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Research Article

Rate of dispersal of spotted knapweed biocontrol beetles (*Larinus* spp. Curculionidae) in Wisconsin

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ABSTRACT: Eurasian seed-head weevils, *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), were released annually from 2004-2013 across Wisconsin for biological control of the invasive forb spotted knapweed (*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek). In 2014, *Larinus* spp. presence/absence surveys were conducted at 18 previous releases sites between 7/18/2014 – 8/16/2014 from 2007 (n=3), 2008 (n=3), 2009 (n=3), 2010 (n=6), 2011 (n=1) and 2012 (n=2). We found a significant, linear relationship between time since release and the natural log of the observed dispersal rates of *Larinus* spp. ($F_{1,10}$ = 18.8, P = 0.002, R^2 = 0.65), suggesting an increasing dispersal rate through time. Modeled result suggested the following relationship: LN (Dispersal distance in km) = 0.258*time since release + 0.741. Because the model intercept (i.e. dispersal rate) did not pass through zero at age zero, which was expected from a biological basis, I warn against extrapolating the modeled dispersal rate to the period from release (t=0) to the end of Year One. Applying these dispersal rates to all 326 previous *Larinus* spp. releases in Wisconsin we predict that by 2017 low level populations of *Larinus* spp could be found state-wide.

KEY WORDS: Biological control, dispersal, *Larinus minutus, Larinus obtusus*, spotted knapweed

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INTRODUCTION

Spotted knapweed (*Centaurea stoebe* L. spp. *micranthos* (Guler) Hay/*synonym C. biebersteinii* L. formerly *C. maculosa* Lam.) is a Eurasian plant that has spread across most of North America since the first report in 1893 (Groh, 1944). With few natural enemies in North America, and being undesirable to livestock, the abundance of this invasive forb quickly increases in fallow grassland systems, often forming monocultures (Wilson and Randall, 2005). Centaurea dominance has been associated with increased erosion (Jacobs and Sheley, 1998; Lacey *et al.,* 1989; Lutgen and Rillig, 2004), the loss of desirable flora (Tyser and Key, 1988), and reduced forage for wildlife (Thompson, 1996). Spotted knapweed populations in the state of Wisconsin have been reported in all but three counties (WDNR, 2015).

Spotted knapweed biological control efforts in Wisconsin have included the introduction of a total of 6 Eurasian insect species (WDA, 2013), including two seed-head flies, *Urophora affinis* Frauenfeld and *U. quadrifasicata* (Meigen) (Diptera: Tephritidae), a root mining moth, *Agapeta zoegana* (Lepidoptera:Cochylidae) introduced in 1991 by United States of Department of Agriculture (USDA), a root mining weevil, *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) which was introduced by the United States Army at Fort McCoy, Wisconsin in 2002 and two root mining weevils, *L. minutus* (Coleoptera: Curculionidae) and *L. obtusus* (Coleoptera: Curculionidae), which were introduced by the United States Army at Fort McCoy, Monroe County, Wisconsin in 2002 (WDA, 2013). These releases were supported by releases in 2004 by the Wisconsin Department of Natural Resources (WDNR) of *C. achates, L. minutus* and *L. obtusus* in Burnett, Waushara and Adams County (WDA, 2013). Since then (2004 – 2013), an additional 329 releases of *L. minutus* and *L. obtusus* have been made to a total 38 of the 72 counties in Wisconsin by the WDNR (WDA, 2013).

Larinus minutus and *L. obtusus* are Eurasian seed-head weevils introduced into North America for biological control of knapweed plants. The biology, host specificity, and potential impact of *L. minutus* were described by Groppe *et al.* (1990), Jordan (1995), and Kashefi and Sobhian (1998), and *L. obtusus* biology was described by Groppe (1992). Evidence suggests that these two species may actually be variants of a single species, making it extremely difficult to distinguish between adults of *L. obtusus* and *L. minutus* when they coexist in the field (Story and Coombs, 2004).

