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# Establishment, Impacts, and Current Range of Spotted Knapweed (*Centaurea Stoebe* Ssp. *Micranthos*) Biological Control Insects in Michigan

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# Establishment, Impacts, and Current Range of Spotted Knapweed (*Centaurea stoebe* ssp. *micranthos*) Biological Control Insects in Michigan

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**Abstract**

*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek (spotted knapweed) is an invasive plant that has been the target of classical biological control in North America for more than four decades. Work in the western U.S. and Canada has shown the seedhead-feeding weevils *Larinus minutus* Gyllenhal and *Larinus obtusus* Gyllenhal (Coleoptera: Curculionidae) and the root-boring weevil *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) to be the most effective *C. stoebe* control agents. These three weevils have recently been introduced into the eastern U.S., including sites in Michigan in 2007 and 2009. In 2010, we made additional releases at six sites in Michigan, monitoring them for three years 2011-13. Here we report on the establishment, impact, and current range of *L. minutus*, *L. obtusus*, and *C. achates* in Michigan. We also report on the initial results of native plant overseeding treatments that were applied to biological control release sites with the aim of supplementing the nectar source *C. stoebe* provides. We found that *L. minutus* has established at all of its Michigan release sites and is widespread in the southwestern part of the state, while *L. obtusus* has established at the single site where it was released in 2007 and is spreading to adjoining counties. We also found *C. achates* to be present at four sites and established at one additional site in Michigan, but in all cases abundances are low and dispersal has been minimal (< 10 m). In the three years following the 2010 releases, we found no measurable impacts of these biological control agents on *C. stoebe* growth, demographics, or plant community metrics. We also found little evidence of native flowering plant establishment at seeded sites. These baseline data will be useful in monitoring the spread and potential impacts of biological control agents on *C. stoebe* in Michigan.

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*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek (spotted knapweed) is an herbaceous 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, *C. stoebe* is considered highly invasive in North America and frequently becomes the dominant species in grassland, rangeland, and old field habitats. Populations of *C. stoebe* are now found throughout much of the continental United States, with the exceptions of Texas, Oklahoma, and Mississippi (USDA 2013). Long considered a pest in western U.S. rangelands, it is estimated that *C. stoebe* costs ranchers as much as $155 million in annual gross revenue in Montana alone (Griffith and Lacey 1991). Infestations of *C. stoebe* have also been shown to cause dramatic decreases in plant diversity, ecosystem functioning, and utilization of land by wild and domesticated foraging animals (Watson and Renney 1974, Hakim 1979, Lacey et al. 1989, Sheley et al. 1998, Mummey and Rillig 2006). More recently, *C. stoebe* has also become a serious invader in the eastern and midwestern U.S.,

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threatening the ecological integrity of natural areas. In Michigan, *C. stoebe* has been documented as an invader of rare native ecosystems, including dry sand and 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 *C. stoebe* invasion of open dune habitats had adverse effects on native plant communities and altered insect community composition.

Despite the efforts of land managers, conventional control methods, including herbicides and mowing, have not adequately controlled the spread of *C. stoebe*. In the U.S., western states, notably Montana, Colorado, and Washington, have spearheaded the effort to use classical biological control to slow and reverse the invasion of *C. stoebe* and *Centaurea diffusa* (diffuse knapweed) (Watson and Renney 1974, Story and Anderson 1978, Sheley and Jacobs 1997, Corn et al. 2009). More recently, agencies in the eastern U.S. have begun making their own biological control releases targeting *C. stoebe* (http://www.biocontrol.entomology.cornell.edu/weedfeed/Larinus).

Biological control of *C. stoebe* in Michigan began with the release of two species of seedhead flies, *Urophora affinis* Frauenfeld and *Urophora quadrifasciata* Meigen (Diptera: Tephritidae) 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) showed that both seedhead flies remain widely established in both peninsulas in Michigan. While seedhead infestation rates in Michigan are high (*U. quadrifasciata* averaged 78.1% and *U. affinis* averaged 52.5% in 2009), *C. stoebe* densities also remain high, confirming the reported inefficiency of these two agents in controlling *C. stoebe* (Myers et al. 2009).

The introduction history of the two seedhead-feeding weevils and root-boring weevil for biological control of *C. stoebe* in Michigan is complex. Based on the request of commercial biological control agent suppliers, in 2007, USDA-APHIS issued permits for the interstate transport of the root-boring weevil *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) and the two seedhead-feeding weevils, *Larinus minutus* Gyllenhal, and *Larinus obtusus* Gyllenhal (Coleoptera: Curculionidae), into Michigan. Releases of these insects were subsequently made at two sites in southern Michigan by private and public land managers (Table 1). However, due to the concerns of commercial beekeepers in Michigan, who value *C. stoebe* as a midsummer nectar source, further issuance of biological control agent release permits was suspended in 2008. In 2009, the USDA-Forest Service requested and received a permit to conduct controlled releases of *C. achates* and *L. minutus* in the western part of Michigan's Upper Peninsula.

While performing preliminary searches for *C. stoebe* biological control insects in 2011, we unexpectedly found *L. minutus* in two Michigan counties near the Indiana border, ca. 60 km from any known Michigan release sites. After additional investigation, 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 (Van Driesche et al. 2002). These releases were made in 1996, and we concluded that they are the most likely source of the *L. minutus* population occurring in southwestern Michigan (Carson and Landis, in review).

