Spread of Larinus minutus (Coleoptera: Curculionidae), a Biological Control Agent of Knapweeds, Following Introduction to Northwestern Arkansas

Authors: Alford, Adam M., Kring, Tim, and Raghu, S.

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Spread of *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of knapweeds, following introduction to northwestern Arkansas

Adam M. Alford[1,], Tim Kring[2], and S. Raghu[3]*

Abstract

Spotted knapweed, *Centaurea stoebe* L. (Asteraceae), is an invasive perennial forb that has become economically and ecologically damaging in North America. The weevil *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), a biological control agent of invasive knapweeds, was introduced to 37 sites in northwest Arkansas since 2008 as part of a biological control program for spotted knapweed. In 2011 and 2012, 25 of these release sites were surveyed to monitor how *L. minutus* infestation rates changed in relation to distance and time from release. The initial *L. minutus* introductions at these sites occurred from 2008 to 2011. Transects were used from the point of initial weevil introduction to establish sampling quadrats in which capitula were collected to document weevil presence and infestation rates. The mean maximum distance of weevil colonization and mean local abundance (within the first 50 m from the release point) were calculated in relation to time (yr) since release. Five sites had >10 quadrats in both sampling years and were analyzed with an exponential decay function to model localized population growth and spread. Annual increases in mean local abundance and maximum distance of colonization were observed. These results were substantiated by localized growth (at 3 sites) and spread (at 2 sites) that occurred at the 5 sites analyzed with the exponential decay function. These findings suggest that in years following *L. minutus* introduction, assuming similar release strategies and environmental conditions, consistent increases in weevil infestation rates and spread from the release site may be expected in about half the sites at which populations establish.

Key Words: spotted knapweed; agent spread; post-release evaluation; spread model

Resumen

La *Centaurea* manchada, *Centaurea stoebe* L. (Asteraceae), es una de las herbáceas perennes invasiva que se ha vuelto económicamente y ecológicamente perjudicial en América del Norte. El gorgojo *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), un agente de control biológico de especies de *Centaurea* invasivas, se introdujo en 37 sitios en el noroeste de Arkansas desde el 2008 como parte de un programa de control biológico de la *Centaurea* manchada. En el 2011 y el 2012, se realizo un sondeo de 25 de estos sitios de liberación para monitorear cómo la tasa de infestación de *L. minutus* cambió en relación con la distancia y el tiempo de liberación. Las introducciones iniciales de *L. minutus* en estos sitios sucedieron entre el 2008 y el 2011. Se utilizó transectos desde el punto de introducción inicial del gorgojo para establecer cuadrantes de muestreo en el que se recogieron los racimos para documentar la presencia del gorgojo y la tasa de infestación. La distancia máxima media de la colonización del picudo y la media de abundancia local (dentro de los primeros 50 m del punto de liberación) se calcularon en relación con el tiempo (año) desde el lanzamiento. Cinco sitios tenían > 10 cuadrant es en ambos años de muestreo y se analizaron con una función de decaimiento exponencial para modelar el crecimiento de las poblaciones localizadas y propagación. Se observaron incrementos anuales en la abundancia local media y la distancia máxima de la colonización. Estos resultados se corroboraron por el crecimiento localizado (a los 3 sitios) y la extensión (a los 2 sitios) que se produjo en los 5 sitios analizados con la función de decaimiento exponencial. Estos hallazgos sugieren que en los años siguientes a la introducción de L. minutus, asumiendo estrategias de lanzamiento similares y las condiciones ambientales, los aumentos constantes en los índices de infestación de gorgojos y lo diseminado desde el lugar de la liberacion pueden ser esperados en alrededor de la mitad de los sitios en los que las poblaciones se establecen.

