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Phenology and dispersal of *Larinus minutus* Gyllenhal and *Larinus obtusus* Gyllenhal (Coleoptera: Curculionidae), two biological control agents of *Centaurea stoebe* ssp. *micranthos* (spotted knapweed) in Michigan

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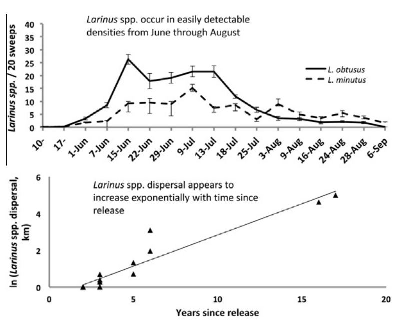
HIGHLIGHTS

- We monitored the phenology and dispersal of *Larinus* spp. populations in Michigan.
- Adults were present in easily detectable densities from mid-June through August.
- Populations of *Larinus* spp. weevils showed little dispersal for 2 years post-release.
- After initial establishment, dispersal rates increased exponentially with time since release.

GRAPHICAL ABSTRACT



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ABSTRACT

Centaurea stoebe L. ssp. *micranthos* (Gugler) (spotted knapweed) is an invasive plant that has been the target of classical biological control in North America for more than four decades. The seedhead-feeding weevils *Larinus minutus* Gyllenhal and *Larinus obtusus* Gyllenhal (Coleoptera: Curculionidae) are two of the most-widely released *C. stoebe* control agents, and have more recently been introduced into the eastern US. While there have been many studies focusing on their ability to impact *C. stoebe* in the western US and Canada, there have been few studies from eastern North America, and basic knowledge of important aspects of their biology is lacking. Here we report on the phenology and dispersal of *L. minutus* and *L. obtusus* in Michigan. We regularly sampled two established *Larinus* spp. populations in southern Michigan in 2012 and 2013, and found that while adult abundance fluctuates during the growing season, they remained at easily detectable levels from mid-June through the end of August. We also used previously established populations of *L. minutus* and *L. obtusus* released in 1996 ($n = 1$), 2007 ($n = 2$), and 2010 ($n = 5$) to determine how dispersal of *Larinus* spp. into the surrounding landscape changes with time since release. Populations of *Larinus* spp. weevils showed little dispersal for 2 years post-release. However, after initial establishment dispersal rates increased rapidly, resulting in average dispersal rates that increased exponentially with time since release. These findings can inform future biological control release and sampling programs for *Larinus* spp. in eastern North America.

1. Introduction

Centaurea stoebe L. subsp. *micranthos* (Gugler) (spotted knapweed) is a highly invasive plant in North America, found throughout most of the lower the 48 United States and parts of Canada (Wilson and Randall, 2005). Infestations of *C. stoebe* can cause decreases in plant diversity, ecosystem functioning, and reduce forage for wild and domesticated animals (Mummey and Rillig, 2006; Lacey et al., 1989; Hakim, 1979; Watson and Renney, 1974), resulting in economic losses (Griffith and Lacey, 1991).

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Despite the efforts of land managers, conventional control methods such as herbicides and mowing have been ineffective at slowing its spread (Wilson and Randall, 2005). Over the last four decades, extensive classical biological control programs to manage infestations of *C. stoebe* and *Centaurea diffusa* (Lam.) (diffuse knapweed) have occurred in western North America (Watson and Renney, 1974; Story and Anderson, 1978; Sheley and Jacobs, 1997; Corn et al., 2009). A more recent invader in the eastern US, *C. stoebe* is impacting the integrity of natural areas (Marshall and Storer, 2008), and several Eastern states have initiated biological control releases. The most common insect species released in the eastern region have been the root-boring weevil *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae), the seedhead weevil *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), and, to a lesser extent, the seedhead weevil *Larinus obtusus* Gyllenhal (Coleoptera: Curculionidae) (Van Driesche et al., 2002).

Adult *L. minutus* and *L. obtusus* are 4–5 mm long brown to black beetles. The larvae are white, C-shaped grubs with brown head capsules (Wilson and Randall, 2005). Both species share a similar life history, overwintering as adults in the leaf litter beneath host plants, and emerging in the late spring. The adults feed on *C. stoebe* stems and foliage when they emerge. Once *C. stoebe* flowers appear, the weevils mate and lay their eggs throughout the summer. Females oviposit from one to five eggs at a time in the flowerhead, and up to 130 eggs in a season (Wilson and Randall, 2005). The larvae hatch three days after oviposition, and begin feeding on the seeds. In their larval stage, the weevils potentially limit recruitment in *C. stoebe* populations by reducing the number of seeds each plant produces. There is also evidence that adult *Larinus* spp. can reduce host plant fitness through foliar feeding (Stephens and Myers, 2013). Releases of *L. minutus* in the Western United States and Canada have effectively reduced *C. diffusa* densities (Myers et al., 2009; Piper, 2003; Smith, 2003), and *L. minutus* has been shown to reduce *C. stoebe* fitness (Wooley et al., 2011).