Following the 2007 and 2009 Michigan releases, interest in *C. stoebe* biological control increased, and the Michigan Department of Natural Resources (MDNR) contracted with Michigan State University to establish biological control research sites on MDNR lands. In 2010, we made releases at six MDNR managed sites in Michigan, monitoring them for agent establishment and

Table 1. Sites and species of *C. stoebe* biological control weevils released in Michigan and bordering states. These include a 1997 release made by the Indiana DNR, two 2007 releases made by private landholders, a 2009 release made by the USFS, and six 2010 releases made by MSU.

| Site Name | County | Year of Release | *Larinus minutus* | *Larinus obtusus* | *Cyphocleonus achates* | Longitude | Latitude |
|---|---|---|---|---|---|---|---|
| | Elkhart (Indiana) | 1996 | + | - | + | W85.77141 | N41.73578 |
| Lake Orion | Oakland | 2007 | + | - | + | W83.29482 | N42.75865 |
| Kalamazoo | Kalamazoo | 2007 | - | + | + | W85.76245 | N42.28951 |
| Ottawa National | 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 | Roscommon | 2010 | + | - | + | W84.89275 | N44.31051 |
| Flat River | Ionia | 2010 | + | - | + | W85.21220 | N43.12400 |
| Sharonville | Jackson | 2010 | + | - | + | W84.14593 | N42.19637 |

impact for three years during 2011–13. Releases of *L. minutus* and *C. achates* at these sites have two goals: 1) to test the efficacy of these *C. stoebe* biological control agents in Michigan, and 2) to investigate the potential for establishing native flowering plant species that can supplement the floral resources *C. stoebe* provides to native and managed pollinators, addressing beekeeper concerns in regard to loss of floral resources. Here, we report the initial establishment and current range of these introduced biological control insects, their impacts on *C. stoebe* populations, and the results of the native plant establishment trials.

## Methods

**Site selection and control agent releases.** In collaboration with MDNR staff, we identified 6 sites (hereafter called 2010 release sites) to test the impacts of biological control agent release on *C. stoebe* and the potential for re-vegetation with native nectar producing plants (Table 1; Fig. 1). These sites were located on state-owned lands and formed a north-south transect allowing for future exploration of latitudinal differences in establishment and effectiveness of biological control agents. At each site, we selected two *C. stoebe*-dominated areas on similar soil types. Weevil release and control (no-release) fields were located ≥ 800 m apart, as Alford (2013) reported that in Arkansas, *L. minutus* spread was only 112.5 m/yr in the first two–three years post release.

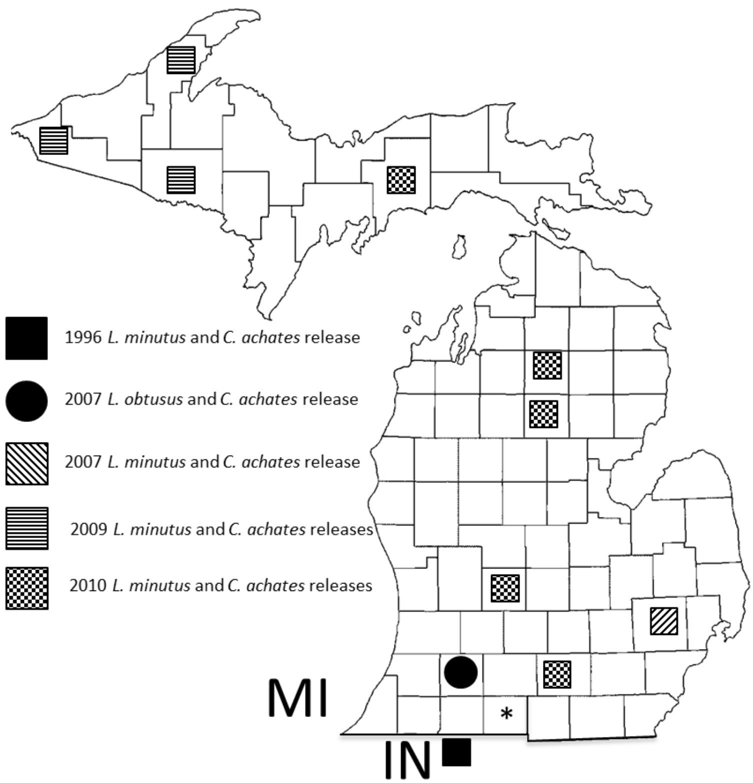


Figure 1. Reported releases of *Larinus* spp. and *C. achates* biological control agents of *C. stoebe* in Michigan and Indiana. * indicates Branch Co., where *L. minutus* weevils from the Bristol, IN release were first detected in MI.

All fields were initially monitored in September–October 2009 to document the presence/absence of *C. stoebe* biological control agents. At this time, we collected *C. stoebe* seedheads and roots, inspecting them for evidence of infestation by *Urophora* spp. (galls), *Larinus* spp. (pupal cases or exit holes) or *C. achates* (larval damage to roots) (Wilson and Randall 2005). In late-July through mid-August 2010, we visited each field to conduct initial monitoring. In release fields, we established a grid of 3 × 3 m plots with 1 m buffers (n = 16) to allow for *C. stoebe* monitoring and replicated trials of native plant overseeding (Fig. 2). In control fields, we established four 3 × 3 m plots to monitor the plant community and potential future dispersal of biological control agents. We collected baseline plant community and *C. stoebe* demographic data using the methods detailed below.

On 11–13 August 2010, releases of 368 *L. minutus* and 41 *C. achates* per field were made at all 6 release sites. Biological control insects were field collected in collaboration with Monika Chandler of the Minnesota Department of Agriculture (MNDA) from established populations near Bemidji, Minnesota. The location where *L. minutus* was collected was known to have *L. obtusus* present in low numbers. After weevil collection, all specimens were examined and putative *L. obtusus* were removed and frozen. Subsequent identification of these specimens in the lab confirmed their identity as *L. obtusus*. The remaining *L. minutus* and *C. achates* were divided equally for release at the six Michigan release fields. At the time of release, containers with weevils were opened at the base of *C. stoebe* plants at four equidistant locations within each release field (Fig. 2) and the weevils allowed to disperse naturally.