Palabras Clave: *Centaurea* manchada; agente de propagación; evaluación pos-liberación; modelo de propagación

The knowledge gained from the analysis of a species' invasion process into a novel habitat is of great importance; in the case of invasive pestiferous species, it can enable us to anticipate problems it may cause, whereas for introduced beneficial species, it can enable us to augment its benefits (Andow et al. 1990). In the case of beneficial invaders like biological control agents, this information can be used for predicting the consequences of variable factors such as release numbers, frequency of release, and spatial proximity of release sites on the impacts on the targeted weed/pest in subsequent years (Shea & Possingham 2000). The goal of our research is to document the local spread of the weevil *Larinus minutus* Gyllenhall (Coleoptera: Curculionidae), a biocontrol agent for spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek; Asteraceae), with a view to understanding dynamics of colonization across various sites.

Spotted knapweed is an invasive short-lived Eurasian perennial forb that is problematic in North America. The plant was initially introduced to the North American Pacific Northwest in the 1890s and is now found in all but four states (USDA 2016). Spotted knapweed pro-

[1]Purdue University, Department of Entomology, West Lafayette, Indiana 47907, USA; E-mail: AdamMAlford@gmail.com (A. M. A.)
[2]Virginia Polytechnic Institute and State University, Department of Entomology, Blacksburg, Virginia 24061, USA; E-mail: timkring@vt.edu (T. K.)
[3]CSIRO Biosecurity Flagship & USDA-ARS Australian Biological Control Laboratory, Brisbane, QLD 2001, Australia; E-mail: Raghu.Sathyamurthy@csiro.au (S. R.)
*Corresponding author; E-mail: AdamMAlford@gmail.com (A. M. A.)

duces 350 to 10,000 small, dry indehiscent fruits (hereafter referred to as achenes) per plant annually depending on growing conditions (Watson & Renney 1974). Achenes can be dispersed by wind, as contaminants of soil and hay, by attachment to vehicles or animals (Sheley et al. 1998), and by defecation following ingestion by animals such as sheep and mule deer (Wallander et al. 1995). Once an achene sprouts, the plant spends the remaining growing season as a basal rosette accumulating biomass and ultimately overwinters at this stage. Bolting of the rosette typically occurs the following growing season along with achene-producing capitula.

Spotted knapweed is a pioneer species and a quick invader of disturbed areas (Watson & Renney 1974). Knapweed invasions have resulted in substantial negative ecological and economic impacts. The direct and indirect effects of spotted knapweed and two other knapweeds, *Centaurea diffusa* Lam. and *Rhaponticum repens* (L.) Hidalgo, are estimated to cost Montana $42 million dollars annually based on an infestation of over 2 million acres (Hirsch & Leitch 1996). A 30,000 ha infestation of diffuse and spotted knapweed in British Columbia reduced available forage up to 88% (Harris & Cranston 1979), and ingestion of a large amount of either plant can lead to toxic symptoms in horses (Maddox 1979). Additionally, spotted knapweed–dominated sites experience reductions in plant community composition (Tyser & Key 1988) as well as increased surface water runoff, soil sedimentation yields, and interrill erosion (Lacey et al. 1989).

Spotted knapweed control options include herbicide application (Müller-Schärer & Schroeder 1993), hand pulling, and mowing (Sheley et al. 1998). These approaches can impede or ameliorate knapweed infestations in certain situations and are often cost and time prohibitive. Most regional knapweed management programs thus include a biological control component. Spotted and other invasive knapweeds have been the target of classical biological control with 13 natural enemies imported and established in North America (Müller-Schärer & Schroeder 1993). Of these, *L. minutus* was first introduced to North America in 1991 (Lang et al. 1996). The weevil has since become established in Arkansas, Colorado, Indiana, Michigan, Minnesota, Montana, Oregon, Washington, Wyoming, Alberta, and British Columbia (Lang et al. 1996; Story 2002; Myers et al. 2009; Minteer et al. 2011; Van Hezewijk & Bourchier 2012; Carson & Landis 2014). Addition of *L. minutus* to sites in British Columbia and Colorado, areas in which other natural enemies have already been established, resulted in a decrease of diffuse and spotted knapweed density (Seastedt et al. 2003; Myers 2008).

