This was at least in part because the total period of time of floral resource availability overlapped with the active period of more bee species.

Common garden experiment

Overall, our common garden revealed several important trends. *C. stoebe* was substantially used by a wide range of bee species, and was visited by more total bees and more bee species than any other plant. These trends are probably the result of two of the plant's characteristics: a long bloom period, and high relative attractiveness. With the exception of *R. hirta*, *C. stoebe* had a longer bloom period than any other plant species in our study. Even after its peak bloom had passed, each plant produced blooms through the end of the season. By remaining in bloom for a longer period of time, *C. stoebe* was more likely to interact with a greater number of bee species. This is because the active period for some bee species is less than the total bloom period of *C. stoebe,* so the active stage of many bee species fall within *C. stoebe's* bloom period. The greater species richness of bees captured on *C. stoebe* may thus be partially explained by its long bloom period. The probability of observing a given bee species at a plant increases with the amount of time spent observing that plant species. Because *C. stoebe* was in bloom for a long period of time, it accumulated more observation time, which could have led to us capturing a greater number of species on the plant. However, this sampling effect should be proportional to the actual number of bee species that used *C. stoebe* during our study.

In the common garden, *C. stoebe* blooms received the most visits per cm^2, indicating that each flower is inherently attractive, and it is not simply the quantity of blooms each plant produces attracting more bees. On a per plant basis, *C. stoebe* was also the most attractive species. However, in September, when most knapweed blooms had senesced, *S. speciosa*, *S. nemoralis*, and *A. laevis* were the most attractive species on a per plant basis. Each of these four plant species had high floral densities when they were in bloom, showing that total floral area interacts with each bloom's attractiveness/cm^2 to drive overall attractiveness.

C. stoebe is valued by commercial beekeepers in Michigan as a nectar source for honey bees. However, somewhat surprisingly, we did not observe heavy use of *C. stoebe* by honey bees in our study. When honeybees were most abundant, we observed more visits to *S. speciosa* and *A. laevis* than to *C. stoebe*. We cannot conclude that honeybees showed a preference for these two species, because they were in bloom after *C. stoebe*'s peak bloom. However, it is evident from this study that some of the native plant species that bloom in September are used heavily by honey bees.

This last finding highlights the important role that plant diversity plays in supporting bee communities. While *C. stoebe* combines high attractiveness with a long bloom period, it is not able to support an entire pollinator community by itself. Plant species that were in full bloom after *C. stoebe*'s peak bloom period were heavily visited by an abundance of bee species. In September, when knapweed had largely finished blooming, we captured five new bee species on late-blooming native plants. By extending the length of time blooms were available, these late-blooming native plants increased the total number of bees and the overall bee species richness observed in the common garden experiment.

The common garden did not reveal any single native plant species capable of completely replacing *C. stoebe*'s floral resource provisioning. However, several native plant species did stand out as being attractive to a range of bee species, and together they may be able to provide greater floral resource provisioning than *C. stoebe* can by itself. In order of bloom period, these were *P. hirsutus*, *C. lanceolata*, *M. fistulosa*, *H. ocidentalis*, *A. laevis*, *S. speciosa*, and *S. nemoralis.*

Field-level floral diversity experiment

Diverse fields had greater bee species richness, diversity, and abundance than knapweed-dominated fields throughout this study. This is not surprising, as many studies have linked floral diversity to bee community diversity (Goulson and Darvill, 2004; Grundel et al., 2010; Potts et al., 2003; Wilson et al., 2009; Moron et al., 2009), and there is a large body of literature linking increases in diversity to the overall productivity of a system (eg.Tilman et al., 1997; Tilman et al., 2001). However, our results show that the mechanism behind this finding is not simply a matter of more plant species supporting more specialist bee species. Instead, we found that while many bee species in the study were generalists that used *C. stoebe* as a resource while it was available, diverse fields contained plant species that bloomed before and after *C. stoebe's* bloom period, extending the period of time floral resources were available at these sites. Because of this temporal resource extension, diverse fields were able to support greater bee abundances and species richness.

Before *C. stoebe's* peak bloom, knapweed-dominated fields contained almost no floral resources, while diverse fields had significant levels of floral resource abundance. Commonly occurring blooming plants included *M. fistulosa*, *E. strigosus*, *A. tuberosa*, *A. millefolium*, *B. incana*, and *H. perforatum*. Of these, *M. fistulosa* was by far the most heavily used by bees. During late-season sampling, *C. stoebe* had almost completely senesced, with only a few individual blooms found on any transect. Diverse fields still contained an abundance of floral resources, with the primary contributors being *S. speciosa*, *S. nemoralis*, and *S. ericoides*. All of these species were heavily used by honey bees, solitary bees, and bumblebees, with *S. speciosa* receiving the most visits.

Total floral abundance was the best predictor of the total bee abundance, as well as the abundances of honey bees, bumblebees, and solitary bees independently. Diverse field types had greater season-long total floral abundance, and also had higher bee abundances. However, during *C. stoebe*'s peak bloom, floral abundances in knapweed-dominated fields were comparable to those in diverse fields. Because bee abundance at our site was more strongly influenced by floral abundance than floral diversity, we saw similar bee abundances in diverse and knapweed-dominated fields during *C. stoebe*'s peak bloom. The higher season-total bee abundance in diverse fields were the result of an extension of floral resource availability, and not an inherent increase in productivity driven by increasing floral diversity.

In a study investigating the impact climate change will have on efforts to improve bumblebee population stability via floral strip plantings in agricultural landscapes, Memmott et al. (2010) found that shifts in floral bloom phenology have the potential to disrupt plant-pollinator networks by causing a phenological mismatch. They conclude that increasing the diversity of conservation floral plantings can help mitigating climate change's impact on bees by increasing the likelihood that there will be plant species in bloom in the beginning and end of the growing season. Our study extends this finding to natural systems, showing that increasing floral diversity does extend the period of floral resource availability, and could therefore help mitigate climate-change induced mismatches in plant-pollinator networks.

If *C. stoebe* had an even more extended bloom phenology, would it be able to support a bee community with as much total diversity and abundance as that supported by a diverse plant community? Our findings support this conclusion to a certain extent. We found no differences in the overall bee communities in diverse and knapweed-dominated fields. There would likely be a loss of some bee species that never utilize *C. stoebe*, but the majority of bees we observed foraging during *C. stoebe*'s bloom period did use it to some extent. However, we cannot discount the possibility that a diversity of flowering plant species enables more abundant and diverse bee communities to persist in the long term, even if the majority of these are generalists.

Some generalist bee species require pollen from a subset of plant species to successfully develop through pupation (Sedivy et al., 2011), and some generalist bee species preferentially seek out the pollen of native plant species (Kleijn and Raemakers, 2008; Williams and Kremen, 2007). Thus, although many species use *C. stoebe*, some might have additional obligate relationships with co-occuring native plant species. A varied diet may also be important for maintaining health in bees. Alaux et al. (2010) found that honeybee larvae that were fed a diet of polyfloral pollen had increased levels of glucose oxidase, leading to an increased baseline immuno-competence compared to larvae that were fed a monofloral pollen diet. Increases in floral diversity on a landscape scale could therefore increase the overall health of a honey bee colony by increasing bee immuno-competence. Furthermore, honey bees overwinter as an entire colony, so they need to store honey to feed workers throughout the winter. The presence of late-season flowering plants in diverse landscapes allows them to gather nectar for an extended period of time. Many commercial beekeepers take their colonies to warmer climates during the winter, and thus are less reliant on late-season plants. However, with concerns over declining commercial honeybee populations (Steffen-Dewenter et al., 2005; Cane and Tepedino, 2001), maintaining robust local honey bee populations will help ensure sustainable pollination services in the future.

The results of both experiments demonstrate that *C. stoebe* is a highly attractive plant that is used by an abundance of bee species during its bloom period. This finding differs somewhat from that of Moran et al. (2011), which reported on the effect of invasive goldenrods in wet meadows in Poland. In their study, sites that were invaded by goldenrod had drastically lower native bee abundance and diversity. While we did see reductions in both of these variables in knapweed-dominated fields, this was primarily the result of a truncated period of floral resource availability. Our results also differ from those of Williams et al. (2011), who found that while bees in disturbed habitats often use exotic plant species, they do not usually exhibit preference for them. Instead, bees use them in proportionately to their relative abundance in the plant community. In our common garden study, many native bees chose to use *C. stoebe* in the presence of flowering native plants. This was despite each species being present in similar abundances, as we located our common garden in a landscape devoid of spotted knapweed. We also saw heavy use of *C. stoebe* by many native bee species in our field observations, even when a diversity of native plant species were available. These findings indicate that the effects invasive flowering plant species have on bee communities are likely to vary based on traits the invasive plant exhibits, as well as the composition of the native plant and pollinator communities.

Many studies have investigated the effect invasive plants have on native plant pollination through competition for pollinators (Morales and Traveset, 2009). The majority conclude that invasives have a negative effect on native plant seed set (Dietzch et al, 2011), though some studies show invasives facilitating native plant pollination by attracting more pollinators to the area (McKinney and Goodall, 2011). While we did not collect data on pollination, we seldom observed bees visiting native plants when *C. stoebe* was available in high abundances, indicating that *C. stoebe* may have a negative effect on native plant pollination. High numbers of bee visits to *C. stoebe* also have the potential to increase its seed production, facilitating further invasion. Honey bees were shown to increase seed set in a closely related invasive plant, *Centaurea solstitialis* (Barthell et al., 2001). This effect may be amplified by the fact that beekeepers place their hives next to spotted knapweed fields to produce a honey crop.