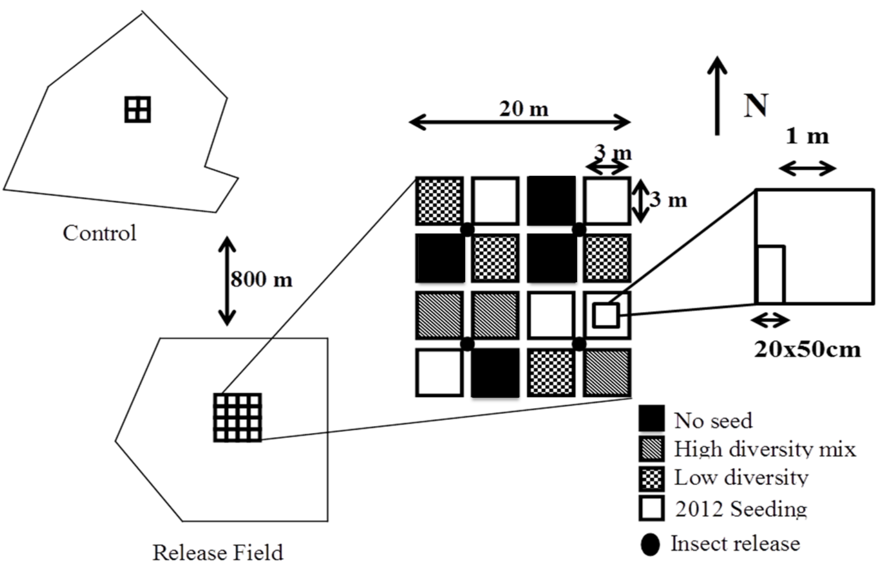


Figure 2. The experimental design of 2010 *C. stoebe* biological control release and control fields in Michigan. Each release field had subplots which either received no seed additions, high diversity 2011, low diversity 2011, or high diversity 2012 seed addition treatments.

**Native plant seed additions.** We developed "low" and "high" diversity mixes of native plants for re-vegetation of *C. stoebe* 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 *C. stoebe* infestations (i.e., dry to dry-mesic soils, full to partial sun, generally low fertility). Our low diversity mix contained eight forb species and three grass species, and our high diversity mix contained 16 forb species and three grass species. All seeds were obtained from Michigan Wildflower Farm in Portland, Michigan. The total weight of grass and forb seed was kept approximately equal in both treatments (Table 2).

Native plant seed mixes were overseeded into 3.0 m × 3.0 m experimental plots (Fig. 2) in the fall of 2011, with four plots receiving the high diversity mix, four plots receiving the low diversity mix, four designated as unseeded controls, and four plots held in reserve for future use. We used a completely randomized experimental design. 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 plot. The seed mix for each plot was evenly mixed with sand and 100 mL of water, and this mixture was hand-sown evenly within each plot receiving seed treatments. In 2012, the reserved plots were seeded with the high diversity mix. During the 2012 seeding, the western half of each plot was raked to increase opportunity for seed-soil contact. All seeding was conducted after the first frost between 15 October and 15 November in 2011–12. Plots in control fields received no seed additions.

**Weevil establishment at 2010 release sites.** To monitor the establishment and growth of *L. minutus* and *C. achates* populations, each release field was surveyed annually from 2011–2013. Establishment was defined as survival of an open release for two or more years (Harris 1991). Surveys occurred during mid-July and mid-August, when the greatest number of adult weevils was expected to be present. This expectation was verified by the results of a phenological study (Carson 2013). When possible, insect populations were sampled during warm days, when insects are most active.

At each field, 200 sweeps with a standard 37 cm diameter sweep net were made within 10 m of the north, south, east, and west sides of the research plots, with 50 sweeps taken from each side. The sweeps were focused on dense *C. stoebe* patches and were aimed at the top half of the plants, where adult *Larinus* spp. and *C. achates* are reported to occur (Stinson et al. 1994, Wilson and Randall 2005). 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 field to determine whether biological control insects had yet spread to the control field from the release field. 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 fields because the research plot area was smaller than that at the release fields.

**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 × 3 m plot in the release fields for signs of establishment of the native plant mixes. Near the end of *C. stoebe*'s peak bloom period in early-mid August, we took measurements of plant community diversity and *C. stoebe* population demographics at each site in 2011–2013. In each year, we began sampling at southern sites, progressing northward.

Within each 3 × 3 m research plot, we established a permanent 1 × 1 m sampling quadrat (Fig. 2). Within this quadrat, two researchers independently

Table 2. Species and seeding rates for low and high diversity seed mixes for re-vegetation of 2010 *C. stoebe* biological control sites with native nectar plants in Michigan.