The largest impact of *L. minutus* and *L. obtusus* on spotted knapweed comes from larval feeding on pappus hair and developing seeds (Wilson and Randall, 2005). This feeding reduces total number of viable seeds produced, and in turn reduces the seed bank surrounding spotted knapweed populations (Story, 2008). Since each species have similar impacts on spotted knapweed (Wilson and Randall, 2005), and are difficult to distinguish, spatial observation data do not discriminat between the two species. Adults emerge in late July and early August through holes chewed in the tops of the pupal chambers and vigorously feed on foliage before moving to overwintering sites in the soil. Adult *Larinus* spp. are 4-5 mm long and females lay one to five eggs per spotted knapweed flowerhead, and up to 130 eggs per season (Groppe, 1992). These *Larinus* spp. overwinter as adults in the leaf litter around host plants and emerge the following spring (Wilson and Randall, 2005). Occasionally, adults may hibernate a second time and live for a second season (Groppe, 1992).

There are few reported studies on the dispersal abilities of *L. minutus* and *L. obtusus*. A field study done by Groppe *et al.* (1990) found evidence that *L. minutus* undertakes dispersal flights. A recent publication in Michigan showed an average dispersal rate of 1 km/year for *L. minutus,* and 3.4 km/year for *L. obtusus* on sites 6 years after initial release, and 8.5 km/year for *L. minutus* 17 years after release (Carson and Landis, 2014). In the Nelson Forest Region in British Columbia, Canada in 2001, *L. obtusus* dispersal was estimated at 1.5 km/year 8 years after initial release (Ministry of Forests, 2002). In Arkansas, USA Alford (2013) reported the spread of *L. minutus* was 1.1 km/yr after 2 and 3 years from initial release. Factors such as knapweed distribution and density, *Larinus* spp. population, or sampling protocols may affect discrepancies in reported dispersal rates of *Larinus* spp. (McPeek, 1992).

Collection, rearing, and distribution of *Larinus* spp. for field release is labor intensive and expensive (WDA, 2013). More information on the dispersal rate of *Larinus* spp. would offer an estimation of time and resources needed to devote to future release efforts. The objective of this research was to determine the average dispersal rate for *L.* spp. in Wisconsin in order to predict current and future potential distributions. Since 2002 a large number of *Larinus* spp. release sites have been made throughout Wisconsin (n=326), so to achieve maximum potential distance surveyed, in this study we sampled only isolated or perimeter release sites (n=18). Sites were sampled in a linear manner in the direction leading away from previous release sites.

MATERIALS AND METHODS

Eighteen study sites were chosen from 326 previous release locations of *Larinus* spp. in Wisconsin (WDA, 2013) (Fig 1). Distribution grouping of current release locations, known concentrations of spotted knapweed populations, and time constraints limited the number of survey areas to 18, indicated on figure 1 by the yellow stars. Maximizing potential survey distance was accomplished by using the spatial locations of the previous *Larinus* spp. releases and creating a multiple ring buffer in ArcGIS™10.3.1 (ESRI; Redlands, CA) in increments of 0.8 km, out to 100 km. Study sites with low buffer overlap from surrounding previous releases were then chosen. Survey directions were created to lead toward areas of little to no overlap from surrounding releases in order to maximize potential survey transect distance. From these 18 sampling areas a total of 113 field samples were taken during September & October, 2014 (Table 1).

To determine the rate of spread of *Larinus* spp., surveys were conducted along roadsides intersecting predetermined survey transects (described above) that were infested with spotted knapweed. The sampling was conducted in September and October to capture both newly emerged and previous year adult *Larinus* spp. on spotted knapweed (Wilson and Randall, 2005), which is still in bloom in Wisconsin at that time. Also during this time period in Wisconsin, the presence of exit holes from emerging *Larinus* spp. are still visible on spotted knapweed plants and can be used to aid in detection (Wilson and Randall, 2005) (Fig. 2).

Previous work on *L. obtusus* estimated an annual dispersal rate of 1.5 km/year (Ministry of Forests, 2002). For efficiency of sampling, I used a 1.5 km/year buffer from selected release locations as a starting point for all treatment searches. Sampling took place on the edge of that radius and continued outward until no *Larinus* spp. were found at 3 consecutive stops moving in increments of approximately 0.8 km, or until no spotted knapweed could be found within 20 km of previous positive location. If no *Larinus* spp. were found at the edge of the starting 1.5 km/year buffer, sampling continued toward the original release location until *Larinus* spp. were detected. Also on some of the transects a maximum extent of sampling could be reached if *Larinus* spp. were continually found, but any further linear sampling distance would bring the transect nearer to a different release site.