The common garden and field experiment demonstrate the important role flowering plant diversity plays in supporting robust bee communities. Together they suggest that increases in the season-total floral abundance on a landscape scale should have a positive effect on pollinator abundance in the region. Because habitats with more flowering plant diversity provide greater total floral abundance and a more consistent flow of floral resources throughout the season, they are able to support a greater season-total abundance and diversity of bees.

Even though our study illustrates the phenological importance of diverse flowering plant communities, it is clear that from a bee’s perspective, spotted knapweed is an attractive plant that is able to provide more nectar and pollen over a longer period of time than many native flowering plant species. This finding should prompt ecologists and land managers to begin to think of exotic species in a more nuanced way, and to recognize that there is more to land management than simply labeling plants as “good” and “bad”. It follows that any attempt to remove an invasive species should consider what ecological services that species is providing, and should coincide with the restoration of species that are able to provide similar services.

APPENDIX

APPENDIX

Table 9.

Scientific and common names of the plant species included in the common garden experiment. 2012-2013, Ingham Co, Michigan

Scientific Name	**Common Name**
Coreopsis lanceolata	lanceleaf coreopsis
Penstemon hirsutus	hairy beardtongue
Anenome canadensis	Canada anenome
Euphorbia corollata	flowering spurge
Centaurea stoebe	spotted knapweed
Asclepias syriaca	common milkweed
Monarda fistulosa	wild bergamot
Helianthus occidentalis	western sunflower
Rudbeckia hirta	black-eyed susan
Gnaphalium obtusifolium	sweet everlasting
Solidago nemoralis	gray goldenrod
Solidago speciosa	showy goldenrod
Symphiotrichum laevis	smooth aster

Table 10.
List of bee species captured at the common garden experiment, 2012-2013, including the months present, total number captured, and % of observed visits that were to *C. stoebe*. Ingham Co, Michigan

	Months Present					
Bee Species	**June**	**July**	**Aug.**	**Sept.**	**Total**	**% Visits to *C. stoebe***
Agapostemon sericeus			■		1	100
Andrena asteris				■	5	0
Andrena simplex				■	1	0
Anthideum manicatum			■		1	0
Apis mellifera	■	■	■		133	7
Augochlora pura			■		1	0
Augochlorella aurata		■			1	100
Bombus bimaculatus		■	■		16	19
Bombus fervidus	■	■		■	4	25
Bombus impatiens		■	■	■	118	13
Ceratina calcarata	■	■	■		9	33
Ceratina dupla	■	■	■		8	25
Ceratina mikmaqi	■	■	■		14	7
Colletes americanus			■		1	0
Colletes kincaidii			■		1	0
Halictus ligatus	■	■	■	■	35	40
Halictus rubicundus		■	■		3	33
Hoplitis pilosifrons	■	■			7	0
Hylaeus affinis			■	■	15	53
Lasioglossum admirandum			■		1	100
Lasioglossum albipenne		■		■	2	0

Table 10. (cont’d)

Lasioglossum anomalum		■			2	0
Lasioglossum hitchensi		■	■		5	40
Lasioglossum leucozonium		■	■		9	33
Lasioglossum lineatulum		■			1	0
Lasioglossum paradmirandum		■	■		21	90
Lasioglossum pectorale		■		■	6	67
Lasioglossum perpunctatum		■	■		6	17
Lasioglossum pilosum		■			1	0
Lasioglossum versatum			■		1	100
Megachile latimanus				■	1	100
Megachile mendica		■			1	100
Melissodes bimaculata			■		2	0
Melissoides agilis		■	■		6	0
Mellisodes druriella			■		1	0
Meslissodes denticulata		■	■		5	100
Pseudopanurgus compositarum				■	1	0

Table 11.
The total number of bee species, the number of unique bee species*, total season-long visitation of all bees, total season-long floral abundance, and the total number of bee visits/total floral abundance for each plant species observed in 2013 transects (Oakland Co, Michigan). *We considered bee species that visited two or fewer plant species to be unique to those plants in our study.

	Sampling Date						**Diverse Fields**			**Knapweed-Dominated Fields**		
Plant Species	**10 Jul**	**30 Jul**	**19 Aug**	**11 Sep**	**# Bee Species**	**# Unique Bee Species**	**Season Total Floral Area $(cm^2)/m^2$**	**Total Bee Visits**	**Bee Visits/ Floral Area**	**Season Total Floral Area $(cm^2)/m^2$**	**Total Bee Visits**	**Bee Visits/ Floral Area**
Centaurea stoebe	░	■	▒		22	14	437	125	0.009	1382	235	0.005
Berteroa incana	■	■	░	░	4	0	25	4	0.005	109	9	0.003
Hypericum perforatum	■	▒	░	░	2	1	28	5	0.006	12	3	0.008
Achillea millefolium	■	▒	░		5	1	290	7	0.001	18	0	0.000
Erigeron strigosus	■	■	▒		3	1	51	6	0.004	10	1	0.003
Vicea villosa	■	▒	░		2	1	21	7	0.010	10	0	0.000
Dianthus armeria	■				1	0	1	0	0.000	1	1	0.031
Monarda fistulosa	▒	■	░		11	7	449	106	0.007	5	1	0.007
Leucanthemum vulgare	■				0	0	3	0	0.000	0	0	0.000
Solidago juncea	░	▒	■		7	3	420	18	0.001	1	0	0.000
Solidago canadensis	▒	■	░		0	0	5	0	0.000	0	0	0.000
Solidago nemoralis				■	9	5	276	48	0.005	0	0	0.000
Symphyotrichum ericoides				■	3	3	39	6	0.005	0	0	0.000
Solidago speciosa				■	9	4	471	96	0.006	19	0	0.000
Asclepias tuberosa	■	░			2	0	194	5	0.001	0	0	0.000
Potentilla recta	■	░			3	1	0	0	0.000	10	4	0.000

Table 11. (cont’d)

Medicago lupilina	■	░			0	0	0	0	0.000	3	0	0.000
Asclepias syriaca	■				0	0	3	0	0.000	0	0	0.000
Euphorbia corollata	▒	■	░		1	1	0	0	0.000	59	1	0.001
Verbascum thapsis		■			0	0	0	0	0.000	1	0	0.000
Daucus carota	■	▒	▒		0	0	7	0	0.000	6	0	0.000
Euthamia gramnifolia				■	0	0	1	14	0.747	0	0	0.000
Total							2721	447		1646	255	

Table 12.

List of bee species found at Orion Oaks County Park (Oakland Co, Michigan) during 2013 transect sampling, including the sampling dates on which each species was collected, the number of plant species each species was collected off of, the total number of individuals of each species, and the percent of that species' observed floral visitations that were on spotted knapweed.

	Sampling Dates Present				**Diverse Fields**		**Knapweed-Dominated Fields**		
Bee Species	**10-Jul**	**30-Jul**	**19-Aug**	**11-Sep**	**# Plant Species Visited**	**Total Captured**	**# Plant Species Visited**	**Total Captured**	**% Visits to *C. stoebe***
Agapostemon sericeus			■		1	3	1	1	100
Agapostemon virescens			■		1	1	0	0	100
Andrena asteris		■			1	3	0	0	0
Andrena hirticincta				■	1	1	0	0	0
Andrena placata		■			1	1	0	0	0
Anthophora terminalis	■				1	1	0	0	0
Apis mellifera		■	■	■	6	115	1	7	17
Augochlora pura		■		■	1	1	1	1	50
Augochlorella aurata				■	2	4	0	0	0
Bombus auricomus		■			1	1	0	0	0
Bombus bimaculatus	■	■	■		2	5	2	2	0
Bombus citrinus			■		1	2	0	0	100
Bombus fervidus		■			2	2	0	0	0
Bombus griseocollis	■	■	■		2	4	1	2	33
Bombus impatiens	■	■	■	■	7	191	3	177	69
Ceratina calcarata	■		■	■	6	12	1	2	36
Colletes americanus				■	2	2	0	0	0

Table 12. (cont'd)

Colletes compactus				■	1	2	0	0	0
Colletes simulan armatus				■	1	1	0	0	0
Halictus confusus	■		■	■	6	8	3	7	27
Halictus ligatus	■	■	■	■	6	21	2	12	61
Halictus parallelus	■		■	■	0	0	1	1	100
Halictus rubicundus	■		■	■	0	0	1	1	100
Heriades carinata	■				1	1	0	0	0
Hylaeus affinis	■		■		1	1	2	4	0
Hylaeus mesillae			■		0	0	1	1	0
Hylaeus modestus				■	0	0	1	1	0
Lasioglossum anomalus	■				1	3	0	0	0
Lasioglossum ellisiae			■		1	1	0	0	0
Lasioglossum leucocomum	■	■	■	■	1	1	3	3	25
Lasioglossum pectorale	■				3	3	0	0	0
Lasioglossum punctatum		■			1	1	0	0	0
Lasioglossum vierecki		■			0	0	1	1	100
Megachile latimanus		■	■		1	2	2	6	88
Megachile mendica			■		0	0	1	1	100
Megachile pugnata			■		0	0	1	1	100
Megachile relativa			■		1	1	0	0	100
Melissodes bimaculata		■	■		2	5	1	1	50
Melissodes nivea				■	1	1	0	0	0
Melissodes subillata		■			1	3	2	8	91
Perdita octomaculata				■	1	2	0	0	0
Sphecodes davisii				■	2	2	0	0	0
Xylocopa virginica		■	■	■	2	12	1	1	8