Despite widespread releases of *Larinus* spp. in the western US and Canada since 1991 (Müller-Schärer and Schroeder, 1993), there is still a dearth of information regarding several aspects of their biology that are relevant to biological control planning and efficacy, especially in eastern North America. Wilson and Randall (2005) stated that adults of both species are generally active from May or June until August but did not address regional differences. Bourchier and Crowe (2011) reported on the phenology of *L. minutus* in southeastern British Columbia, but it is uncertain whether the weevils will follow similar emergence patterns in the rest of their introduced range. Knowledge of biological control agent phenology in a particular region is important to inform decisions of when to sample for population monitoring, or for redistribution efforts.

In addition to understanding phenology, knowledge of *Larinus* spp. dispersal patterns can inform release strategies (Paynter and Bellgard, 2011). To date, there have been no reported studies of the dispersal of *L. obtusus*, and only limited documentation for *L. minutus*. Van Hezewijk and Bourchier (2011) reported an average rate of spread for *L. minutus* of 1.9 km/year (5 years after initial release) in a landscape dominated by *C. diffusa* in Alberta, Canada. Because *C. diffusa* is the 'preferred' host plant of *L. minutus* and may facilitate greater rates of reproduction (Groppe et al., 1990), it is uncertain whether data obtained from a *C. diffusa* system can be extrapolated to *C. stoebe*-dominated landscapes. Recently, Alford (2013) reported that in Arkansas, *L. minutus* spread was only 112.5 m/year in the first 2 and 3 years post release. These wide disparities in the reported dispersal rates for *L. minutus* may be a result of the differing environmental conditions in the study areas or may have arisen from dispersal rates that increased with time. For example, in monitoring dispersal of the fire ant decapitating fly *Pseudacteon tricuspis*, Porter et al. (2004) report that they exhibited dispersal rates that increased dramatically over time, spreading up to 6 km from the release point 2 years post-release, and then increasing to 36 km from the release point 3 years post-release. These findings are likely in part due to sampling effects, i.e. that more distant dispersing individuals become more detectable as the overall population increases but could also arise as localized host depletion increases the need for greater dispersal.

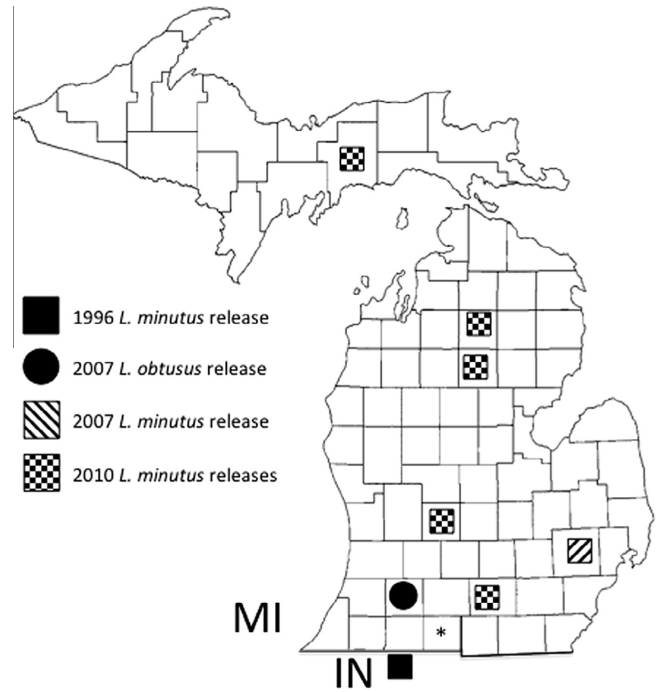

Fig. 1. *Larinus* spp. biological control releases in Michigan and a release site in Indiana from which phenology and dispersal data were collected. (*) Indicates Branch Co, where *L. minutus* weevils from the Bristol, IN release were first detected in MI.

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 (Fig. 1). In 2010, six additional releases of *C. achates* and *L. minutus* were made by Michigan State University in conjunction with the Michigan Department of Natural Resources (MDNR) in 2010. In 2012, we also became aware of a 1996 release of biological control agents against *C. stoebe* at a site 2.5 km south of the Michigan-Indiana border near Bristol, Indiana (R. Dunbar, Indiana Dep. Nat. Resources, pers. comm., Van Driesche et al., 2002), from which *L. minutus* appears to have immigrated into southern Michigan forming a large and expanding population (Carson, 2013). In 2012–2013, we studied *Larinus* spp. populations descended from these 1996, 2007, and 2010 releases (i.e. 16, 17, 5, 6 and 2, 3 years post release respectively) to establish patterns of adult phenology and dispersal rates in the Midwestern U.S.