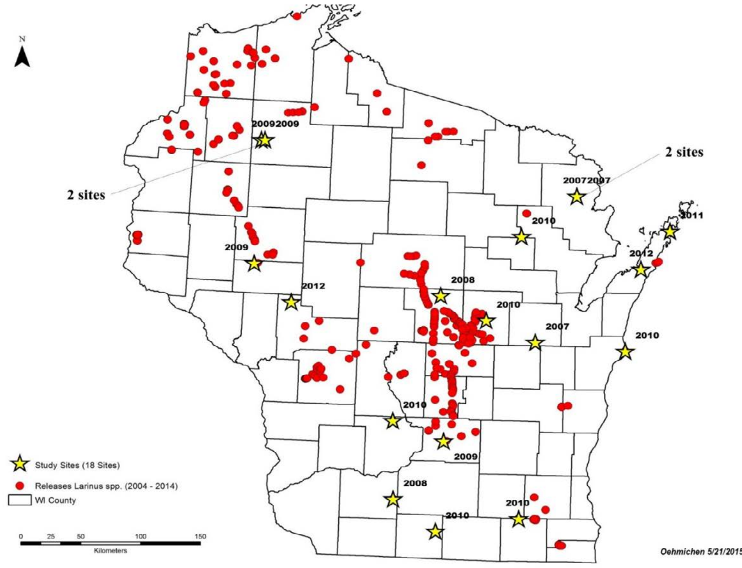

Fig. 1. The 326 previous *Larinus* spp. field releases (2004 – 2014) designated with red round icons and the 18 survey sites designated with yellow star icons.

Table 1. The summary of the 113 field presence/absence survey samples of *Larinus* spp. (Sept – Oct, 2014). Columns are as follows; Site – recorded name of release site from WDNR release records, Site # - labeled site number, Year of release – release year of *L.* spp., # of *L.* – number of adult *L.* spp. released, County Surveyed – Sampling along transect intersected within these counties, # Sample Points – number of samples taken along the transect until termination of survey occurred, Termination of Sampling – ending of survey along transect 1) >20 km – no spotted knapweed could be located within 20 km of previous sample location linear along transect, 2) 3 negative - 3 consecutive negative samples for *Larinus* spp., 3) maximum – maximum extent of transect reached because any positive locations further would be closer to other releases than survey site, Method – method used for detection of *L.* spp. 1) visual – inspection of blooming spotted knapweed head, 2) net – sweep netting of spotted knapweed and inspection of contents, Distance (km) – linear distance from release site to last found Larinus spp. on transect in kilometers, Annual Avg Distance (km) – distance (km) / year since release in kilometers.

Site	Site No.	Year of Release	No. of *Larinus* spp.	Country Surveyed	Sample No. Point	Termination of Sampling	Method	*L.* spp. Distance Detected	
								Distance (km)	Annual Avg. Distance (km)
Hemlock	9n	2007	400	Marinette, Oconto	3	maximum	visual	62.2	8.9
Nikolai_test	9s	2007	300	Outagamine, Brown, Oconto	2	maximum	visual	68.5	9.8
Amberg	10e	2007	550	Marinette, Florence, Michigan	27	3 negative	visual, net	103.7	14.8
Powell	1	2008	250	lowa, Grant, Richland	10	> 20 km	visual, net	60.4	10.1
RRFA	4	2008	250	Columbia, Dane, Waushara, Green Lake	7	> 20 km	visual	57.5	9.6
Oison	13	2008	250	Portage, Shawano	4	> 20 km	visual	51.9	8.7
Woodford 1	15	2009	500	Eau Claire, Buffalo	2	> 20 km	visual	34.1	6.8
Amstrong	16	2009	500	Sawyer, Rusk	6	3 negative	visual, net	51.2	10.2
Amstrong 2	17	2009	500	Sawyer, Price	3	> 20 km	visual, net	55.5	11.1
B Town	2	2010	300	Green	6	3 negative	visual, net	22.6	5.6
Young Rd	3n	2010	300	Walworth, Jefferson	9	> 20 km	visual, net	16.5	4.1
Rotharator	5	2010	400	Sauk	4	> 20 km	visual	17.6	4.4
Guy	6	2010	300	Manitowoc, Sheboygan, Kewaunee	8	> 20 km	visual, net	18.4	4.6
Old W	11	2010	300	Oconto, Menomiee, Shawano	4	maximum	visual	35.2	8.8
Hlaban	12	2010	300	Waupaca, Shawano	3	maximum	visual	35.8	9.0
DOT 2012	14n	2012	300	Jackson, Clark	5	> 20 km	visual	10.1	5.1

Fig. 2. Pupal chambers left in heads of spotted knapweed by *Larinus* spp. Used as a visual evidence of recent occupancy by *Larinus* spp. larva. One of the observation methods used to determine presences of *Larinus* spp.