| **Species** | **kg/ha** | **seeds/m²** |
|---|---|---|
| **Low Diversity Mix** | | |
| FORBS | | |
| *Anemone cylindrica* | 0.14 | 13 |
| *Asclepias syriaca* | 0.98 | 14 |
| *Coreopsis lanceolata* | 0.42 | 30 |
| *Euphorbia corollata* | 0.42 | 12 |
| *Liatris aspera* | 0.56 | 32 |
| *Monarda fistulosa* | 0.14 | 44 |
| *Rudbeckia hirta* | 0.14 | 45 |
| *Solidago nemoralis* | 0.04 | 37 |
| Total Forbs | 2.84 | 227 |
| GRASSES | | |
| *Andropogon gerardii* | 0.84 | 30 |
| *Koeleria macrantha* | 0.28 | 198 |
| *Schizachyrium scoparium* | 1.12 | 47 |
| Total grasses | 2.24 | 275 |
| Total seed | 5.08 | 502 |
| **High Diversity Mix** | | |
| FORBS | | |
| *Anemone cylindrica* | 0.07 | 6 |
| *Asclepias syriaca* | 0.49 | 7 |
| *Aster laevis* | 0.14 | 27 |
| *Coreopsis lanceolata* | 0.21 | 15 |
| *Desmodium canadense* | 0.56 | 11 |
| *Euphorbia corollata* | 0.21 | 6 |
| *Gnaphalium obtusifolium* | 0.01 | 12 |
| *Helianthus divaricatus* | 0.35 | 6 |
| *Liatris aspera* | 0.28 | 16 |
| *Monarda fistulosa* | 0.07 | 22 |
| *Monarda punctata* | 0.14 | 44 |
| *Penstemon digitalis* | 0.14 | 64 |
| *Rudbeckia hirta* | 0.07 | 23 |
| *Solidago juncea* | 0.02 | 18 |
| *Solidago nemoralis* | 0.02 | 19 |
| *Solidago speciosa* | 0.07 | 23 |
| Total forbs | 2.84 | 320 |

Table 2. Continued.

| Species | kg/ha | seeds/m$^2$ |
|---|---|---|
| GRASSES | | |
| *Andropogon gerardii* | 0.84 | 30 |
| *Koeleria macrantha* | 0.28 | 198 |
| *Schizachyrium scoparium* | 1.12 | 47 |
| Total grasses | 2.24 | 275 |
| Total seed | 5.09 | 595 |

evaluated the percent cover of each plant species. The average of the two estimates was recorded. Within one 20 × 50 cm microplot located in the southwestern corner of each 1 × 1 m quadrat, we counted the number of *C. stoebe* rosettes, stems, adult plants, and seedheads and measured the height of the five tallest *C. stoebe* plants. Adult stems rising from the same basal rosette were considered to belong to the same plant. To investigate biological control impacts on *C. stoebe* populations, we compared the percent cover, rosette density, and mean plant height of *C. stoebe* across years at the release and control fields at each site using repeated measures ANOVA. For all sites and measures, ANOVA assumptions were checked using the Shapiro-Wilk test for normality and the Equal Variance test. Because these assumptions were both met for the majority of analyses (13/15 analyses, with one site where percent cover was non-normal and mean plant height was observed to have unequal variance), and no one analysis violated both assumptions, ANOVA was applied to all comparisons for consistency. The Holm-Sidak method for multiple comparisons was applied to compare responses within treatments and between years. For all statistical tests, alpha = 0.05.

**State-wide distribution of *C. stoebe* biological control insects.** To evaluate the statewide distribution, density, and rate of spread of *C. stoebe* 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 20 June and 25 July (Carson and Landis, in review). Surveys for *C. stoebe* 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 systematic 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 *C. stoebe* along the roadside or at highway off-ramps. To qualify for sampling, patches had to be a minimum of 50 square meters in area and contain at least 70% cover of *C. stoebe*. Once a location was chosen for sampling and its longitude and latitude were recorded (Garmin GPS 2 Plus), we took 60 sweeps in the *C. stoebe* patch using the methodology described above. After sampling, we returned to driving along the designated route. After traveling 15 km, we began searching for another patch of *C. stoebe* to sample. This process was repeated until we completed the route.

During the roadside sampling in 2011–2013, it became apparent that there was a large and nearly contiguous population of *L. minutus* in southwestern Michigan that may have originated from a 1996 release made in Bristol, Indiana. To delineate the northern edge 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. Here we also report *C. stoebe* biological control monitoring data from the USFS that was taken from three counties in the western Upper Peninsula of Michigan.

## Results

**Weevil establishment at 2010 release sites.** In 2011, adult *L. minutus* were recovered at Grayling and Houghton Lake and adult *C. achates* were recovered at the two Seney release fields (Table 3). In 2012, adult *L. minutus* were recovered at all six release fields 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 from release fields at Sharonville and again at Seney North. In all, *C. achates* was recovered from every release field except Flat River over the 3 year sampling period. Additionally, with the exception of Grayling in 2013, the observed density of *L. minutus* increased in the release fields each year. In contrast, *L. minutus* were not found at any control fields, with one exception. In 2013, a single *L. minutus* was found just outside of the control field at Houghton Lake. It is unclear if this insect dispersed to the site on its own or was inadvertently transported there by individuals conducting the sampling. Individual beetles identified as *L. obtusus* were found at Sharonville and Grayling release fields in 2012, and Flat River, Houghton Lake, and Sharonville release fields in 2013, suggesting that low numbers of *L. obtusus* were present in the stock of *L. minutus* released at these sites.

**Native plant establishment and biological control impacts on the plant community.** The yearly plant community and *C. stoebe* 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 fields, these differences did not follow a consistent pattern, and the release fields never showed a decrease in these metrics over time that was not also matched by similar decreases at control fields. For example, significant differences in mean *C. stoebe* cover at Seney (Fig. 3) and in mean rosette number and plant height at Flat River (Fig. 4) probably reflect preexisting field-level conditions. Although we observed a net decrease of rosettes at the Seney release site while the number of rosettes increased in the control field, and this interaction was statistically significant, this result was not generalizable across sites. While rosette number declined at the Flat River release field, similar changes also occurred at the control field. Thus, there was no indication that these differences were caused by the effect of newly introduced biological control weevils and could be a result of natural year-to-year variation in the *C. stoebe* community at each site. Plant metrics and demographic results from all 2010 releases are available in Carson (2013).