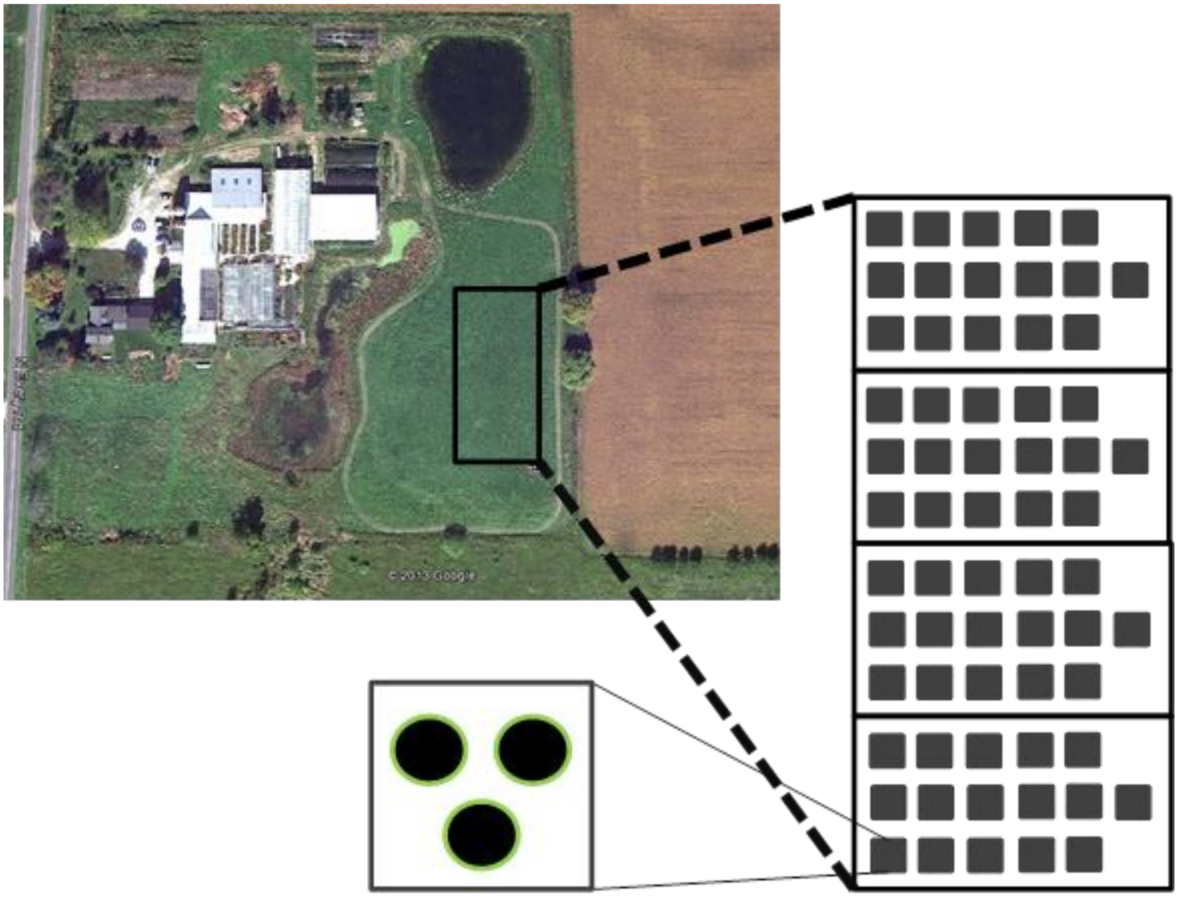


Figure 20. The layout of the common garden study (2012-2013) in Ingham Co, Michigan. Each circle represents a cluster of three plants of the same species, and each grouping of 16 plants a replicate block.

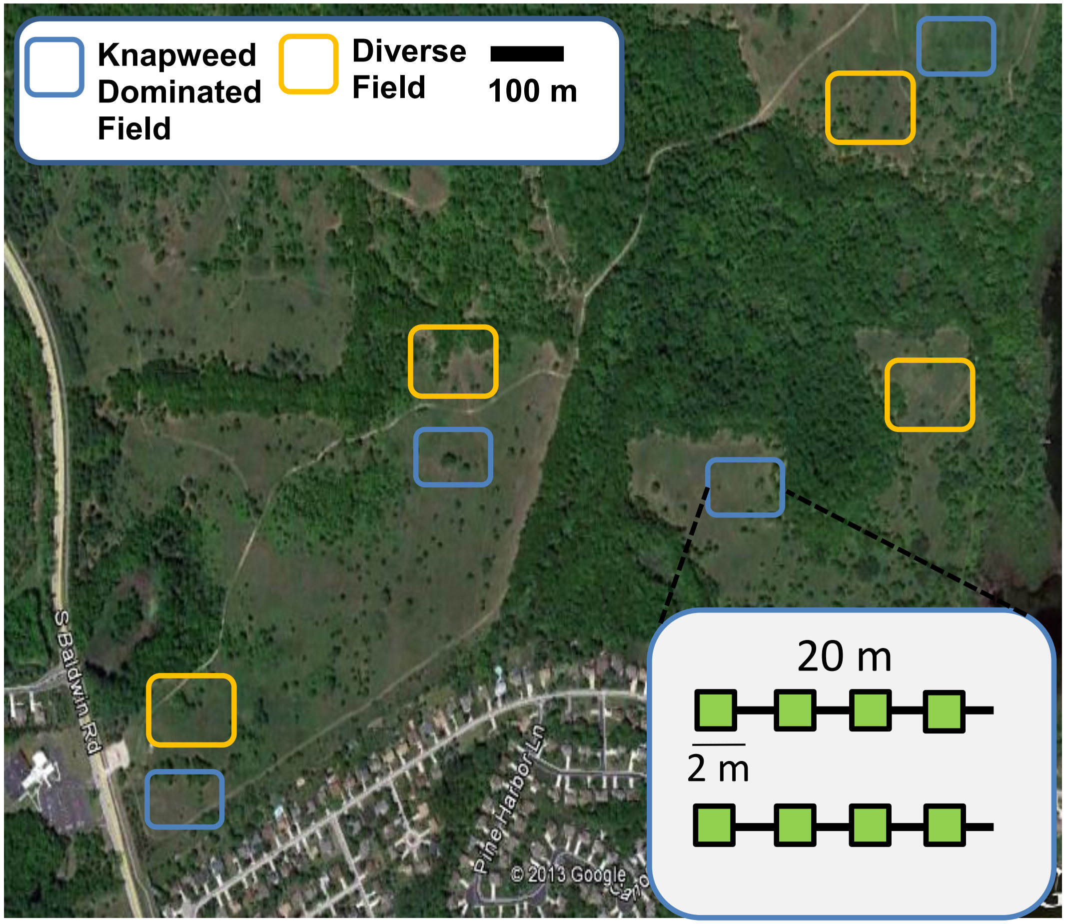


Figure 21. Locations of diverse and knapweed-dominated field pairs, Orion Oaks Study Site (Oakland County, MI). Inset shows two 20 m transects in each field, with 2 x 2 m floral resource sampling quadrats placed at 0, 5, 10, and 15 meters.