At each sample point, the presence/absence *Larinus* spp. was visual determined by observation of adults on flowering knapweed plants, or using a 15" sweet net to capture and identify adults. Absence was recorded if no *Larinus* spp. individuals were found after 10 a minute search per stop, and after searching at 3 consecutive stops, spaced at a minimum distance of 0.8 km increments, from the previous positive *Larinus* spp. location, or if no spotted knapweed could be located within 20 km of previous positive location, or after the maximum distance for a site was achieved. Coordinates of all sampled locations and outlines of transects were imported into the GPS program Oruxmap© on an Android Galaxy S4 (version 4.4 Mountain View, CA); this information was also used to determine survey direction and incremental movement between samples.

The relationship between time since release and observed dispersal rate was evaluated in PROC REG (SAS 9.3 Cary, NC). As noted previously (Table 2) our data suggested an exponential relationship between time since release and dispersal rate, so all data was natural log transformed prior to analysis.

Table 2. The annual dispersal rate of *Larinus* spp. for the 12 sites where positive Larinus spp. were found and maximum survey extent was not reached as the termination method for survey.

Release year (n)	Years post release	Average dispresal rate (km/year since release)
2012 (1)	2	5.1
2010 (4)	4	6.1
2009 (3)	5	9.4
2008 (3)	6	9.5
2007 (1)	7	11.2

RESULTS AND DISCUSSION

In 2014, a total of 113 samples were taken between the months of September and October. *Larinus* spp. were detected within 16 of the 18 original release locations. The only 2 sites that yielded no *Larinus* spp. presence, were both found in Door County, WI and were releases made in 2011 and 2012. Both these sites had high initial release numbers (400 and 500 *Larinus* spp.) relative to other study sites, so the absence of Larinus spp. did not appear correlated with release density.

There were three 2007 release sites, two of which recorded positive *Larinus* spp. presence past the maximum survey extent, which limited the distance possible to be recorded without approaching previous releases. These two transects were excluded from the dispersal model, as sampling was stopped at 68.5 km and 62.2 km. The one 2007 site that was stopped by 3 negative observations recorded a total of 103.7 km for an annual dispersal rate of 14.8 km/yr (Table 2). For the three 2008 release sites, *Larinus* spp. dispersed a minimum of 51.9 km, and a maximum of 60.4 km (Table 2). The rate of annual dispersal across all 2008 release sites was 9.5 km/yr (Table 2). For the three 2009 releases, *Larinus* spp. dispersed a minimum of 34.1 km, with a maximum of 55.5 km (Table 2). The rate of annual dispersal for all 2009 releases was 9.4 km/yr (Table 2). For two of the six 2010 release sites, two of the transects recorded positive *Larinus* spp. past the maximum survey extent, so sampling was stopped at 35.2 km and 35.8 km. At the four remaining 2010 release sites, *Larinus* spp. dispersed a minimum of 10.1 km, and a maximum of 22.6 km (Table 2). The average rate of dispersal of the four 2010 releases was of 6.1 km/yr (Table 2). At the one 2012 release site, we detected *Larinus* spp. 10.2 km (Table 1) from original release location, with an annual dispersal rate of 5.1 km/yr (Table 2).

I found a significant, linear relationship between time since release and the natural log of the observed dispersal rates of *Larinus* spp. ($F_{1,10}$ = 18.8, P = 0.002, R^2 = 0.65; Fig. 3), suggesting an increasing dispersal rate through time (Table 2). Modeled result suggested the following relationship: LN (Dispersal distance) = 0.258*time since release + 0.741. Because the model intercept (i.e. dispersal rate) did not pass through zero at time zero (i.e. the time of release), which was expected from a biological basis, I warn against extrapolating the modeled dispersal rate to the period from release (t=0) to the end of Year Two.