We detected little germination of native plants introduced to the release fields, with the exception of *Monarda punctata* L. found in one 2011-seeded plot in 2013. This species was not found elsewhere in this field and it is highly likely these seedlings originated from our seed mix. Apart from this single occurrence, none of the seeded species were found growing in the seed addition plots, including those that received a raking treatment.

**State-wide distribution of *C. stoebe* biological control insects.** 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. In 2011, we found *C. achates* at the Seney North and Seney South sites in Schoolcraft County in the Upper Peninsula. In 2012, we found *C. achates* at two additional sites, Camp Grayling and Houghton Lake. By 2013, C. *achates* was recovered at Sharonville and at the 2007 Oakland County release site, as well as at 2009 USFS release sites in Gogebic and Houghton Counties. Thus, *C. achates* is present at sites in Schoolcraft, Crawford, Roscommon, Jackson, Oakland, Gogebic, and Houghton Counties (Fig. 5). All of the sites of *C. achates* recovery were at known release sites for the species, and *C. achates* was never observed more than 10 m from a release point.

Table 3. Spotted knapweed biocontrol insect recovery at release sites during 2011, 2012, and 2013.

| Site | Sample Year | **Total *Larinus* sp. recovered** | ***L. minutus* in subsample** | ***L. obtusus* in subsample** | ***Larinus* spp. per sweep avg.** | **Total *Cyphocleonus* recovered** |
|---|---|---|---|---|---|---|
| Seney North | 2011 | 0 | na | na | 0.00 | 1 |
| Seney North | 2012 | 1 | 1 | 0 | 0.01 | 0 |
| Seney North | 2013 | 5 | 5 | 0 | 0.03 | 2 |
| Seney South | 2011 | 0 | na | na | 0.00 | 1 |
| Seney South | 2012 | 3 | 3 | 0 | 0.02 | 0 |
| Seney South | 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 |

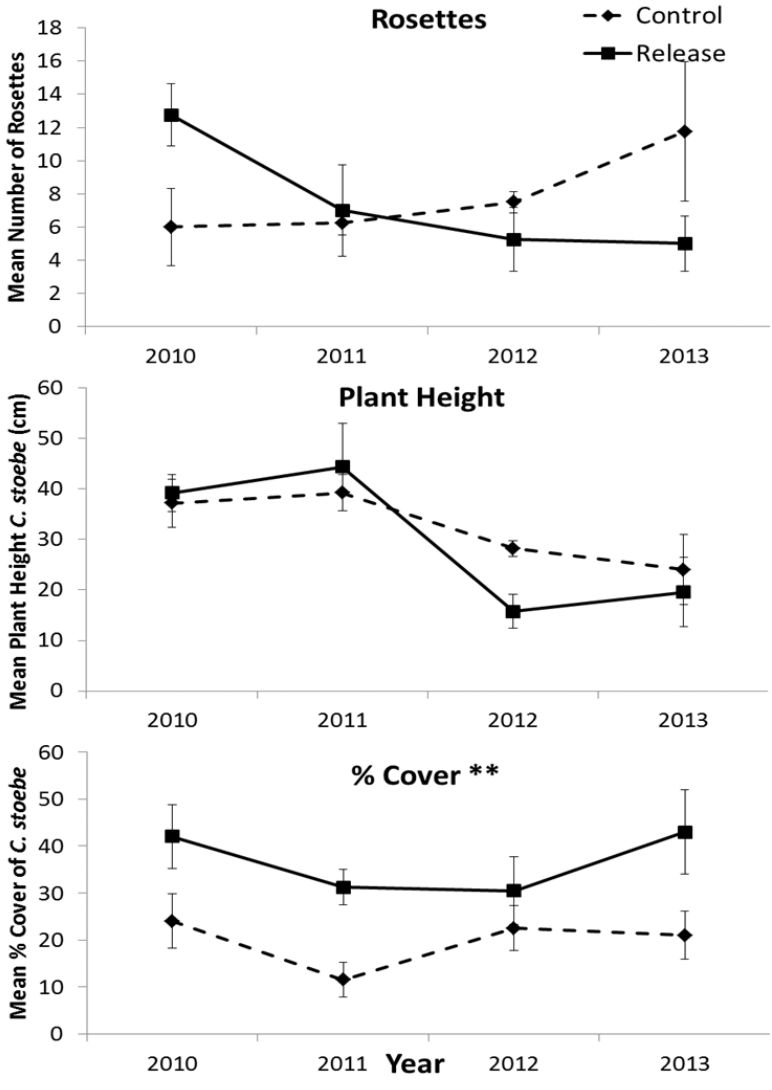


Figure 3. Mean (± SEM) of *C. stoebe* plant response variables at Seney Release 1 in year of biological control agent release (2010) and three subsequent years. Data were collected from 20 cm × 50 cm subplots. ** indicates a consistent overall significant difference between release and control treatments. Rosettes refer to seedlings and juvenile plants.