Larinus minutus is univoltine and overwinters as an adult stage in the debris of knapweed sites (Kashefi & Sobhian 1998). Weevils leave overwintering sites in the spring and preferentially feed on the flowers of nearby knapweed. Adults mate approximately 4 wk after this feeding period (Groppe 1990), and females oviposit between knapweed florets. Upon hatching, larvae bore into the capitulum and consume the developing knapweed achenes (Kashefi & Sobhian 1998). Weevil larvae pupate within the capitulum, and the adult form emerges soon thereafter, leaving a characteristic emergence hole in the capitulum. The overall egg-to-adult development period occurs within a month in northwest Arkansas. Larval *L. minutus* feeding destroys 100% of achenes in infested capitula (Kashefi & Sobhian 1998), and adult feeding may kill bolting stems (Myers et al. 2009).

Larinus minutus was initially introduced at 40 sites in Arkansas from 2008 to 2011 (Minteer et al. 2014). As of yet, relatively little has been reported on the infestation and spread rates of *L. minutus* in the early years following introduction to a new area. In Colorado, after about 6 yr, infestation rates of about 40 to 60% have been reported (Knochel & Seastedt 2010); however, supplementations of about 3,000 weevils were made at the study site in the years preceding infestation calculation. *Larinus minutus* has been reported to spread up to 100 m and 1.8 km away from the release site 2 and 3 yr post-release, respectively, in Michigan (Carson & Landis 2014). Expansions of 140 m at 1 yr post-release in Washington State (Whaley 2002), and about 2 km at 2 yr after a single release in California have also been documented (Woods & Popescu 2001). However, less has been published on the long-term spread patterns of *L. minutus* post-release. An exception is the work of Carson & Landis (2014), who found *L. minutus* to disperse 10.5 km and 145 km, at 6 and 17 yr post-release, respectively. The aim of our study was to expand our understanding of *L. minutus* colonization and spread patterns through a multi-year study spanning 25 sites across northwestern Arkansas.

Materials and Methods

RELEASE SITES AND SAMPLING PROCEDURE

Larinus minutus spread and infestation rates were documented by surveying transects in spotted knapweed fields from late fall to late winter in 2011 to 2012 and 2012 to 2013 (hereafter 2011 sampling and 2012 sampling, respectively) in northwest Arkansas (Table 1). Because *L. minutus* is univoltine, a late fall/winter sampling period allowed us to ensure all adults had emerged from capitula. The year of initial release of *L. minutus* varied among release sites (2009 to 2011) allowing us to collect data on *L. minutus* spread and population increase 0 to 3 yr post-introduction. Sites ranged in size from 30 to 700 m at their largest length, and were mostly in ruderal habitats like road margins and areas in urban development. Mowing, urban development, and yearly variation in knapweed patch size limited our ability to sample across all 40 of the release sites; data were collected from 20 release sites in 2011 and 23 sites in 2012 (Table 1).

Sampling transects were initiated at the most dense knapweed patch at each site. A second transect, oriented approximately 90° to the first transect was similarly established when knapweed populations allowed. Four transects were established in this manner, such that transects were initiated in areas of dense knapweed and progressed outward to areas with less knapweed. Additional transects were established between the initial 4 transects when patches were sufficiently large in order to ensure a more complete description of *L. minutus* density across the knapweed-infested site.

The total number of transects was generally limited by spotted knapweed abundance, as the weed is patchily distributed. Standardized circular sampling quadrats with a 7 m radius were established every 15 m along each transect. A quadrat was established at the next available knapweed patch along that transect in the event no knapweed occurred at the next interval. The coordinates of the center of each quadrat were recorded with a GPS device (Garmin Nüvi 500, Garmin Ltd., Kansas City, Missouri) and later used to determine the distance of a quadrat from the release point at a given site.

Within each quadrat, visual searches were conducted for 3 min or until an emergence hole was observed, whichever came first. A transect was terminated when no emergence holes were observed during the visual searches of two successive quadrats during 2011 sampling. In 2012, sampling transects were terminated when knapweed was no longer available along the transect, or when further sampling was not possible because of the extension of the transect into posted private property. This change was made after observations from 2011 suggested weevil distribution at a given site could occur in a non-continuous manner and that the weevil could spread further than what was expected.