2. Materials and methods

2.1. Study sites

Data were collected on *Larinus* spp. phenology and dispersal from release sites in Michigan and northern Indiana. The Indiana release occurred in 1996 using *L. minutus* weevils that were sourced from USDA APHIS in Bozeman, Montana. In 2007 *L. minutus* was released in Oakland County, Michigan, using weevils sourced from the Minnesota Department of Agriculture, and *L. obtusus* was released in Kalamazoo County, Michigan, using weevils from a commercial source in Bozeman, Montana. In 2010, *L. minutus* was released at sites in Michigan by MSU researchers in conjunction with the Michigan Department of Natural Resources. The weevils for this release were collected from sites near Bemidji, Minnesota with the help of Monika Chandler of the Minnesota Department of Agriculture (Carson, 2013). The MSU release sites used in this study were Seney North in Schoolcraft Co; Camp Grayling, Crawford Co; Houghton Lake, Roscommon Co; Flat River, Ionia Co; and Sharonville, Jackson Co (Fig. 1).

2.2. Phenology

To determine the phenology of *L. minutus* and *L. obtusus* in Michigan, studies were conducted in 2012 and 2013 at the Lake Orion and Kalamazoo sites (Table 1). During our study, the Kalamazoo site experienced an influx of *L. minutus* (not present in 2010 sampling) presumably from an expanding population resulting from the 1996 Indiana release. The co-occurrence of both *L. minutus* and *L. obtusus* presented the opportunity to compare their phenology in the same location. The abundance of active adult *Larinus* weevils was monitored weekly at Kalamazoo and bi-weekly at Lake Orion.

At Kalamazoo monitoring occurred between May 10 and September 5 in 2012 and between May 2 and September 17 in 2013. At Lake Orion monitoring occurred between May11 and October 3 in 2012 and between May 2 and September 24 in 2013. Sampling was conducted between 10:00 and 17:00. We used 37 cm diameter sweepnets to collect adult weevils from *C. stoebe* patches, targeting the upper portion of the canopy. After one set of 20 sweeps, *Larinus* spp. weevils were counted and a subset of five weevils was retained for identification in the laboratory. This procedure was repeated 10 times, with each set of sweeps taken from a different *C. stoebe* patch. All sampling was performed within 100 m of the original release points.

At Lake Orion, sampling was conducted by members of the Oakland County Parks Natural Resources staff. To ensure consistency, personnel were trained at the beginning of each field season by one of us (BDC). Sampling at Lake Orion took place bi-weekly due to scheduling limitations but was identical to sampling at Kalamazoo in all other respects. The subsamples of 50 weevils collected on each sampling date were identified to species.

To distinguish *L. minutus* and *L. obtusus*, we used a key published by Draney and Oehmichen (2007) and had the characters in the key verified by Gary Parsons, the collections manager of MSU's A.J. Cook Arthropod Research Collection. The characters used in separating the species were as follows: *L. minutus*: longer, more dense hair patterns on the 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. We did not find any individuals that did not fit one of these morphological types. Voucher specimens were deposited in the MSU A.J. Cook Arthropod Research Collection in 90% ethanol.

The first occurrence of teneral adults appeared in the subsamples, as indicated by a red tint throughout the integument, was also recorded. The resulting time series abundance data are displayed by sample date, with the corresponding degree day (DD_{10C}) accumulation presented in the text. Because no physiological development data are available for *Larinus* spp., DD_{10C} was selected as a value that has commonly been used to measure arthropod emergence in the Midwest region (Preuss, 1983).

To examine changes in species dominance at the Kalamazoo site, we compared the per sweep abundances of *L. minutus* and *L. obtusus* from June 1 to July 25, 2012, from August 3 to September 6, 2012, and from May 30, 2013 to September 17, 2013 using one-way ANOVA. ANOVA assumptions were checked using the Shapiro–Wilk test for normality and the Equal Variance test. For all statistical tests, alpha = 0.05.

2.3. Dispersal

To determine the rate of spread of *C. achates* and *Larinus* spp. populations, we conducted surveys of *C. stoebe*-infested fields in the landscape surrounding two 2007 and five 2010 biological control release sites (Table 1). The prevailing wind direction at all release sites was from the West. 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 *C. stoebe* in each cardinal direction and conducted 60 sweeps at each site, counting the number of each species of knapweed seedhead 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 *L. minutus* or *L. obtusus* 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. If we found weevils further than 3000 m, we continued sampling at intervals of approximately 8 km, and then finally in 16 km intervals. It was not always possible to find *C. stoebe* 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. We measured the maximum apparent dispersal at each site as the distance from the original release points to the furthest point where *Larinus* weevils were detected in each cardinal direction. Because low-density populations probably existed at distances beyond those at which we detected weevils, our estimates are a conservative measure of the actual population-level dispersal rate. We also inferred dispersal rates of the *L. minutus* population from the 1996 Indiana release (Table 1) using presence/absence sampling conducted approximately every 20 km, coupled with finer scale sampling to delineate the population's northern edge in both 2012 and 2013 (Carson, 2013).

Table 1
The maximum dispersal detected and mean (±SEM) maximum dispersal detected from 2012 to 2013 for *Larinus* spp. on *C. stoebe* at different sites in Michigan.