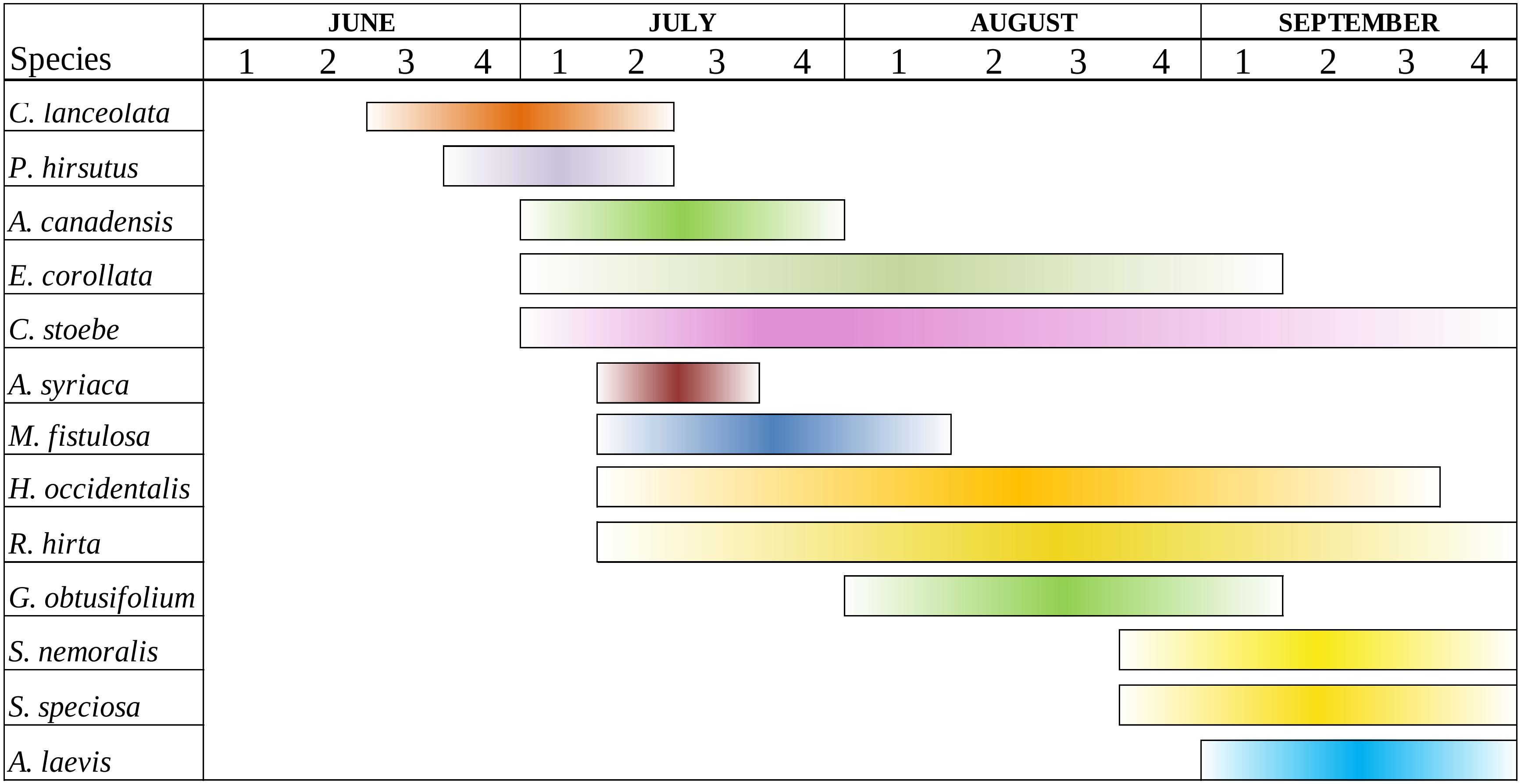


Figure 22. The bloom period of each plant species in the common garden experiment (2012-2013, Ingham Co, Michigan).

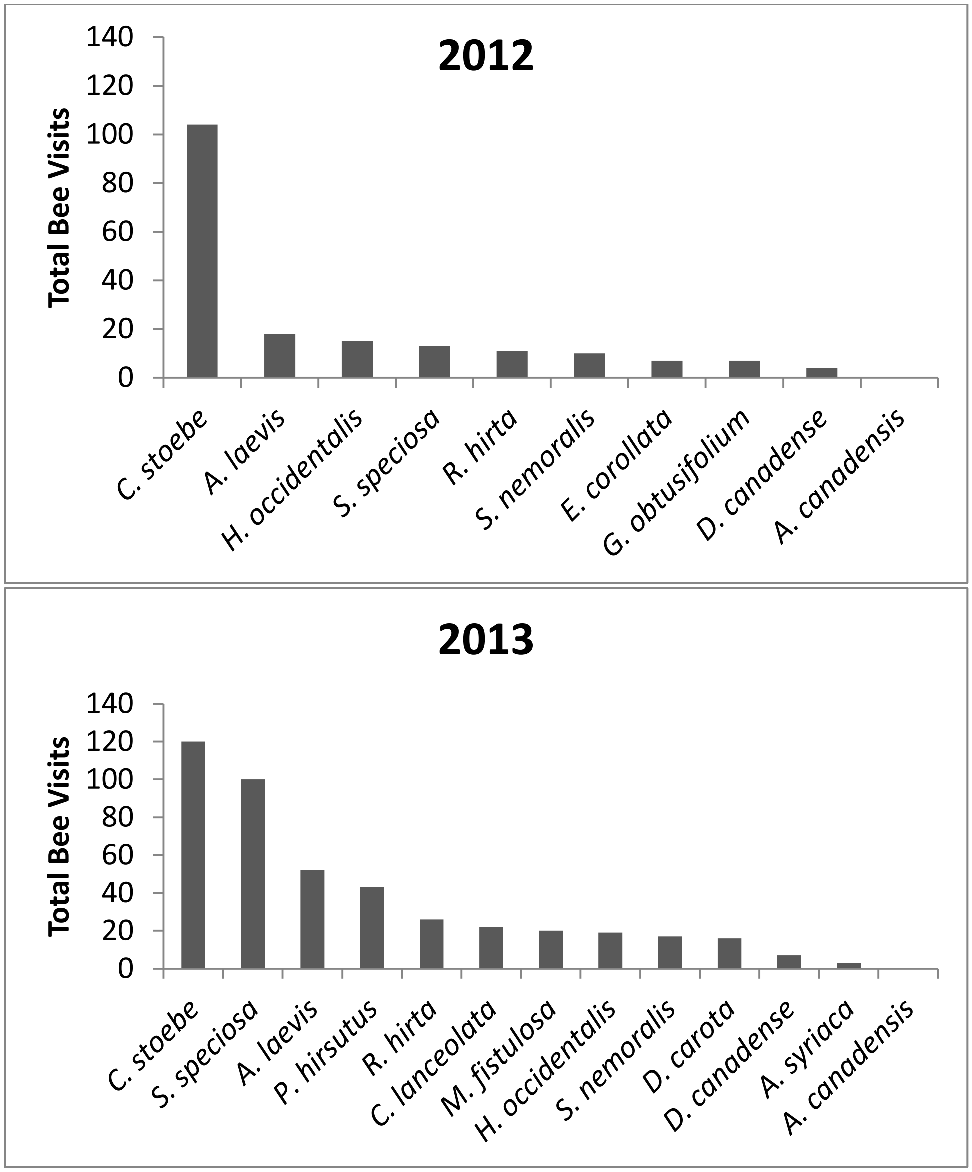


Figure 23. The total number of bee (wild and managed) visits to each common garden plant species in 2012 and 2013(Ingham Co, Michigan).

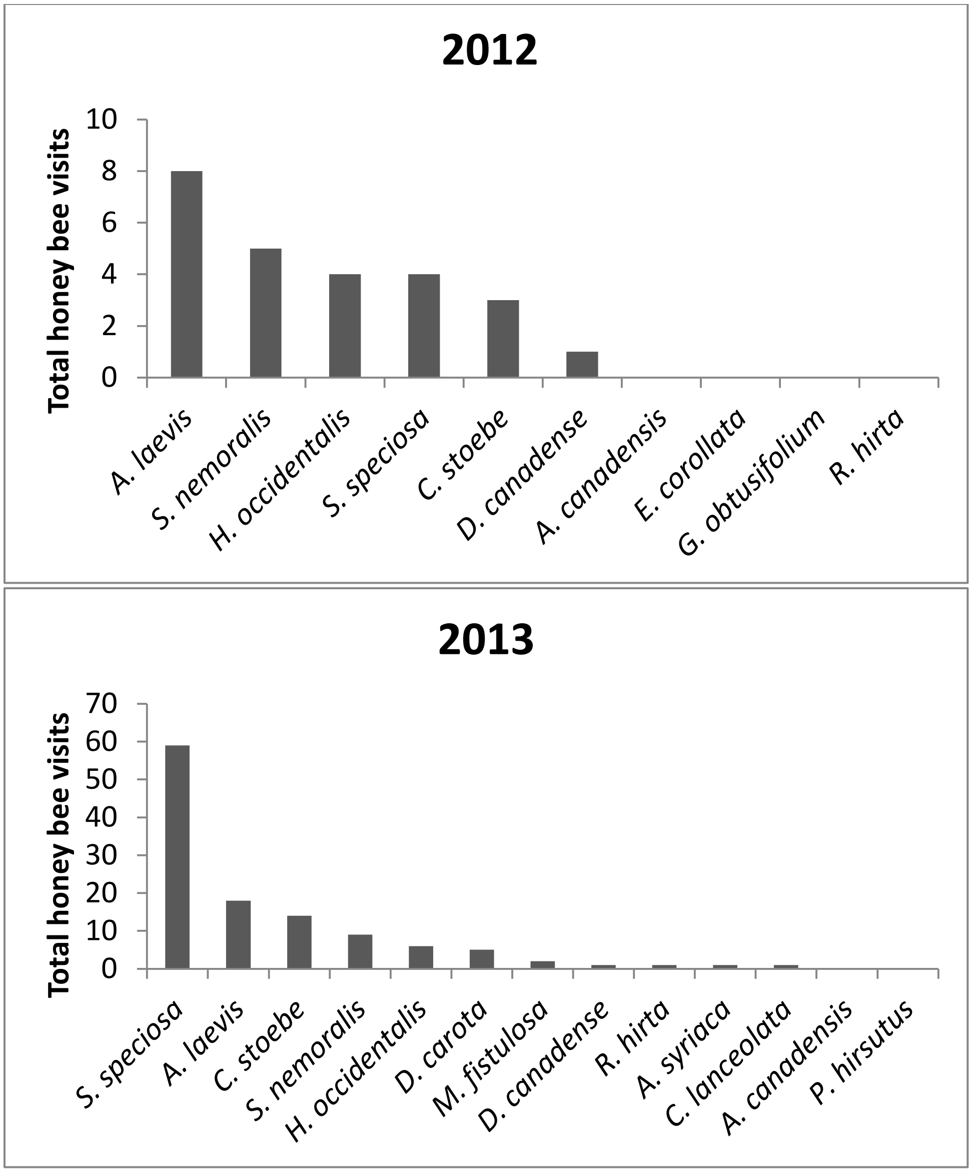


Figure 24. The total number of honeybee (*Apis mellifera*) visits to each common garden plant species in 2012 and 2013 (Ingham Co, Michigan).