Greater than 100 fully developed capitula were collected randomly from each quadrat and saved for subsequent dissection to determine

Table 1. *Larinus minutus* release sites in spotted knapweed in northwest Arkansas sampled over the 2 yr course of study.

					Sampling year			
					2011		2012	
Site ID	Latitude	Longitude	Initial weevil release date	# weevils released	Sampled +/−	Recovery +/−	Sampled +/−	Recovery +/−
1	36.31833	−94.18419	6/29/10	600	+	—	+	+
2	36.30461	−94.18047	6/29/10	600	+	+	+	+
3	36.32233	−94.18543	6/29/10	600	+	+	+	+
4	36.29850	−94.16965	6/25/10 & 7/2/10	1,000 & 800	+	+	+	+
5	36.35461	−94.17526	6/25/10	700	—	N/A	+	+
8	36.33401	−94.16293	6/24/11	1,500	+	+	+	+
9	36.20656	−92.99887	7/6/10	400	—	N/A	+	—
11	36.17681	−93.53625	7/1/10	300	—	N/A	+	—
12	36.23018	−93.53030	7/1/10	600	+	+	—	N/A
13	35.90324	−93.93130	6/25/11	600	+	+	+	+
14	35.91032	−93.93620	6/25/11	600	+	+	+	+
15	36.25772	−93.63195	7/1/10	1,200	+	+	+	+
16	36.25634	−93.63939	7/1/10	1,200	+	+	+	+
20	36.17105	−93.91383	6/25/10	700	+	—	+	—
21	36.05995	−94.12745	6/29/10	900	+	+	+	+
22	36.10334	−94.00610	7/1/10	800	—	N/A	+	+
23	36.10069	−94.05145	7/1/10	400	—	N/A	+	+
25	36.12047	−94.15321	7/2/10	400	+	+	—	N/A
27	36.03448	−94.18479	6/13/09	700	+	—	+	—
29	35.92496	−94.19784	6/14/09	700	+	+	+	+
30	35.96508	−93.99424	6/25/11	800	+	—	+	+
31	36.10053	−94.18552	6/16/09	700	+	+	+	+
33	35.98450	−94.19894	6/15/09	700	+	+	+	+
34	36.07678	−94.19767	6/13/09	600	+	+	+	+
35	36.11140	−94.16240	6/26/11	700	+	+	+	+

percentage of infestation by *L. minutus*. Capitula were approached and collected from an angle to inhibit observation of potential emergence holes in order to minimize sampling bias. Percentage of infestation was determined from a maximum of 100 dissected capitula, even if more than 100 were collected for each quadrat. If fewer than 100 capitula were present within a quadrat, all fully developed capitula were collected and an infestation percentage was determined. Capitula were classified as infested if an *L. minutus* emergence hole was observed, or if dissection revealed evidence of *L. minutus* pupation. Presence of larval remains was not counted as infested. To control for variation as a result of multiple samplers (Morris 1960), all transects and dissections were conducted by the same person.

DATA ANALYSES

We calculated 2 variables for each release site: (1) the maximum infestation distance (the distance of the sampling quadrat furthest from the release point at which *L. minutus* infestation was observed) and (2) the mean *L. minutus* infestation within the first 50 m from the release point (hereafter also referred to as the area of release or release area). The mean *L. minutus* infestation was calculated by averaging the percentage of *L. minutus* infestation of all sampling quadrats within the area of release. Utilizing the 2 variables calculated for each release site, we then grouped sites by years post-introduction and calculated an overall average for each variable.

R was used for all statistical analyses (R Development Core Team 2012). Data were analyzed with a 1-way ANOVA with maximum infestation distance or percentage of infestation at the release area as the response variables and years since release as the fixed factor. Data on maximum infestation distance were log transformed and data on percentage of infestation were arcsine square root transformed to ensure that they conformed to the assumptions of the ANOVA. Post hoc pairwise comparisons of means across the fixed factor were made using Tukey's HSD tests. Statistical differences between means were evaluated at the α level of 0.05.