Site	Year of release	Species	Maximum dispersal detected (km) Sampling year		Maximum dispersal detected (km) Sampling year	
			2012	2013	2012	2013
Bristol	1996	*L. minutus*	100.00 (North)	145.00 (North)	NA	NA
Lake Orion	2007	*L. minutus*	1.40 (West)	10.50 (North)	1.06 ± 0.21	6.05 ± 2.07
Kalamazoo	2007	*L. obtusus*	3.27 (West)	43.30 (West)	2.75 ± 0.34	20.87 ± 8.50
Seney North	2010	*L. minutus*	0	0	0.00 ± 0.00	0.00 ± 0.00
Camp Grayling	2010	*L. minutus*	0	0.1 (North)	0.00 ± 0.00	0.03 ± 0.03
Houghton Lake	2010	*L. minutus*	0	1.8 (South)	0.00 ± 0.00	0.48 ± 0.44
Flat River	2010	*L. minutus*	0.1 (East)	1.0 (South and West)	0.03 ± 0.03	0.33 ± 0.13
Sharonville	2010	*L. minutus*	0.1 (North)	0.1 (East)	0.03 ± 0.03	1.00 ± 0.00

We evaluated dispersal patterns 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 ($n = 4$). The "average maximum dispersal rate" was calculated as the "average maximum dispersal detected" divided by the years since release. We also calculated weevil densities per sweep for the two 2007 release sites, and evaluated population densities at the release site, <800 m from the release site, 801–3000 m from the release site, and >3001 m from the release site. To compare per sweep weevil abundances at the release sites to those occurring 800 m from the release sites we used one-way ANOVA. ANOVA assumptions were checked using the Shapiro–Wilk test for normality and the Equal Variance test.

3. Results

3.1. Phenology

In 2012, low numbers of *Larinus* spp. adults were observed at Kalamazoo on May 10 (242 DD_{10C}), which was the first sampling date of that year (Fig. 1). Their abundance increased rapidly, peaking between June 15 (404 DD_{10C}) and July 19 (911 DD_{10C}). Thereafter, adult abundance steadily declined until the end of sampling on September 6. Teneral adults first appeared on August 3, and were present through our last observation on September 13. Both species exhibited broadly similar trends. *L. obtusus* was more abundant than *L. minutus* ($F_{(1,14)} = 7.30$, $p = .02$) until late July when the trend reversed, and *L. minutus* became the more abundant species ($F_{(1,10)} = 5.33$, $p = .04$). In 2013, we observed the first weevils emerging on May 14 (125 DD_{10C}) (Fig. 2). Both *L. minutus* and *L. obutsus* showed rapid increases beginning June 6 (298 DD_{10C}) and peaking between June 14 (369 DD_{10C}) and July 12 (673 DD_{10C}). Populations of both weevil species showed a steady decline after this, and by August 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 contrast to 2012, *L. minutus* was more abundant than *L. obtusus* throughout the season ($F_{(1,30)} = 4.50$, $p = .04$).

At Lake Orion, *L. minutus* was the only species observed in both years of the study. In 2012, *L. minutus* was observed in low abundance on the first sampling date on May 11 (210 DD_{10C}), and its population generally increased until June 28 (600 DD_{10C}) (Fig. 3). The population then underwent a decline before peaking again on September 7 (1473 DD_{10C}). Subsequently, there was a steep decline and the population then remained at low levels 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 September 7 through September 27. In 2013, *L. minutus* adults were observed in low abundance on the first sampling date on May 2 (104 DD_{10C}) (Fig. 3). Weevil abundance remained low until a sharp increase beginning on July 3 and peaking on July 15 (945 and 1222 DD_{10C}, respectively). Thereafter, *L. minutus* abundance declined abruptly and remained at low levels for the remainder of the season. Teneral adults first appeared on August 28, and we continued to find them in our subsamples through the last sampling date on September 24.

3.2. Dispersal

At the five 2010 release sites, we detected *Larinus* spp. dispersal of at least 100 m at two sites in 2012, and at four sites in 2013 (i.e. 2 and 3 years post-release) (Table 1). 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