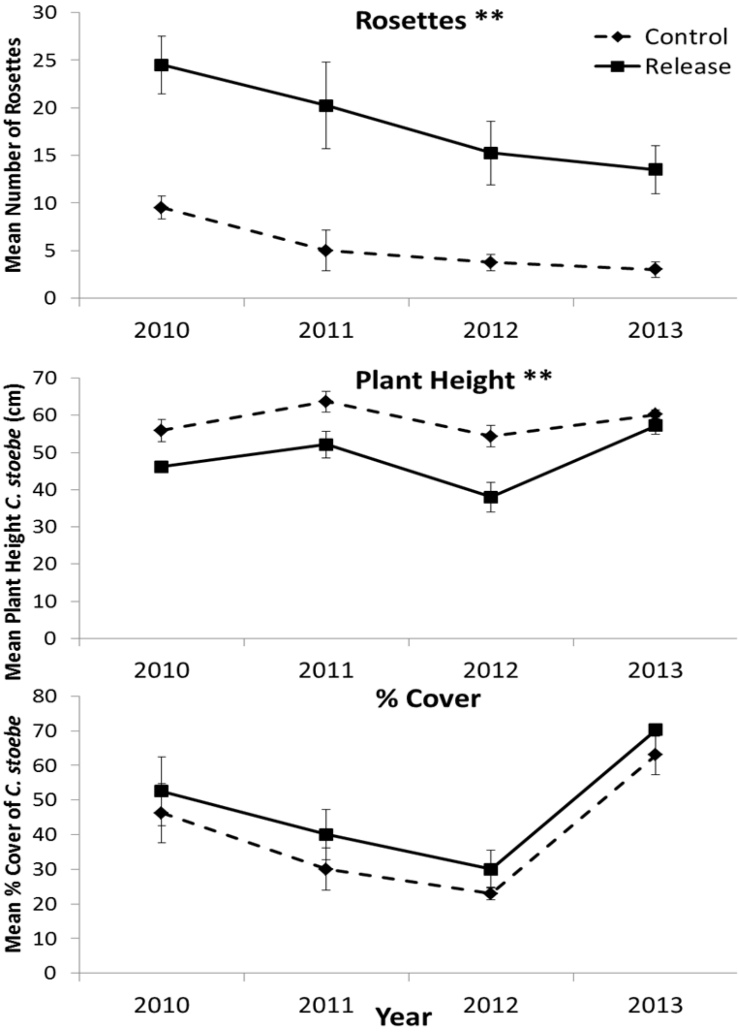


Figure 4. Mean (± SEM) of *C. stoebe* plant response variables at Flat River in year of biological control agent release (2010) and three subsequent years. Data were collected from 20 cm × 50 cm subplots. ** indicates a consistent overall significant difference between release and control treatments. Rosettes refer to seedlings and juvenile plants.

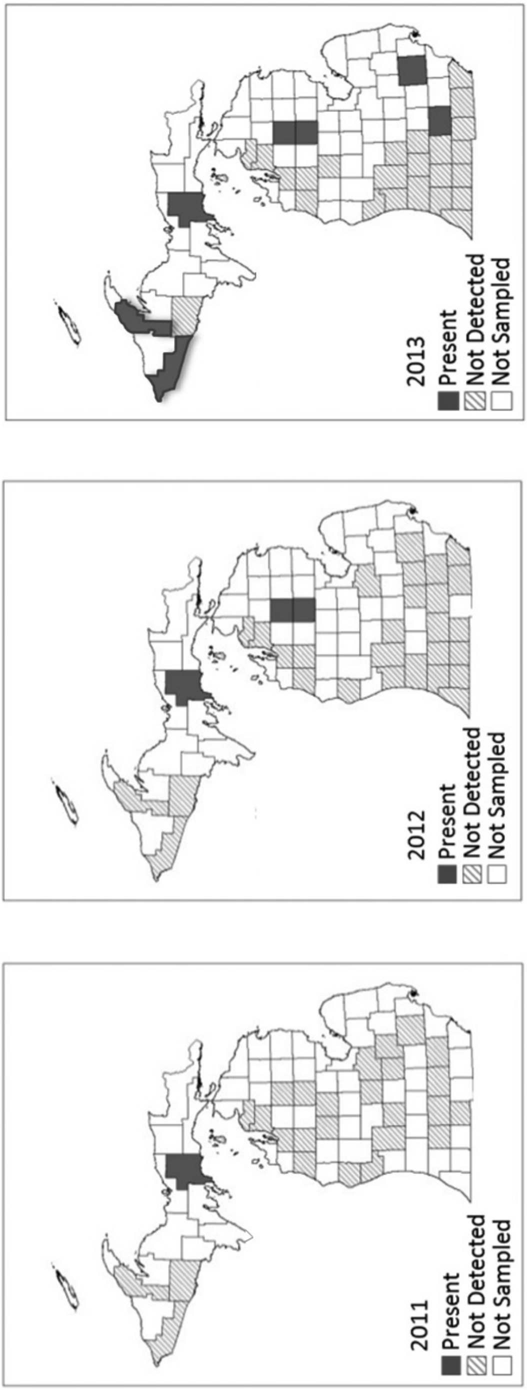


Figure 5. Occurrence of the *C. stoebe* biological control weevil *C. achates* in Michigan in 2011–2013, by county.

In 2011, we found *L. minutus* in eight counties (Schoolcraft, Crawford, Roscommon, Ionia, Jackson, Oakland, Kalamazoo, and Branch), and it was also recovered by the USFS in three additional counties (Gogebic, Iron, and Houghton) in the Upper Peninsula (Fig. 6). Seven of the counties where it was found were known to be release sites of the species, but two counties in southwestern Michigan (Kalamazoo and Branch) did not contain any known release sites. During 2012, we found *L. minutus* in 17 counties. Again, the species was found in seven southwestern counties that had no known releases (Berrien, Cass, Van Buren, St. Joseph, Branch, Calhoun, Kalamazoo, Jackson, and Allegan). In these counties *L. minutus* occurred in abundance, often exceeding one weevil per sweep. It seemed unlikely that these *L. minutus* populations had spread and grown from any release site in Michigan, because weevils at other known release sites remain locally distributed for many years (Carson 2013) and most likely arose from the 1996 Indiana release.