In addition to the above analyses, we modeled the spread of *L. minutus* at our study sites. Knapweed is patchily distributed and *L. minutus* is univoltine. Both of these factors place limits on the distance *L. minutus* can move within a season. Therefore, we predicted/expected high levels of infestation at the point of release with decreasing levels of infestation at locations more distal to the release point. We postulated that an exponential decay function should adequately describe both this expected local population increase (i.e., at the release point) and spread of *L. minutus* following introduction, based on analogous spread models in the literature (Rudd & Gandour 1985; Andow et al. 1990).

The exponential decay function was fitted to transect sampling data with a Levenberg–Marquardt nonlinear least-squares algorithm in R to quantitatively describe spread at each of the release sites (R Development Core Team 2012). In the above equation, y is the predicted percentage of infested capitula, and x is the distance from the release point. A is the estimate of percentage of infested capitula at the initial release location (i.e., $x = 0$). B represents the rate at which density of *L. minutus* declines relative to the distance from the initial release point ("decay in weevil abundance" hereafter). The smaller the absolute value of B, the more gradual is the decay in abundance from the initial release point, i.e., evidence of spread from release point. An initial approximate value of A and B based on the data were used as starting values for the iterative fitting of the exponential decay function using the algorithm mentioned earlier; the analysis provides the

best-fit parameter estimates for *A* and *B* given the data. Differences in fit between years for a given site were evaluated by overlap of 95% confidence intervals of the parameter estimates.

Release sites had to meet the following criteria to be included in the exponential decay model: 1) more than 10 quadrats were sampled per year, and 2) sampling occurred in both collection years. These criteria ensured that only sites with adequate data were used to describe the spread patterns, and enabled us to examine differences in spread patterns across sampling years at a given site. Five sites (Sites 3, 4, 15, 16, and 31) met both of these requirements.

Results

Larinus minutus infestation (based on dissection of collected capitula) was recorded at all but 4 of the 20 release sites sampled in 2011 (1, 20, 27, 30) and all but 4 of the 23 sites sampled in 2012 (9, 11, 20, 27) (Table 1). Larval remains were discovered in only 2 capitula in separate sampling quadrats and were consequentially not included in calculations. Of the aforementioned 6 sites (1, 9, 11, 20, 27, 30), timed visual searches confirmed presence of *L. minutus* emergence holes at 3 of the sites.

Release sites sampled in 2012 generally had *L. minutus* established at further distances from the release point than in 2011. Capitula from which *L. minutus* emerged were documented up to 309 m and 622 m from release points during the 20v11 and 2012 sampling periods, respectively. The average maximum distance of *L. minutus* infestation from the release point increased by about 60 m and about 100 m between 0 and 1 yr and between 1 and 2 yr from *L. minutus* introduction, respectively, but decreased about 25 m between 2 and 3 yr after release (Table 2). There was a marginal effect of years since release on the average maximum distance that *L. minutus* was recorded at a site ($F = 2.946$; df = 3,30; $P = 0.049$), although there were no discernible differences observed between any 2 given years by the post hoc Tukey's HSD test ($P > 0.05$). There was no effect of years since release on the average percentage of infestation ($F = 2.607$; df = 3,25; $P = 0.074$) (Table 2).

Of the sites analyzed with the exponential decay model, a significant increase in percentage of infested capitula was observed at the release point at Sites 3, 4, and 15 whereas an increase was not observed at Sites 31 and 16 ($P < 0.05$; Table 3). The decay in weevil abundance (*B*) at Sites 3 and 15 were lower between years ($P < 0.05$; Table 3). *Larinus minutus* releases were made in 2009 for Site 31, whereas releases in Sites 3, 4, 15, and 16 were made in 2010. The percentages of infested capitula at the initial release point were about 5 to 15% based on the exponential decay model (Table 3) for 2010 release sites sampled in 2011. Infestation levels were higher for these sites from 2012 sampling 2 yr after release (about 38 to 48% at initial release point, Table 3). The exponential decay model explained between 3 and 72% of the variation in *L. minutus* abundance as a function of distance from release point across 2011 and 2012 (Fig. 1).