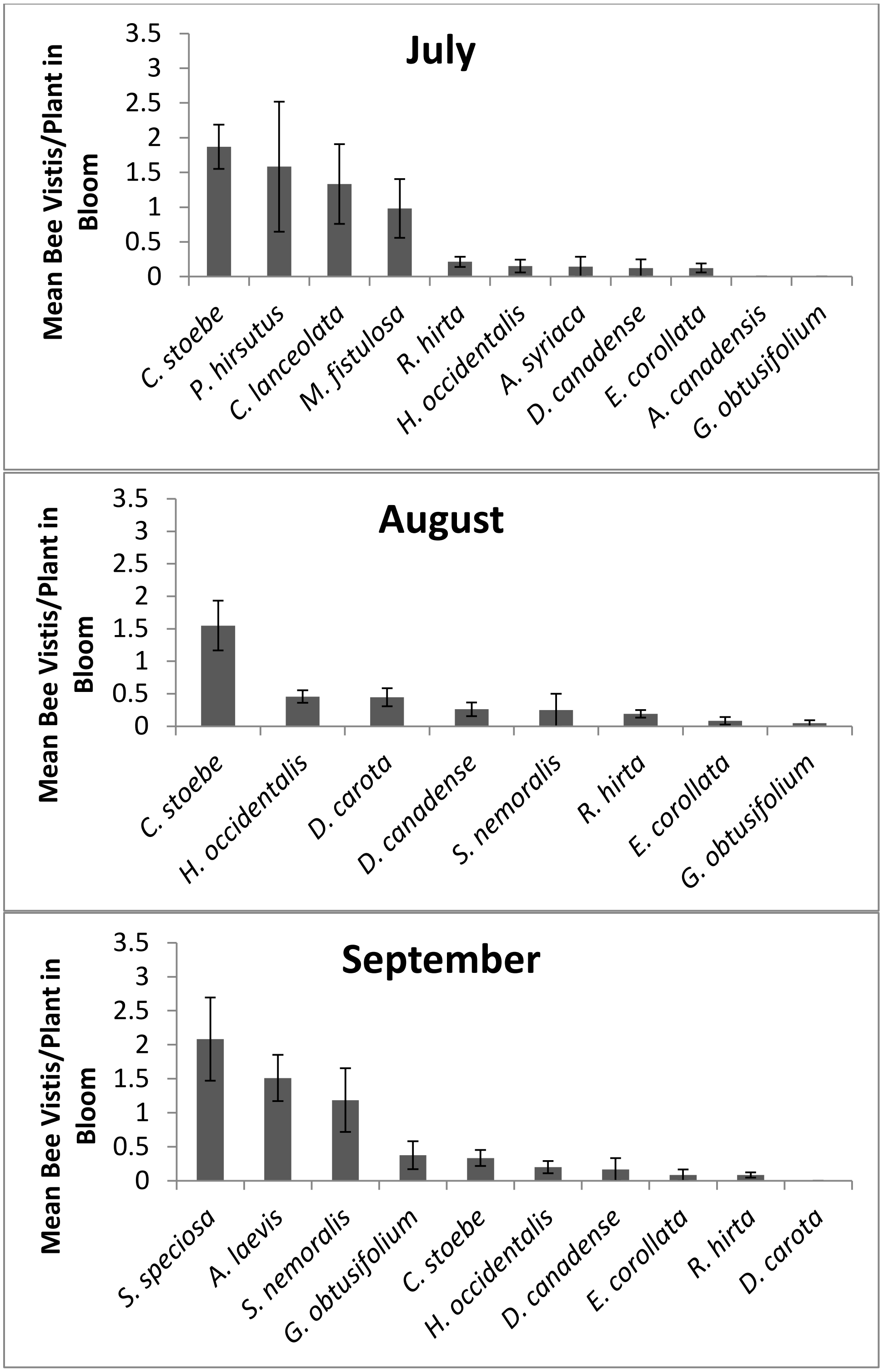


Figure 25. The mean number of bees per plant in bloom (±SE) for each common garden species in July, August and September. 2012 and 2013 observations are combined (Ingham Co, Michigan).

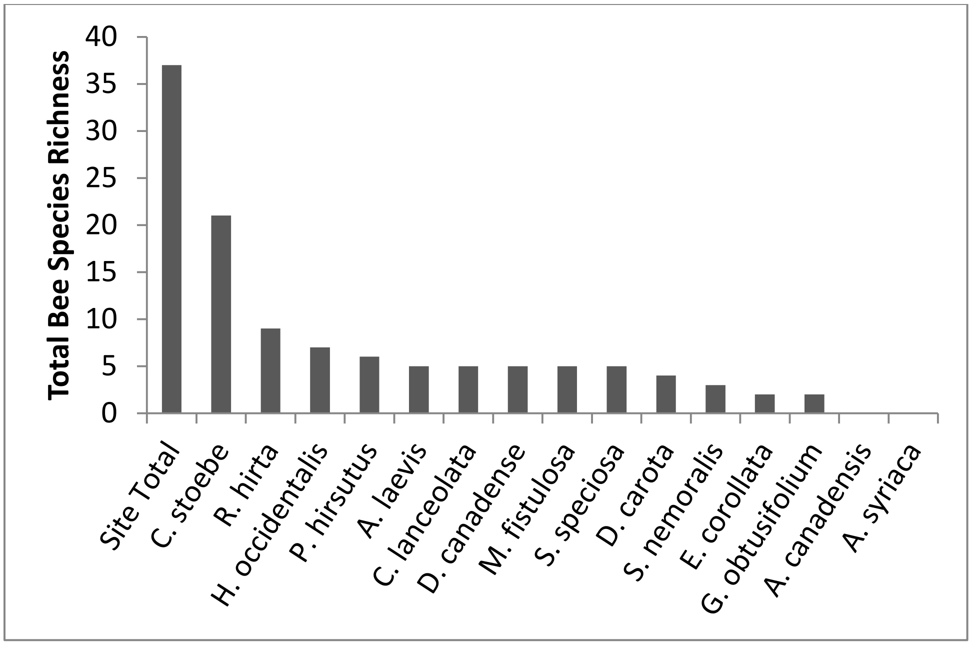


Figure 26. Total species richness of bees visiting each plant in the common garden (2012-2013, Ingham Co, Michigan).

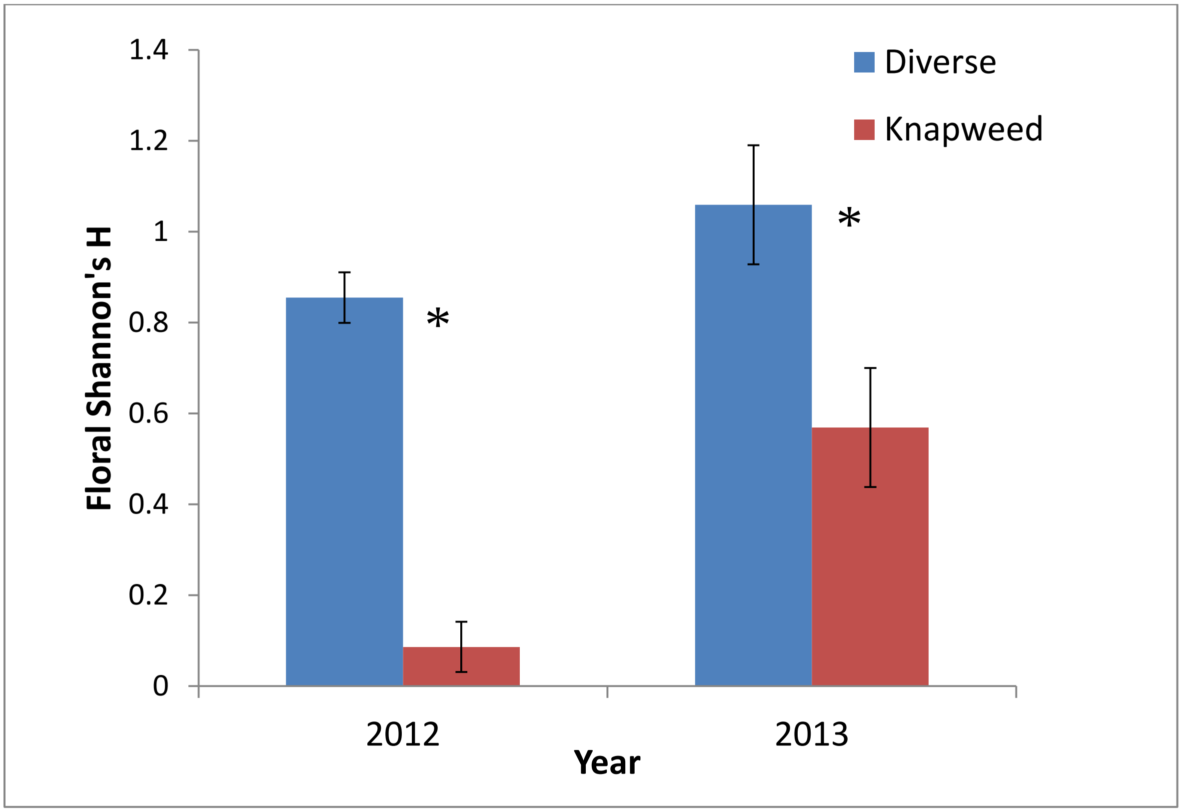


Figure 27. Average season-long floral diversity (Shannon's H) in diverse and knapweed-dominated fields (2012-2013, Oakland Co, Michigan). Asterisks indicate significant differences between treatments.