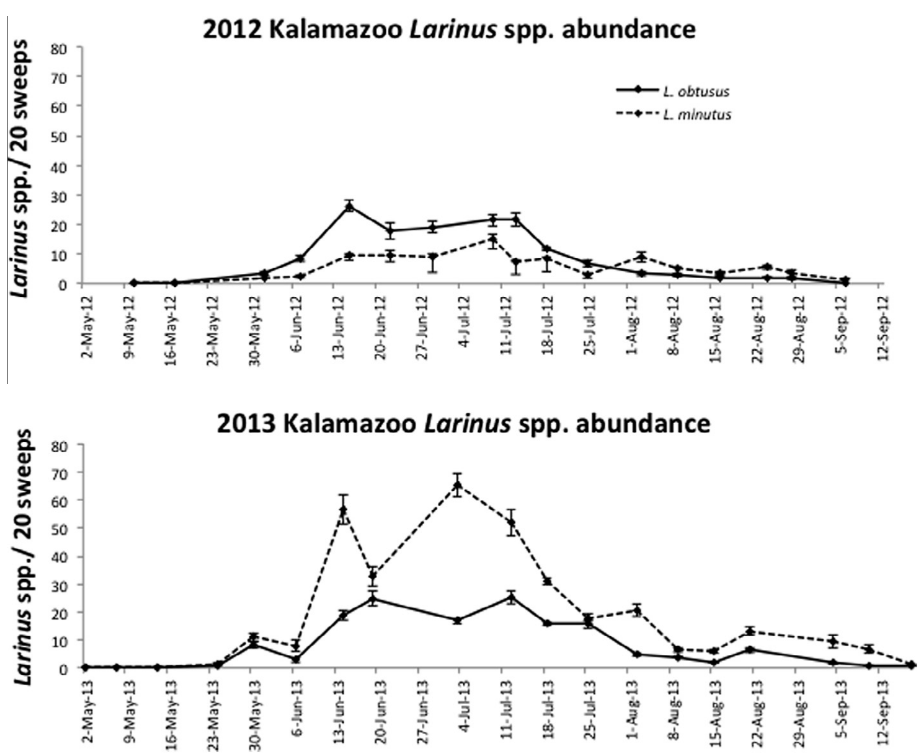

Fig. 2. The mean (±SEM) numbers of *L. minutus* and *L. obtusus* adults by sampling date in each set of 20 sweeps ($n = 10$) from 2012 to 2013 sampling at a 2007 spotted knapweed biological control release site in Kalamazoo Co, Michigan.

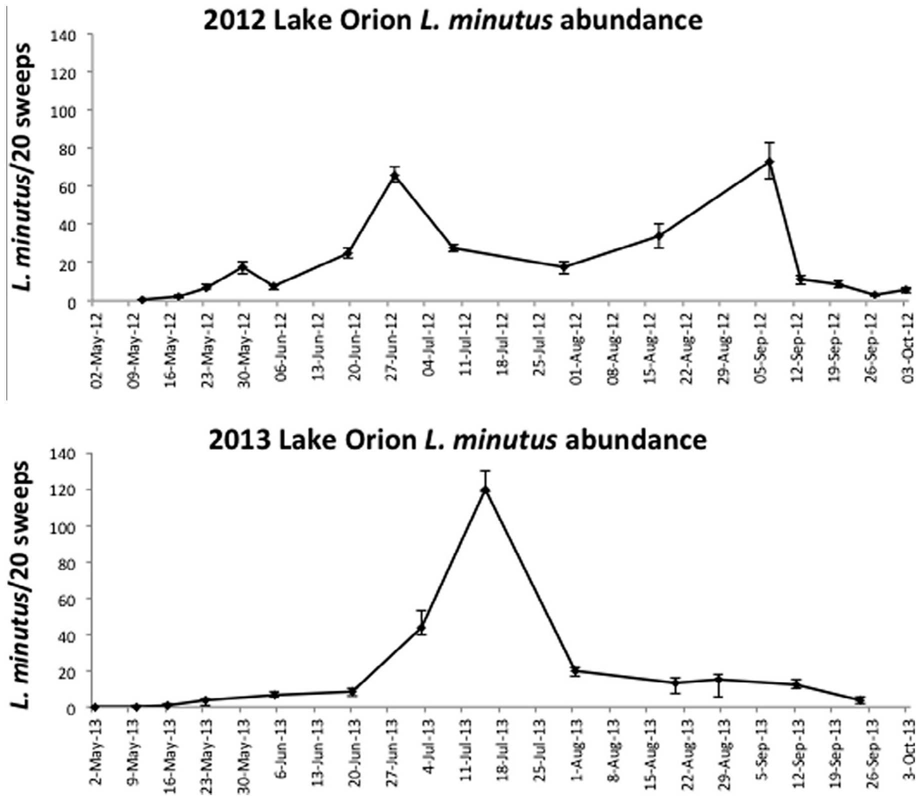

Fig. 3. The mean (±SEM) number of *L. minutus* adults by sampling date in each set of 20 sweeps ($n = 10$) from 2012 to 2013 sampling at a 2007 spotted knapweed biological control release site in Oakland Co, Michigan.

and Flat River. Additionally, at Flat River we detected weevil dispersal 1000 m to the South and 1000 m to the West. No weevil dispersal was detected from the Seney release site. Between 2012 and 2013, the rate of spread averaged across all 2010 release sites was 175 m ± 92 (SEM). All adults of both *Larinus* spp. recovered beyond the release points occurred in low densities, usually less than 0.05 weevils per sweep.

At the two 2007 release sites, we detected *Larinus spp.* dispersal from both sites on a local and landscape scale (Table 2). In 2012 at Lake Orion, we found local *L. minutus* dispersal up to 800 m from the release point in each cardinal direction. At 800 m per sweep weevil abundances were significantly less than those at the release point ($F_{(1,6)} = 16.82$, $p = 0.006$). By 2013, the densities of *L. minutus* up to 800 m from the release point were not significantly different from densities at the release ($F_{(1,6)} = 2.11$, $p = 0.19$), indicating that the population was relatively evenly distributed up to this distance (Table 2). In the landscape surrounding the Lake Orion release, we detected a maximum dispersal of *L. minutus* 1.4 km from the release site in 2012. In 2013, *L. minutus* was consistently found up to 1 km in each direction from the release, and we detected a maximum dispersal distance of 10.5 km (Table 1).