Discussion

The combination of timed visual searches and sampling of capitula used in this study was effective at confirming weevil establishment in the first few years following introduction to a novel habitat. Whereas capitula sampling alone failed to record *L. minutus* infestation at 6 sites, infestation was confirmed for 3 of these sites during the timed visual search portion of sampling. This result may be due to either a clumped distribution of *L. minutus* such that 100 capitula per quadrat represented an inadequate sample size to capture establishment, a low level of weevil abundance throughout the site, and/or a reduced knapweed density. Regardless, the large number of sites at which *L. minutus* infestation was recorded supports the conclusions of prior research reporting successful weevil establishment at the vast majority of the release sites (Minteer 2012).

The transect sampling method was also effective in describing the colonization and spread patterns of *L. minutus* on a small scale (<2 km). Transects never extended beyond about 1 km from the release point in both sampling years, and most terminated within the first 300 m

Table 2. The average maximum distance and average infestation (calculated from number of emergence holes observed in dissections) of *Larinus minutus* within 50 m of the release point at varying durations (yr) from release. Reductions in sample size between average maximum distance and average infestation are a result of some sites not having spotted knapweed within the first 50 m.

Years since *L. minutus* introduction	Average maximum distance (m) *L. minutus* recorded (mean ± SE)	Average infested capitula (%) in first 50 m (mean ± SE)
0	64.38 ± 37.49 ($n = 4$)	2.83 ± 0.83 ($n = 4$)
1	126.1 ± 25.53 ($n = 13$)	5.27 ± 1.28 ($n = 11$)
2	225.5 ± 53.41 ($n = 13$)	20.76 ± 5.80 ($n = 12$)
3	205.7 ± 47.72 ($n = 4$)	19.75 ± 1.79 ($n = 2$)

Table 3. Percentage (mean ± SE) of infested capitula (*A*) and percentage (mean ± SE) of spread rate (*B*) of *L. minutus* populations on spotted knapweed from the release point at each of 5 release sites, as estimated by the exponential decay function, . For a given parameter, means in the same row of the same variable followed by the same letter are not significantly different as determined by the lack of overlap of 95% confidence intervals (i.e., $P > 0.05$). Initial release was made in 2010 for sites 3, 4, 15, and 16, and in 2009 for site 31.

	A		*B*	
Site number	2011–2012	2012–2013	2011–2012	2012–2013
3	15.33 ± 2.48 a	38.58 ± 4.69 b	$1.6 \times 10^{-2} \pm 3.9 \times 10^{-3}$ a	$9.6 \times 10^{-4} \pm 1.4 \times 10^{-3}$ b
4	5.57 ± 2.08 a	44.40 ± 9.85 b	$4.8 \times 10^{-3} \pm 4.9 \times 10^{-3}$ a	$5.6 \times 10^{-3} \pm 2.0 \times 10^{-3}$ a
15	11.81 ± 1.40 a	39.74 ± 6.20 b	$3.1 \times 10^{-2} \pm 4.4 \times 10^{-3}$ a	$1.2 \times 10^{-2} \pm 3.0 \times 10^{-3}$ b
16	14.41 ± 3.78 a	47.83 ± 20.83 a	$1.6 \times 10^{-2} \pm 6.9 \times 10^{-3}$ a	$3.0 \times 10^{-2} \pm 1.4 \times 10^{-2}$ a
31	30.09 ± 4.68 a	24.34 ± 5.52 a	$2.3 \times 10^{-2} \pm 7.1 \times 10^{-3}$ a	$4.0 \times 10^{-3} \pm 3.0 \times 10^{-3}$ a