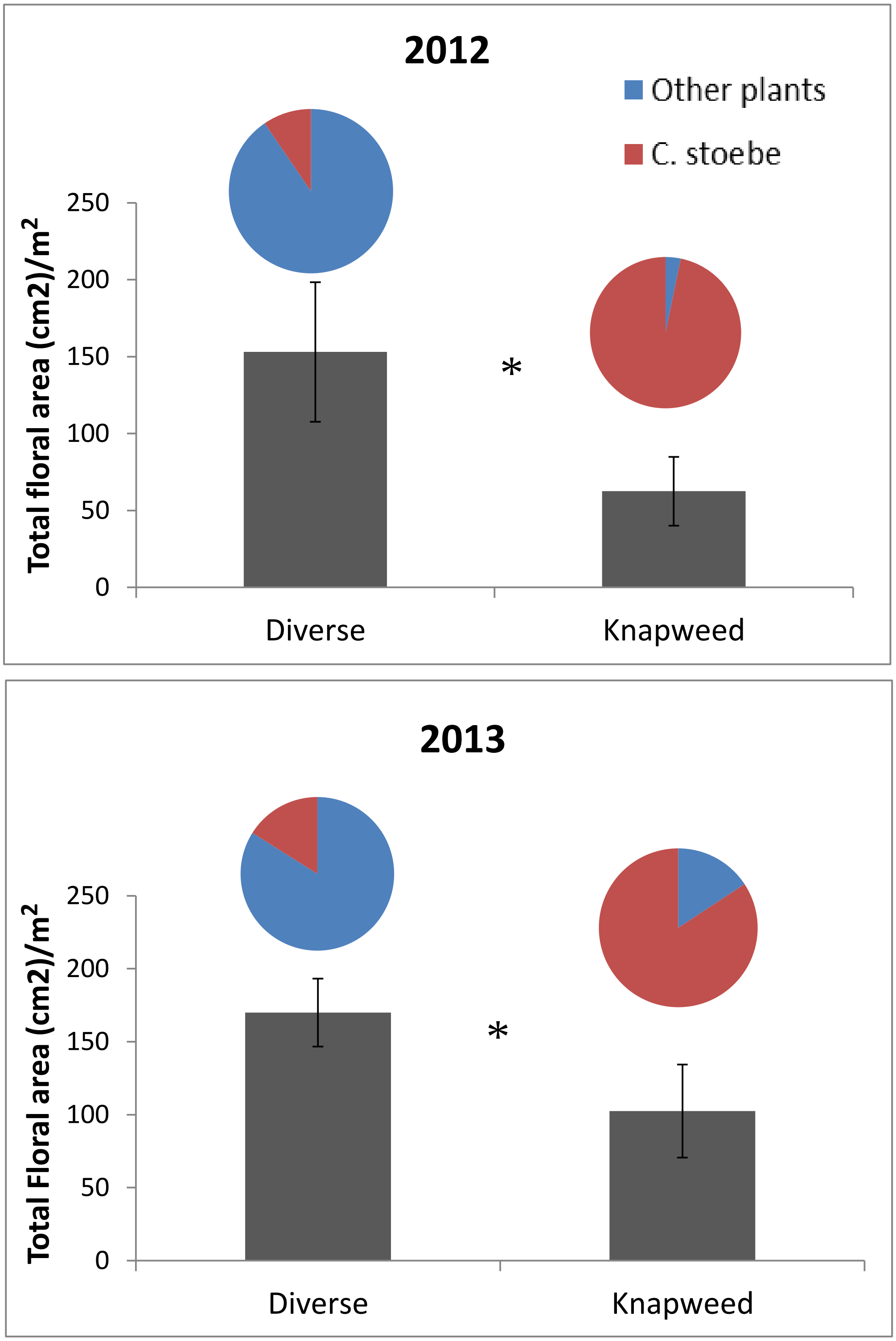
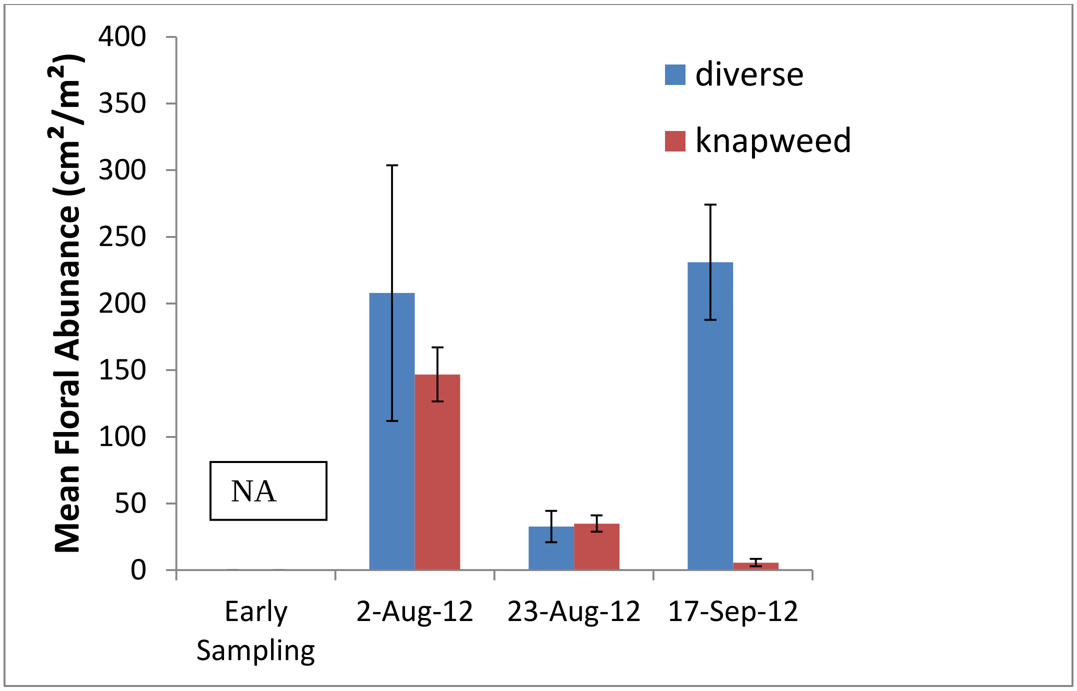


Figure 28. Mean season-long floral abundance (cm^2) in diverse and knapweed-dominated fields in 2012 and 2013, and the proportion *C. stoebe* blooms contributed to total floral abundance in each field type (Oakland Co, Michgan). Asterisks indicate significant differences in floral abundance between treatments.



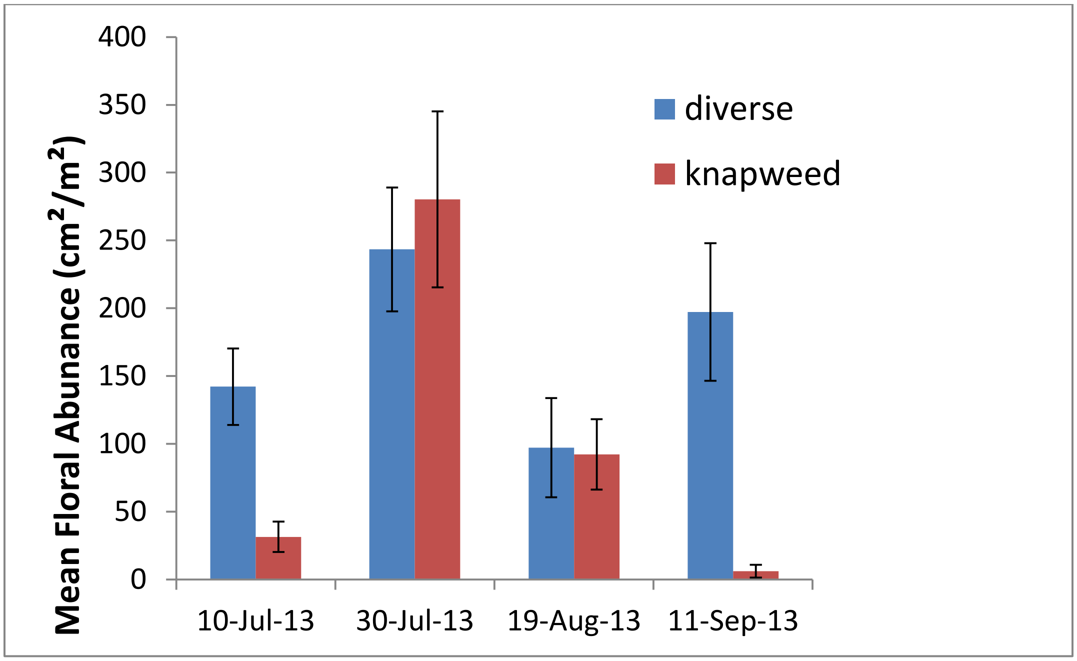


Figure 29. The mean floral abundance in diverse and knapweed-dominated fields by sampling date in 2012 and 2013, respectively (Oakland Co, Michigan).