In 2013, we observed an apparent continued expansion of the Indiana population of *L. minutus*. They were found in five additional counties (Kent, Barry, Eaton, Hillsdale, and Lenawee), and the northernmost 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 (Fig. 7), at the site of the 2007 release of this species. In 2012, we detected populations in two adjacent counties (Allegan and VanBuren). We also recovered *L. obtusus* at two sites (Grayling and Sharonville) where we had made releases of *L. minutus*. These later 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. Populations in Kent, Ottawa, Ionia, Barry, and Eaton counties are adjacent to the original 2007 Kalamazoo County release, and a nearly uninterrupted population of *L. obtusus* now occupies that part of the state. The 2013 detection of *L. obtusus* in Lenawee and Monroe counties in southeastern Michigan was unexpected. The nearest known *Larinus* spp. release to these two recovery sites was in Sharonville, 14 and 38 km, respectively, from the Lenawee and Monroe County detections. 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 the Sharonville release could explain the Lenawee and Monroe County detections.

## Discussion

Of the six 2010 *C. stoebe* biological control release sites, *L. minutus* is well established at the three southernmost sites and seems likely to persist at all six sites. In contrast, *C. achates* has only been recovered in low numbers at five of the six sites and considered established at only one site (Seney North). However, *C. achates* has been reported to have a slow initial population growth rate (Story et al. 1997, Story and Stougaard 2006), and its populations may increase in the future.

While multiple biocontrol agents occur at most sites, our plant data shows that there has not yet been a quantifiable impact on *C. stoebe* populations. This is likely because insects have not been present at release sites for long enough to reach the densities necessary to impact *C. stoebe* recruitment. Sites that are near to the 1996 Bristol, Indiana release still have *C. stoebe* populations that appear robust, despite the presence of *L. minutus* for over a decade (D. Landis, pers. obs.). While *C. achates* was reported to be released at this site, it has not been recovered (R. Dunbar, Indiana Department of Natural Resources, pers. comm.). Studies conducted in the western U.S. and Canada suggest that while

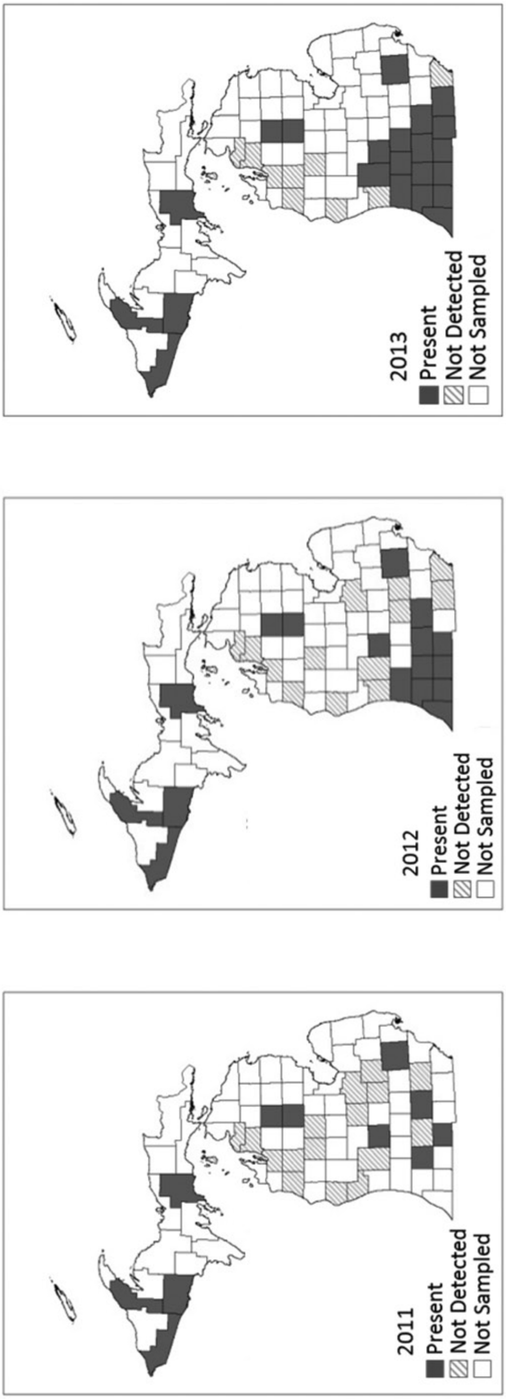


Figure 6. Occurrence of the *C. stoebe* biological control weevil *L. minutus* in Michigan in 2011–2013, by county.