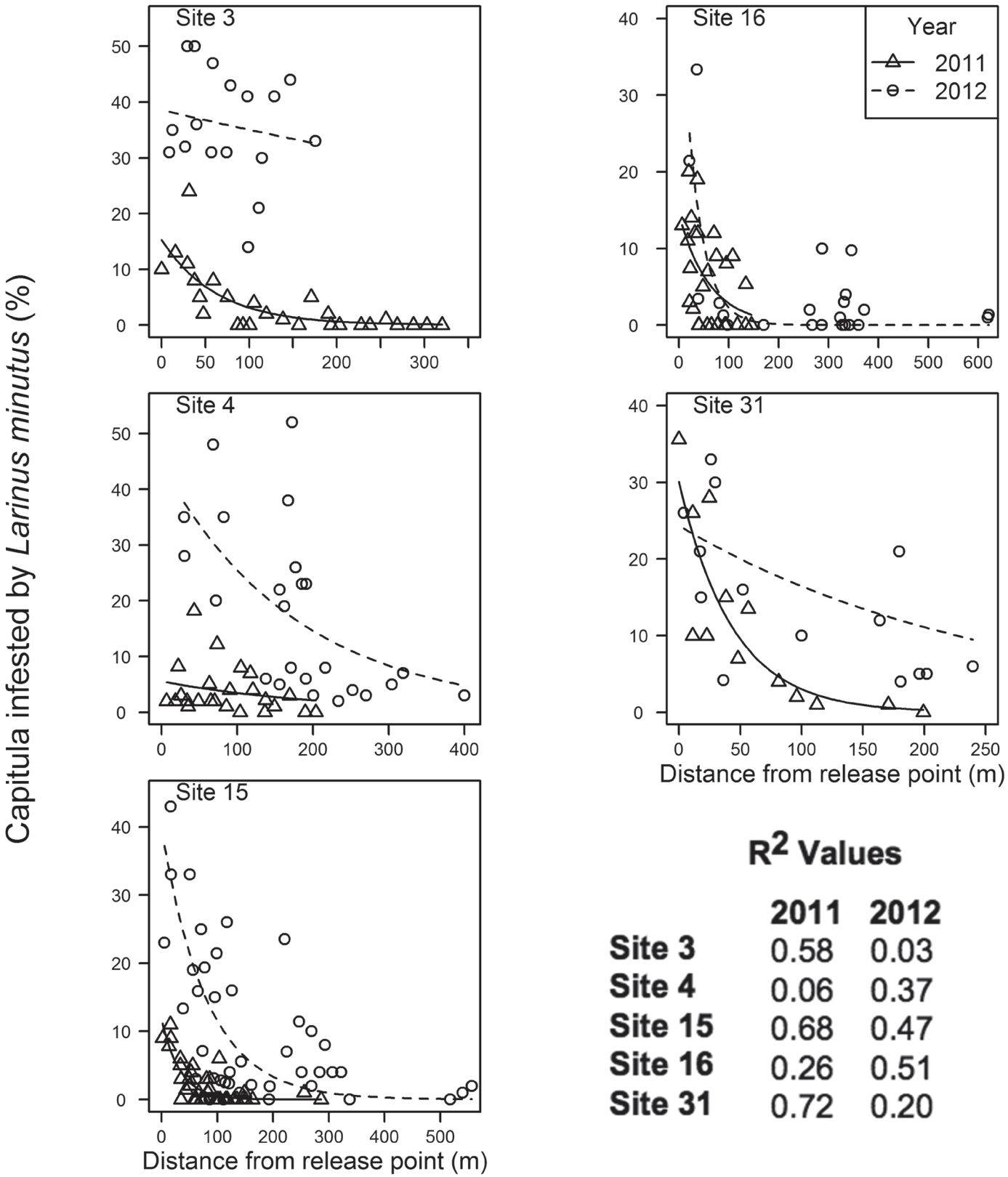

Fig. 1. Percentage of infested capitula (*A*) and spread rate (*B*) of *Larinus minutus* populations on spotted knapweed from the release point at each of 5 release sites in northwest Arkansas as modeled by the exponential decay function: $y = Ae^{-Bx}$