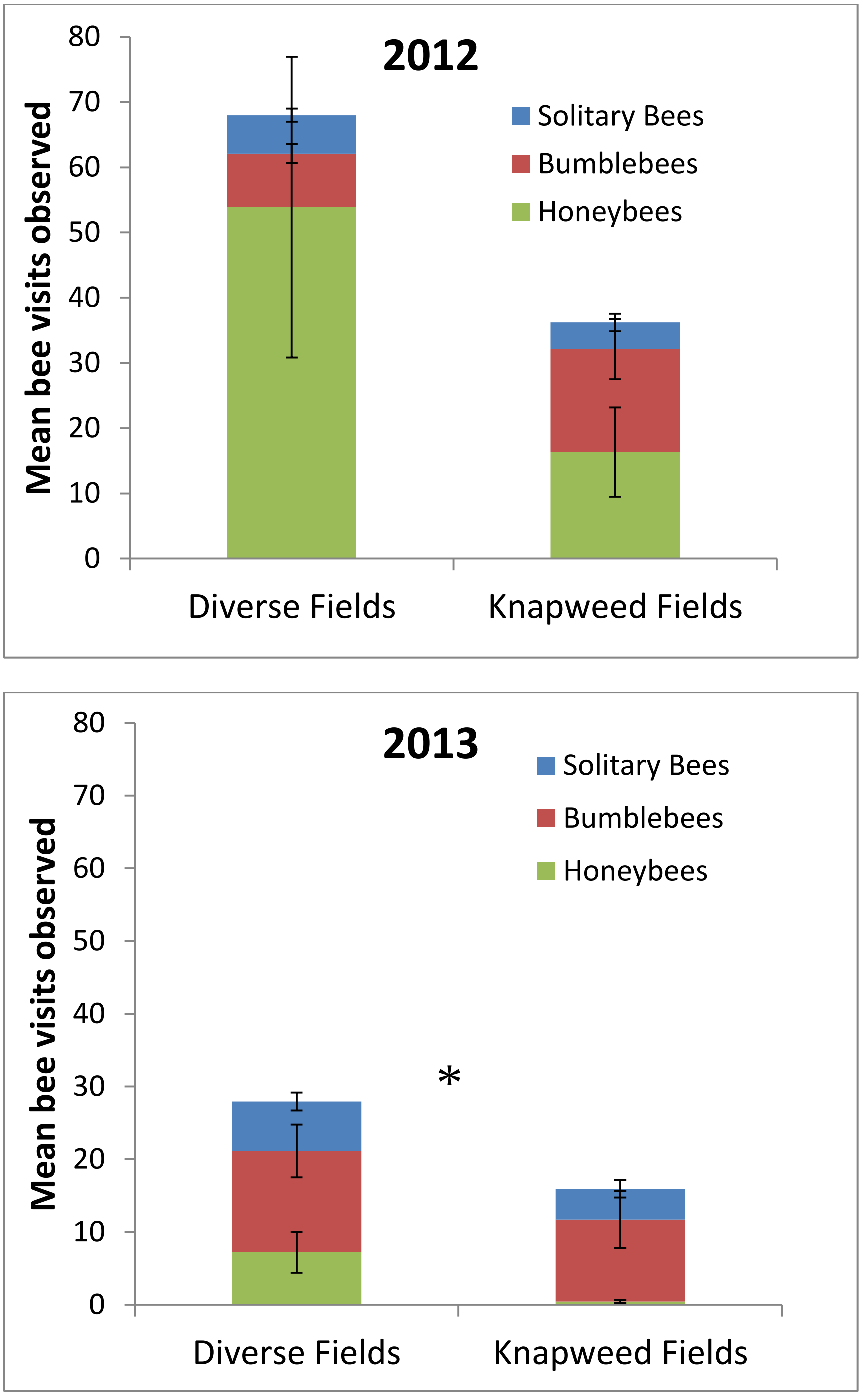


Figure 30. The season-long mean number of bee visits observed per field in 2012 and 2013. Total bees are subdivided into solitary bees, bumblebees, and honey bees, and error bars indicate SE of each group. Asterisks indicate significant differences in total bee abundance between treatments (Oakland Co, Michigan).

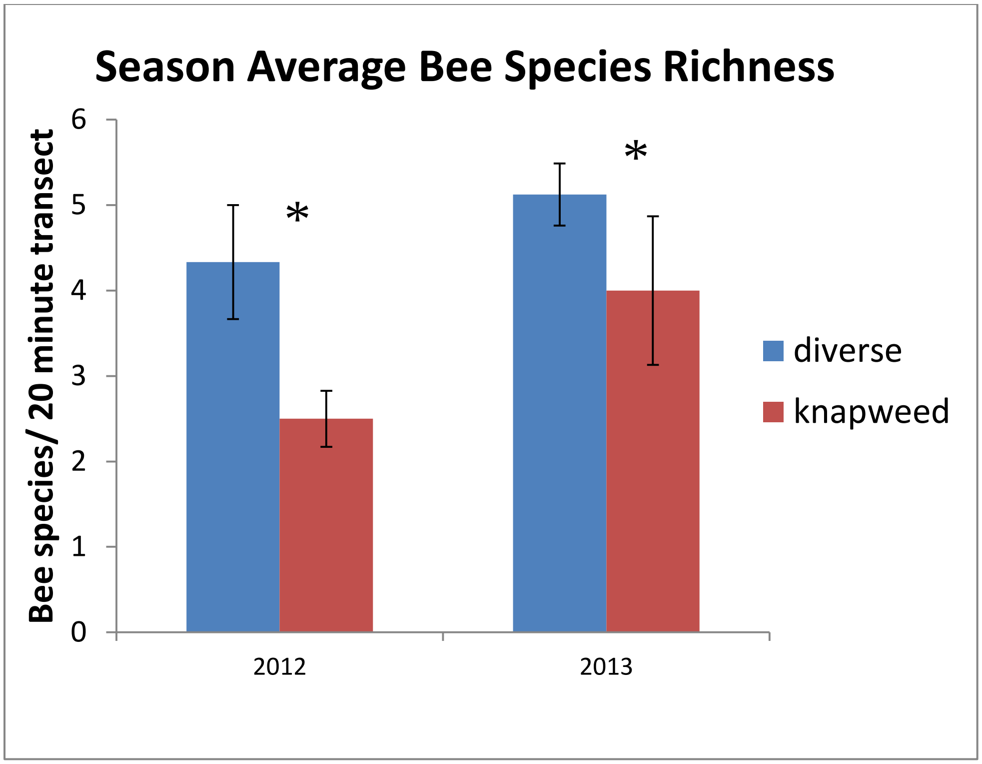


Figure 31. 2012 and 2013 mean season-long bee species richness in diverse and knapweed-dominated fields (Oakland Co, Michigan). Asterisks show significant differences between treatments.

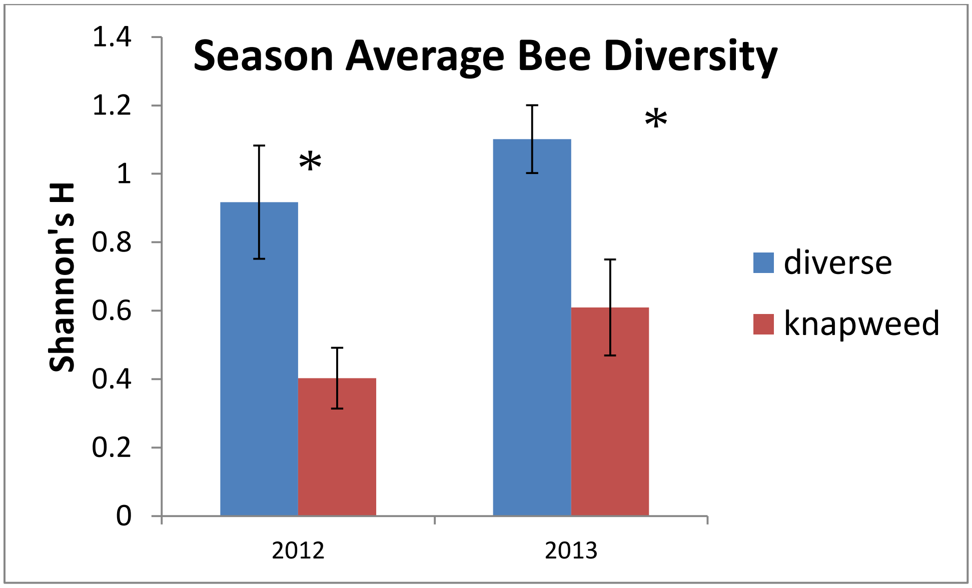


Figure 32. 2012 and 2013 mean season-long bee diversity (Shannon’s H) in diverse and knapweed-dominated fields (Oakland Co, Michigan). Asterisks show significant differences between treatments.

REFERENCES

REFERENCES

Alaux C, Ducloz F, Crauser D, Le Conte Y (2010) Diet effects on honeybee immunocompetence. Biology Letters. 6: 562-565

Albert DA (1999) *Natural community abstract for open dunes* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Open_dunes.pdf

Burnham KP, Anderson DR (1998) *Model selection and multimodel inference*: *a practical information-theoretic approach.* Springer U.S.