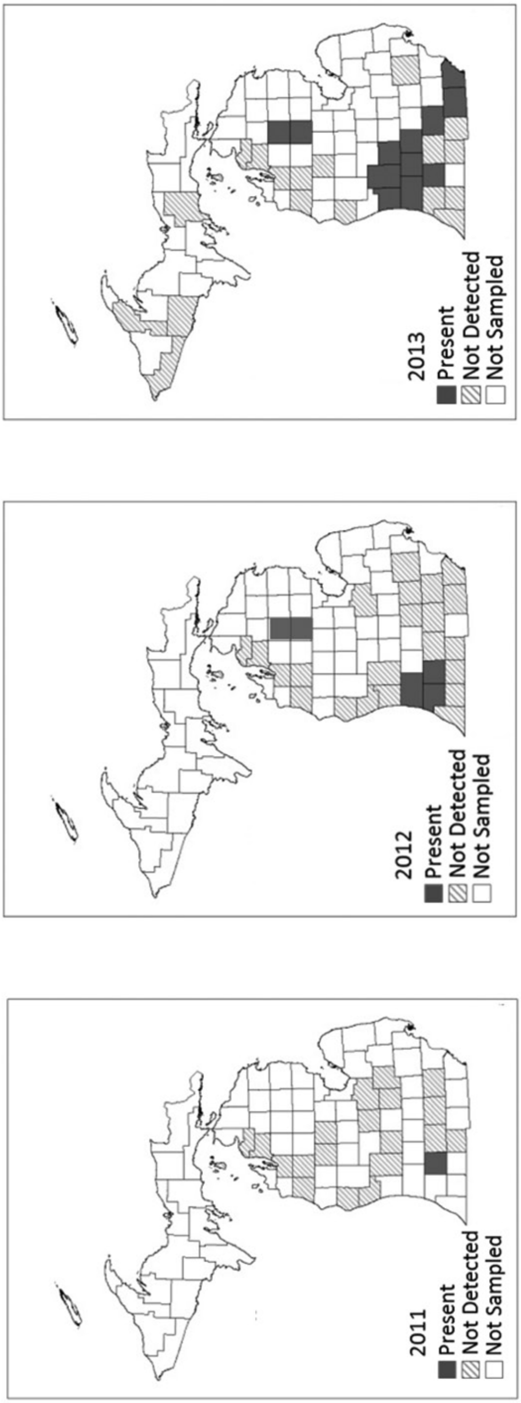


Figure 7. Occurrence of the *C. stoebe* biological control weevil *L. obtusus* in Michigan in 2011–2013, by county.

*Larinus* spp. are able to reduce *C. stoebe* infestations, it is able to persist in the presence of substantial seedhead predation (Story et al. 2008).

Western studies indicate that *C. achates* is the key to reducing *C. stoebe* densities (Clark et al. 2001, Seastedt et al. 2003, Jacobs et al. 2006, Story and Stougaard 2006), and the combination of *L. minutus* and *C. achates* has an even stronger impact on *C. stoebe* populations (Knochel et al. 2010). 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.

During the two years we monitored plant communities at release sites, we found little establishment of the seeded native plants. Allelopathic (Callaway and Ridenour 2004) or competitive effects of *C. stoebe* may have impacted survival of native plant seedlings. However, other research has shown that it is possible to germinate (Emery and Gross 2006) and establish (MacDonald et al. 2013) similar native plant species into *C. stoebe* stands in Michigan with minimal site preparation. The summer of 2012 was characterized by unusually high temperatures and drought conditions which likely decreased survival of seedlings germinating from the fall 2011 seeding. However, conditions were more favorable in 2013 and yet we still observed little germination from either 2011 or 2012 seed additions. It is also possible that our seeding rate (≈ 5 kg/ha) was too low for native seeds to compete with *C. stoebe* seeds, which were already present in the soil in high numbers. More research is needed to develop reliable methods for native plant introductions into *C. stoebe* stands.

Throughout the three years of sampling, we documented multiple expanding populations of *L. minutus* in Michigan. The six populations derived from the 2010 MSU release sites are spreading locally and individuals can be detected up to 2 km from the original release points (Carson 2013). The population of *L. minutus* arising from the 2007 release at Lake Orion has spread up to 10.5 km but is still contained within Oakland County (Carson and Landis, in review). In contrast, the southwest Michigan population, which we believe arose from the 1996 release made in Bristol, Indiana, now covers parts of 14 counties. This population appears to be expanding both north and eastward (Fig. 6). The rate at which this population is expanding is much greater than that of the more recent Michigan releases and points towards increasing dispersal rates with time since release (Carson and Landis, in review).

In 2011, *L. obtusus* was only known to occur at a single release site in Kalamazoo County but by 2013 was found in 13 Michigan counties (Fig. 7). Most of the occurrences of *L. obtusus* can be explained by either dispersal from the Kalamazoo release or by the presence of low numbers of *L. obtusus* in the 2010 releases of *L. minutus*. However, the detection of *L. obtusus* in southeast Michigan (Monroe and Lenawee counties) in 2013 was unexpected. It is possible that these weevils came from an unreported release in Michigan or Ohio.

In addition to natural dispersal, it is also possible that human activity is aiding in the spread of these biological control agents. For example, 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. Similarly, we have observed *C. stoebe* seedheads trapped in the bumper of vehicles after driving in fields or two-track roads containing *C. stoebe* infestations. At certain times of the year, these seedheads could contain *Larinus* or *Urophora* spp. that could potentially be transported long distances.

Human activity also has the potential to negatively affect knapweed weevils. Destructive *C. stoebe* management techniques, such as mowing, herbicide use, or burning, could slow or potentially prevent the initial establishment of both *C. achates* and *L. minutus* populations at release sites. However, our results show that established *L. minutus* populations occupy most knapweed stands within a landscape. Even if local populations are exterminated by *C. stoebe* management, those *C. stoebe* stands will quickly be re-populated by dispersing weevils. Because of its slower growth and low dispersal rates, *C. achates* would be less likely to re-populate managed sites. This should be taken into consideration when combining *C. stoebe* biological control with other management techniques.

In summary, *L. minutus* has established at every site at which they were released in Michigan, and *L. obtusus* has become established in the counties surrounding its initial release in Kalamazoo County, and is also present in small numbers at several *L. minutus* release sites. The abundance of *L. minutus* is increasing at release sites and these populations are expanding spatially, while *C. achates* populations are showing little sign of growth or expansion. There has not yet been a measurable impact of biological control on *C. stoebe* population demographics on the sites we studied, but as *C. achates* density increases, we anticipate potential reductions in *C. stoebe* density. Our efforts to establish native flowering plants at *C. stoebe* biological control sites have not yet been successful, though it is still possible that we will see germination in later years. Overall, these data provide a baseline for future studies of the expansion and potential impacts of *C. stoebe* biological control agents in Michigan. We expect that, over time, the effects of these three biological control agents will accumulate, and the density of *C. stoebe* will be reduced, improving the efficacy of restoration efforts involving native plants.

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