In 2012 at Kalamazoo, the densities of *L. minutus* up to 800 m from the release point were not significantly different from densities at the release ($F_{(1,6)} = 0.02$, $p = 0.89$) (Table 2) and *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 except to the South where we found *L. obtusus* in similar densities up to 3.4 km away along a power line, which may have served as a corridor for dispersal. In the landscape surrounding the Kalamazoo release, we detected a maximum dispersal of *L. obtusus* 3.3 km from the release site in 2012. In 2013, *L. obtusus* was consistently found up to 3.3 km in each direction from the release, and we detected a maximum dispersal distance of 43.3 km (Table 1).

4. Discussion

4.1. Phenology

Observations at the Kalamazoo site allow us to directly compare the phenology of *L. minutus* and *L. obtusus.* In both 2012 and 2013, the trend for each species followed a very similar pattern. Weevils first appeared in early to mid-May, and the adult *Larinus* spp.

Table 2
Mean (± SEM) number of *L. minutus* or *L. obtusus* per sweep recovered at different distances from release points.

Site	Species	Sampling year	*Larinus* spp./sweep, at release	*Larinus* spp./sweep, <800 m from release	*Larinus* spp./sweep 801–3000 m from release	*Larinus* spp./sweep > 3001 m from release
Lake Orion	*L. minutus*	2012	2.15	0.37 ± 0.16	0.02 ± 0.01	0.00 ± 0.00
Lake Orion	*L. minutus*	2013	5.42	4.28 ± 0.35	0.21 ± 0.06	0.14 ± 0.13
Kalamazoo	*L. obtusus*	2012	0.74	0.76 ± 0.22	0.49 ± 0.13	0.02 ± 0.02
Kalamazoo	*L. obtusus*	2013	1.36	1.48 ± 0.55	0.89 ± 0.27	0.25 ± 0.15

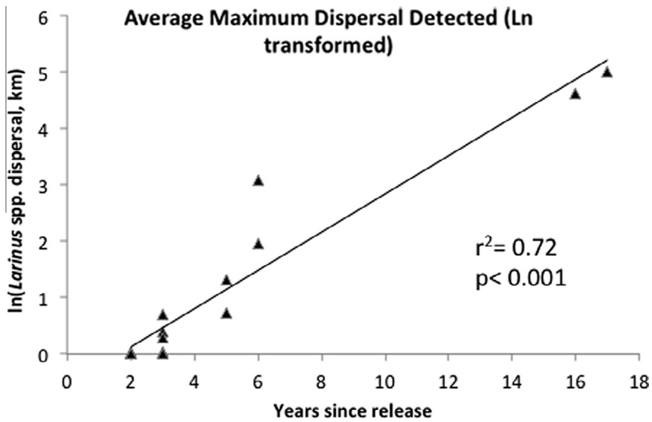

Fig. 4. The mean maximum dispersal (ln transformed) of *Larinus* spp. by years since release, based on data collected in 2012 and 2013 from spotted knapweed biological control releases made in 2010 ($n = 5$), 2007 ($n = 2$) and 1996 ($n = 1$).

populations were abundant and easily detectable by the middle of June. The weevils remained at easily detectable densities through the beginning of August in both years. The total number of weevils observed varied throughout the season. Initially we hypothesized that this variation was due to the emergence of the second generation of weevils later in the season. However, after searching for freshly emerged teneral weevils in our subsamples, we discovered that this new generation of weevils did not appear until the beginning of August in either study year, well after the total adult population level had begun to decline.

The results of our phenology study at Lake Orion were similar to those of Kalamazoo. In both years adult *L. minutus* appeared in early to mid-May, reaching easily detectable levels by the end of June. Weevils remained at relatively high abundance through early September. Total observed weevil abundances varied from sampling date to sampling date. Days with high levels of *Larinus* spp. weevils did not correspond to trends in temperature or cloud cover (Carson, 2013) at either site, and thus there seems to be a degree of natural variability in *Larinus* spp. weevil activity that is not explainable by any of the parameters that we measured. *Larinus minutus* phenology at the two Michigan sites was similar to that observed by Bourchier and Crowe (2011) in southeastern British Columbia, where adults were abundant by the end of June, and populations had declined by the end of August.

Land managers or researchers in the Midwestern US who merely wish to survey for the presence/absence of *L. minutus* or *L. obtusus* could do so between mid-June and the end of August. Researchers who want to determine the maximum population density at a site should do so between mid-June and late July, when weevils are at their highest abundance. 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.