Bush RT, Seastedt TR, Buckner D (2007) Plant community response to the decline of diffuse knapweed in a Colorado grassland. Ecological Restoration 25:169-174

Barthell JF, Randall JM, Thorp JM (2001) Promotion of seed set in yellow star-thistle by honey bees: Evidence of an invasive mutualism. Ecological Application. 11:6 1870-1883

Cane JH, Tepedino VJ (2001) Causes and extent of declines among native North American invertebrate pollinators: Detection, evidence, and consequences. Conservation Ecology 5:1

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2:436-443

Clark SE, Van Driesche RG, Sturdevant N, et al. (2001) Effect of root feeding insects on spotted knapweed (*Centaurea maculosa*) stand density. Southwestern Entomologist 26:129-135

Cohen JG (2000) *Natural community abstract for oakpine barrens* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Oak-pine_barrens.pdf

Cohen JG (2001) *Natural community abstract for oak barrens* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Oak_barrens.pdf

Dietzsch AC, Stanley DA, Stout JC (2011) Relative abundance of an invasive alien plant affects native pollination processes. Oecologia 167**:**469-479

Garibaldi LA, Steffan-Dewenter I (2011) Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecology Letters 14**:**1062-1072

Goulson D, Darvill B (2004) Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? Apidologie 35**:**55-63

Griffith D, Lacey JR (1991) Economic Evaluation of Spotted Knapweed (*Centaurea maculosa*) Control Using Picloram. Journal of Range Management 44:1 43-47

Grundel R, Jean RP, Frohnapple KJ, Glowacki GA, Scott PE, Pavlovic NB (2010) Floral and nesting resources, habitat structure, and fire influence bee distribution across an open forest gradient. Ecological Applications 20:1678-1692

Jacobs JS, Sing SE, Martin JM (2006) Influence of herbivory and competition on invasive weed fitness: Observed effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and grass-seeding treatments on spotted knapweed performance. Environmental Entomology 35:1590-1596

Kleijn D, Raemakers I (2008) A retrospective analysis of pollen host plant use by stable and declining bumble bee species. Ecology 89**:**1811-1823

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B-Biological Sciences 274**:**303-313

Kost MA (2004) *Natural community abstract for dry-mesic prairie.* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Dry-mesic_prairie.pdf

Kost MA, Slaughter BS (2009) *Natural community abstract for mesic sand prairie* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Mesic_sand_prairie.pdf

Lee JG (2007) *Natural community abstract for dry-mesic southern forest.* [Online]. Michigan State University, Michigan Natural Features Inventory. Available: http://web4.msue.msu.edu/mnfi/abstracts/ecology/Dry-mesic_southern_forest.pdf

McKinney AM, Goodell K (2011) Plant-pollinator interactions between an invasive and native plant vary between sites with different flowering phenology. Plant Ecology 212**:**1025-1035

Memmott J, Carvell C, Pywell RF, Craze PG (2010) The potential impact of global warming on the efficacy of field margins sown for the conservation of bumble-bees. Philosophical Transactions of the Royal Society B-Biological Sciences 365**:**2071-2079

Morales CL, Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. Ecology Letters 12:7:716-728

Moron D, Lenda M, Skorka P, et al. (2009) Wild pollinator communities are negatively affected by invasion of alien goldenrods in grassland landscapes. Biological Conservation 142:1322-1332

Potts SG, Vulliamy B, Dafni A, Ne’eman G, Willmer P (2003) Linking bees and flowers: How do floral communities structure pollinator communities? Ecology 84: 2628-2642

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution 25**:**345-353

Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120**:**321-326

Reznicek AA, Voss EG, Walters B S (2011) *MICHIGAN FLORA ONLINE* University of Michigan. Web. 11-14-2013. http://michiganflora.net/home.aspx. (Accessed 15 November 2013)

Seastedt TR, Gregory N, Buckner D (2003) Effect of biocontrol insects on diffuse knapweed (*Centaurea diffusa*) in a Colorado grassland. Weed Science 51:237-245

Sedivy C, Muller A, Dorn S (2011) Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: evidence for physiological adaptations to digest pollen. Funtional Ecology. 25: 718-725

Shannon, C. E. (1948) A mathematical theory of communication. The Bell System Technical Journal 27:379-423 and 623-656

Sheley RL, Jacobs JS, Carpinelli MF (1998) Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technology 12:353-362

Stout JC, Morales CL (2009) Ecological impacts of invasive alien species on bees. Apidologie 40:388-409

Stephens AEA, Krannitz PG, Myers JH (2009) Plant community changes after the reduction of an invasive rangeland weed, diffuse knapweed, *Centaurea diffusa*. Biological Control 51:140-146

Story JM, Callan NW, Corn JG, et al. (2006) Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biological Control 38:227-232

Tilman D, Lehman CL, Thomson KT (1997) Plant diversity and ecosystem productivity: Theoretical considerations. Proceedings of the National Academy of Sciences of the United States of America 94:1857-1861

Tilman D, Reich PB, Knop J, Wedin D, Mielke T, Lehman C (2001) Diversity and productivity in a long-term grassland experiment. Science 294:843-845

Steffan-Dewenter I, Potts SG, Packer L (2005) Pollinator diversity and crop pollination services are at risk. Trends in Ecology & Evolution 20:651-652

Vanbergen AJ, Baude M, et al. (2013) Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment 11**:**251-259

Watson AK, Renney AJ (1974) Biology of Canadian weeds *Centaurea diffusa* and *Centaurea maculosa*. Canadian Journal of Plant Science 54:687-701

Williams, NM, Cariveau D, Winfree R, Kremen C (2011) Bees in disturbed habitats use, but do not prefer, alien plants. Basic and Applied Ecology 12:332-341

Williams NM, Kremen C (2007) Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. Ecological Applications 17**:**910-921

Wilson JS, Messinger OJ, Griswold T (2009) Variation between bee communities on a sand dune complex in the Great Basin Desert, North America: Implications for sand dune conservation. Journal of Arid Environments 73:666-671

Wilson LM, Randall CB (2005) Biology and biological control of knapweeds. U.S. Forest Service Publication

SUMMARY

Spotted knapweed has caused significant damage to ecosystems and ecosystem services in North America by reducing plant community diversity, reducing food availability for livestock, birds, and other wildlife, and altering physical processes such as water sedimentation and fire regimes. In Michigan and much of the surrounding Midwestern United States, concerns over increasing spotted knapweed populations have led natural area managers to look for alternative control methods, including biological control. Borrowing knowledge accumulated from biological control releases in Western North America, land managers have concluded that the most effective combination of biological control insects is the root feeding weevil, *Cyphocleonus achates,* and the seedhead feeding weevils, *Larinus minutus* and *Larinus obtusus*.

Here I reported the findings of seven data collection efforts related to the biological control of spotted knapweed in Michigan. These included monitoring the establishment and impacts of *L. minutus* and *C. achates* at release sites and documenting each insects' current distribution in Michigan, establishing the dispersal rates and phenology of *L. minutus* and *L. obtusus*, and documenting the relative importance of spotted knapweed in supporting bee communities in old field habitats.

We observed the successful establishment of every biological control species that was released in Michigan, with *L. minutus* and *L. obtusus* establishing and reaching easily detectable populations much faster than *C. achates*. No measurable impacts on plant communities at release sites have been found, but the biological control insects are still in their establishment phase and may take several decades to have a quantifiable impact. After 2 yr, we saw no establishment of the native plant species we attempted to seed into spotted knapweed biological control release sites. This may have been influenced by the severe drought of 2012 and more research on means to reliably establish native nectar plants into spotted knapweed stands is needed

In our study of *Larinus* spp. weevil phenology, we found that *L. minutus* and *L. obtusus* have very similar phenologies. Both species become very active once degree day accumulations have reached about 350 DD_{10C}, and remain in large abundances for approximately 2 mo after this point. This finding is relevant because it will help inform decisions about when future sampling efforts should be made.

We found that *L.minutus* and *L. obtusus* are both very capable dispersers, and are able to re-distribute themselves on a regional level in less than a decade after their initial release. In contrast, *C. achates* does not appear capable of significant dispersal, and is thus a good candidate for more targeted biological control releases. While it is still uncertain what the long-term impacts of spotted knapweed biological control will be in the Midwestern United States, it seems likely that *L. minutus* will occupy most knapweed-dominated habitats in Michigan's Lower Peninsula within the next decade. As *Larinus* spp. populations approach saturation and operate on a regional scale, it is likely that recruitment rates of spotted knapweed will decrease, allowing other plant species to gain or retain their foothold. It will be much longer before *C. achates* is widespread, and barring intentional redistribution its populations may be local in nature for the foreseeable future. Sites where this root-feeder is able to establish at high densities, however, may see stronger decreases in spotted knapweed populations. The apparent influx of *L. minutus* from Indiana illustrates the fact that insects released as biological control agents do not heed political boundaries. This point deserves consideration and perhaps in the future biological control management plans should be collaborations by multiple stakeholders across a region.

The common garden and field experiment demonstrated the important role flowering plant diversity plays in supporting robust bee communities. Together they suggest that increases in the season-total floral abundance on a landscape scale should have a positive effect on pollinator abundance in the region. Because habitats with more flowering plant diversity provide greater total floral abundance and a more consistent flow of floral resources throughout the season, they are able to support a greater season-total abundance and diversity of bees.

Even though our study illustrated the phenological importance of diverse flowering plant communities, it is clear that from a bee's perspective, spotted knapweed is an attractive plant that is able to provide more nectar and pollen over a longer period of time than many individual native flowering plant species. Because of spotted knapweed's prevalence in the region, it is likely to be supporting a large number of wild and managed pollinators. Although it is likely that spotted knapweed will remain a dominant member of the plant community in well-drained, coarse-textured soils where it is most competitive, we expect biological control will decrease spotted knapweed densities over the next few decades. It follows that knapweed-dominated habitats should be managed in a way that will continue provide nectar and pollen, namely by increasing flowering plant diversity at these sites. These findings should prompt ecologists and land managers to begin to think of exotic species in a more nuanced way, and to recognize that there is more to land management than simply labeling plants as "good" and "bad."