as sites were bounded by the patchy distribution of knapweed, natural boundaries impeding knapweed invasion (e.g., woodlots), and anthropogenic features of the landscape (e.g., posted private property, freeways, roadside mowing). Consistent increases in both the average maximum *L. minutus* infestation distance and percentage of infested capitula within the area of release were found for the first 2 yr following weevil introduction. Both factors imply yearly population growth and spread following introduction to a site. This pattern did not hold

for either variable for sites sampled 3 yr post-introduction; however, this is likely due to the low number of knapweed patches available for sampling at these sites. Furthermore, 2012 sampling generally established sampling quadrats at further distances than those sampled in 2011. This is a result of changing the criteria used in deciding a termination point for a given transect after observations made in 2011 suggested abundance of *L. minutus* could occur in a non-continuous manner. Changing the transect termination point is unlikely to impact the average infestation within the area of release estimated for both sampling years as transects generally continued beyond 50 m when possible; however, it may underestimate the maximum infestation distance for release sites sampled in the year of *L. minutus* introduction.

The yearly increase of infestation within the area of release and maximum infestation distance is a conclusion supported by analysis of sites with the exponential decay model. Of the 5 sites for which spread was modeled, localized population increase of *L. minutus* was evident at 3 sites, and at 2 of these sites spread was also observed (Table 3). Of these 3 sites, spotted knapweed was the most dominant at Site 3 for both sampling years, with a consistently high level of knapweed coverage. Mowing from nearby businesses contained knapweed in this site to an absolute distance of about 180 m from the release point in 2012. The absolute value of *B* at Site 3 was significantly smaller in 2012 than in 2011, indicating that *L. minutus* had spread across the extent of the knapweed at this site (Table 3).

Although not as consistently dominant as in Site 3, knapweed levels at both Sites 4 and 15 were robust in both years with reduced competition from other plants. This dominance also likely led to the significant increases in *L. minutus* density observed at the release point at both sites (Fig. 1) through provision of ample food sources and reproduction sites. Although sampling in 2011 extended only to about 200 m at Site 4, there was a marked increase in infestation levels at the same distance in 2012, suggesting successful spread of the weevil from the release site. There was a similar pattern at Site 15, in which a clear increase in weevil infestation was recorded at about 300 m, the edge of that site's sampling in 2011 (Fig. 1). These data indicate an outward expansion of *L. minutus* from the release point after population growth occurred.

Diffusion models like the exponential decay function used in this study are valuable tools for taking a first look at the spread of an invading organism (Andow et al. 1990), and our findings support the utility of the approach. The relatively high R^2 values calculated from the exponential fit of Sites 3, 15, and 31 in 2011 and Sites 15 and 16 in 2012 suggested that simplistic forms of a model may suffice for initial analysis, but it is evident that exponential decay alone is inadequate in describing *L. minutus* movement. The influence of knapweed density on infestation rates warrants further investigation. Specifically, whether *L. minutus* density is positively or negatively influenced by knapweed density (i.e., resource concentration vs. resource dilution, *sensu* Stephens & Meyers 2012) needs verification. This information can be used for predictive purposes and to refine protocols that implement knapweed biological control programs by providing descriptions of how the *L. minutus* invasion process progresses in the first few years following release.

Our study suggests that in the years following introduction of *L. minutus* to a release site, consistent increases in infestation and spread can be expected. The large number of release sites in which *L. minutus* was recovered suggests that *L. minutus* was likely present at all release sites, although sometimes at non-detectable levels with the sampling regime used in this study. Capitula infestations up to about 20% may be expected within the area of release 2 yr post-introduction given an average release of about 750 *L. minutus* individuals. Furthermore, *L. minutus* could be expected to spread at least about 225 m from the release point 2 yr post-release based on our results. Distances between release points could be increased if the near-term management is not a priority, such as in a roadside setting that receives regular mowing. Alternatively, if spotted knapweed infestation is causing an economic loss in a confined area, such as in a pasture setting, increases in both the number of weevil release sites and their proximity to each other may be an appropriate approach for near-term management.

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