4.2. Dispersal

In general, we saw a pattern of increasing dispersal rate with increasing time since release (See Fig. 4 and Table 3). The average annual rate of dispersal since release increased between 2012 and 2013 sampling at every site sampled, with the exception of the Seney North site. 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. While we did not conduct dispersal transects originating from the 1996 release made in Bristol, Indiana, we were able to infer the rate that this population of *L. minutus* has spread northward based on data collected during roadside surveys from 2012 to 2013 (Carson, 2013). Since being released in 1996, this population of *L. minutus* has spread northward at least 145 km over the course of 17 years. Between 2012 and 2013 alone, this population's northern boundary was observed to move 45 km (Carson, 2013). These data, while limited, provide further support for dispersal rates increasing as the time since release increases. This observation is not surprising, as a newly founded population needs time to increase before it can establish satellite populations that exist at detectable levels. Assuming dispersal distances of individuals in a growing population follow a normal distribution, longer dispersing weevils will exist at low densities that are unlikely to be detected for some time. Thus the probability of weevils dispersing to a certain point and the probability of detecting those individuals both increase as the population grows after the initial release. Because newly released *Larinus* spp. 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. Within the timeframe we studied, it appears that *Larinus* spp. populations expanded outward exponentially with time since release.

The overall pattern of increasing dispersal rate with time since release in *L. minutus* and *L. obtusus* is similar to that reported for *P. tricuspis* by Porter et al. (2004). However, while we found that it took 5 and 6 years for *Larinus* spp. populations to be detected more than a few hundred meters from release sites, *P. tricuspis* was dispersing at a landscape scale 3 years 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 the mild climate there.

Our observed rates of *Larinus* dispersal following initial release are lower than those in other published studies. While Alford (2013) reported that *L. minutus* had a mean maximum dispersal of 112 m/year 2 years following release, our mean maximum dispersal rate 2 years post release was only 5 ± 3 m/year. Van Hezewijk and Bourchier (2011) reported a mean maximum dispersal rate for *L. minutus* of 1900 m/year 5 years after initial release, while we calculated a mean maximum dispersal rate 5 years post-release of 212 ± 41 m/year. Compared to the 5 years post-release dispersal that Van Hezewijk and Bourchier (2011) observed in Alberta, the mean annual dispersal rate of the Lake

Table 3
The mean (±SEM) dispersal rate of *Larinus* spp. based on the years since release at four sites.

Site (n)	Release year	Years post release	Species	Average maximum dispersal rate (m/year since release)
MSU (5)	2010	2	*L. minutus*	5 ± 3
MSU (5)	2010	3	*L. minutus*	61 ± 30
Lake Orion (1)	2007	5	*L. minutus*	212 ± 42
Kalamazoo (1)	2007	5	*L. obtusus*	550 ± 68
Lake Orion (1)	2007	6	*L. minutus*	1008 ± 345
Kalamazoo (1)	2007	6	*L. obtusus*	3478 ± 1416
Bristol (1)	1996	16	*L. minutus*	6250
Bristol (1)	1996	17	*L. minutus*	8529

Orion *L. minutus* population is much less. However, by the following year (6 years post-release) *L. minutus* at Lake Orion had undergone further dispersal, with their average annual dispersal rate increasing to 1008 ± 345 m/year. This is closer to the 5 years post-release rate calculated by Van Hezewijk 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 Van Hezewijk and Bourchier (2011) and Alford (2013). The fact that the study in Alberta was conducted in a *C. diffusa*-dominated system 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, 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 years 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.

The evidence for an expansion of the 1996 Bristol, Indiana release of *L. minutus* into Michigan is circumstantial but compelling. Since 2010 we have been conducting roadside surveys for *C. stoebe* natural enemies. Driving pre-planned routes throughout much of the Lower Peninsula, sweepnet samples are taken approximately every 20 km and samples returned to the lab for identification (Carson, 2013). Most of these samples are negative, as would be expected given that prior to 2010, releases in southern Michigan had only occured in Kalamazoo and Oakland counties. However, in 2010 we unexpectedly detected *L. minutus* in samples from sites in Branch Co. near the Indiana border. In 2011, additional recoveries of *L. minutus* were made in Kalamazoo and Jackson Counties. In 2012, we found reference to a release of *L. minutus* in that occurred in 1996 in Indiana (Van Driesche et al., 2002). This release was made in Bristol, IN at a site 2.5 km South of the Michigan-Indiana border (R. Dunbar, Indiana Dep. Nat. Resources, pers. comm.). Subsequent sampling in 2012 confirmed that nine counties in southwest Michigan all had abundant *L. minutus* populations and that the northern edge of this population had reached our study site in Kalamazoo County (Carson, 2013). Notably, the abundance of *L. minutus* at the Kalamazoo County site increased rapidly from 2011 to 2013 representing 6%, 39%, and 70% of the *Larinus* spp. recovered in those years, respectively. As no *L. minutus* were released at that site, and prior sampling in 2010 had detected only *L. obtusus* (Landis unpub. data), we believe that the expanding population of *L. minutus* from the 1996 Indiana release 59 km to the south overtook the site between 2011 and 2013 and now represents the most abundant species. As the number of *L. minutus* in the landscape have increased through immigration and reproduction, their population size has surpassed that of *L. obtusus*, the species that was released at the site in 2007.

Because of the many factors affecting biological control agent dispersal rates, it is likely to prove difficult to predict how fast *Larinus* spp. will expand into the surrounding landscape at other locations. Dispersal is likely to depend on the overall abundance of *C. stoebe* in the landscape and factors like the occurrence of potential dispersal corridors like highways and utility rights of way. However, several relevant patterns have emerged from our work that can inform future biocontrol efforts. First, rates of *Larinus* spp. dispersal consistently increase with time since release. Considering the continued expansion of *L. minutus* from the Indiana release in 1996, it appears that this pattern is maintained for at least 16 and 17 years. 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 6 years after their respective releases, *Larinus obtusus* populations 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 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 was never found more than 10 m from a known release site in Michigan (Carson, 2013). Despite the reported effectiveness *C. achates* as a biological control of *C. stoebe*, it seems that *Larinus* weevils will spread to occupy *C. stoebe*-dominated habitats across the region long before *C. achates* arrives in most places. It remains to be seen whether the seedhead-feeders will have a significant effect on *C. stoebe* populations in the Midwest.

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References

Alford, A.M., 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, pp. 69.

Bourchier, R.S., Crowe, M.L., 2011. Role of plant phenology in mediating interactions between two biological control agents for spotted knapweed. Biol. Control 58, 367–373.

Carson, B.D., 2013. The biological control of spotted knapweed and the conservation of associated pollinator communities (Master's thesis). Michigan State University, pp. 187.

Corn, J.G., Story, J.M., White, L.J., 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. Environ. Entomol. 38, 971–976.

Draney, M.L., Oehmichen, W.T., 2007. Spotted knapweed bioagents reaction to fire. National Fish and Wildlife Foundation Final Programmatic Report 2007-0079-003.

Griffith, D., Lacey, J.R., 1991. Economic evaluation of spotted knapweed (*Centaurea maculosa*) control using Picloram. J. Range Manage. 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, S.A., 1979. Range condition on the Threemile Game Range in western Montana (Masters thesis). University of Montana, Missoula, MT.

Lacey, J.R., Marlow, C.B., Lane, J.R., 1989. Influence of spotted knapweed (*Centaurea maculosa*) on surface runoff and sediment yield. Weed Technol. 3, 627–631.

Marshall, J.M., Storer, A.J., 2008. Comparative analysis of plant and ground dwelling arthropod communities in lacustrine dune areas with and without *Centaurea biebersteinii* (Asteraceae). Am. Midl. Nat. 159, 261–274.

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, D.L., Rillig, M.C., 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant Soil 288, 81–90.

Myers, J.H., Jackson, C., Quinn, H., White, S.R., Cory, J.S., 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biol. Control 50, 66–72.

Paynter, Q., Bellgard, S., 2011. Understanding dispersal rates of invading weed biocontrol agents. J. Appl. Ecol. 48 (2), 407–414.

Piper, G.L., 2003. Biotic suppression of invasive weeds in Washington state: a half-century of progress. In: Proceedings of the XI Symposium on the Biological Control of Weeds, pp. 584–588.

Preuss, K.P., 1983. Day-degree methods for pest management. Environ. Entomol. 12 (3), 613–619.

Porter, S.D., Nogueira, L.A., et al., 2004. Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biol. Control 29 (2), 179–188.

Rondeau, K.J., 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, pp. 86.

Sheley, R.L., Jacobs, J.S., 1997. ''Acceptable'' levels of spotted knapweed (*Centaurea maculosa*) control. Weed Technol. 11, 363–368.

Smith, L., 2003, Impact of biological control agents on *Centaurea diffusa* (diffuse knapweed) in central Montana. In: Proceedings of the XI Symposium on the Biological Control of Weeds, pp. 589–593.

Stephens, A.E.A., Myers, J.H., 2013. Influences of two life-history stages of the weevil, *Larinus minutus*, on its host plant *Centaurea diffusa*. Ecol. Entomol. 38, 40–48.

Story, J.M., Anderson, N.L., 1978. Release and establishment of *Urophora affinis* (Diptera Tephritidae) on spotted knapweed in western Montana. Environ. Entomol. 7, 445–448.

Van Driesche, R. et al., 2002. Biological Control of Invasive Plants in the Eastern United States. USDA Forest Service Publication, New York (FHTET-2002-04, 413).

Van Hezewijk, B.H., Bourchier, R.S., 2011, Dispersal and impact of *Larinus minutus* among *Centaurea diffusa* patches in Alberta, Canada. In: XIII International Symposium on Biological Control of Weeds, pp. 328.

Watson, A.K., Renney, A.J., 1974. Biology of Canadian weeds *Centaurea diffusa* and *Centaurea maculosa*. Can. J. Plant Sci. 54, 687–701.

Wilson, L.M., Randall, C.B., 2005. Biology and Biological Control of Knapweeds. U.S. Forest Service Publication.

Wooley, S.C., Smith, B., King, C., Seastedt, T.R., Knochel, D.G., 2011. The lesser of two weevils: physiological responses of spotted knapweed (*Centaurea stoebe*) to above- and belowground herbivory by *Larinus minutus* and *Cyphocleonus achates*. Biocontrol Sci. Technol. 21, 2.