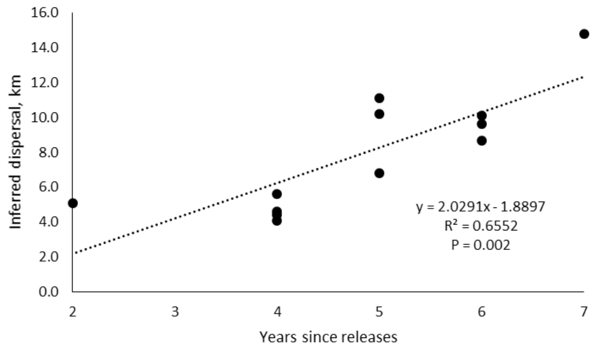

Fig. 3. The dispersal rate of *Larinus* spp. by years since released, based on data collected in 2014 from spotted knapweed biological control releases made in 2007 (n=1), 2008 (n=3), 2009 (n=3), 2010 (n=4) and 2012 (n=1).

Our results suggest an increasing dispersal rate of *Larinus* spp. through time (Table 2 and Fig. 3) and our average annual dispersal rate consistently increased with time. Similarly, Carson and Landis (2014) also found a pattern of increasing *Larinus* spp. dispersal rate as time since release increased; although our observed dispersal rates are higher than those reported in that study and others.

Carson and Landis (2014) observed at a site 6 years post release that *Larinus minutus* moved on average 1.0 km/yr (n=1) and *L. obtusus* moved 3.5 km/yr (n=1). Our results showed an average annual dispersal of 9.5 km/yr (n=3) for *Larinus* spp. 6 years post release (Table 2). Carson and Landis (2014) did acknowledge that low-density populations of *Larinus* spp. probably existed beyond their findings. Van Hezewijk and Bourchier (2011) reported a dispersal rate average of 1.9 km/yr after 5 years from initial release of *Larinus minutus* in Alberta, Canada, our results showed an annual dispersal of 9.4 km/yr (n=3) for *Larinus* spp. 5 years post release (Table 2). In the Nelson Forest Region of British Columbia, Canada in 2001, *Larinus obtusus* dispersal was estimated at 1.5 km/year 8 years after initial release (Ministry of Forests, 2002). In Arkansas, USA Alford (2013) reported the spread of *L. minutus* was 112.5 m/yr after 2 and 3 years from initial release; our study only had one 2 year old post release site, which we found had an average annual dispersal rate of 5.1 km/yr (n=1).

The differences in our observed rates of *Larinus* spp. dispersal and those reported in other published studies could be from a number of factors. First, differences could result from differences in survey methods. Carson and Landis (2014) sampled in all 4 cardinal directions in set intervals of 100 m, 500 m, and 1000 m, and if no *Larinus* spp. were found at those intervals sampling was discontinued, but if they were detected then sampling occurred at 3000 m, then 8 km, and then 16 km. Carson and Landis (2014) acknowledge that their estimated dispersal rates were conservative. Our method was to gradually leave the dense population of *Larinus* spp. and to detect small ranging populations by setting small intervals between sample distances (approximately 0.8 km), and only ending the survey after 3 consecutive negative samples, or only after no spotted knapweed could be located within 20 km from the previous positive *Larinus* spp. location.

A second factor that might have contributed to greater estimated dispersal rates in this study could be the density of the *Larinus* spp. population at the time of survey. As *Larinus* spp. populations increase locally, the greater the potential ranging distance needed for an average individual within the population to find an unoccupied spotted knapweed plant (Kim and Sappington, 2013). In Wisconsin over 120,000 *Larinus* spp. individuals have been released or moved to a total of 326 field insectaries across 39 of the 72 counties (WDNR 2015). This is considerably more than in Michigan, which only has 7 field releases across 7 of the 83 counties (Carson and Landis, 2014), and in Arkansas where 29,000 *Larinus minutus* had been released across 40 sites in only 6 of the 75 counties that Alford (2013) investigated. The Nelson Forest Region in British Columbia, Canada is approximately 24.7 million acres (Ministry of Forestry, 2015), roughly the size of Indiana, USA (Gorte *et al*., 2012). Between 1993 -2001 a total of 150 releases totaling 76,600 *Larinus obtusus* were made. Their dispersal survey for *L. obtusus* was done in 2001; if releases done in 2001 are excluded, because it was not clarified if these releases were done before or after the survey had taken place that year, the total of released *Larinus obtusus* would be only 46 releases of a total of 42,040 *L. obtusus*. This is a larger distribution and population than in Michigan and Arkansas, but much smaller that the *Larinus* spp. population and distribution in Wisconsin at the time of survey.

A third factor could be the differences in the density and distribution of host plants available for *Larinus* spp. in release areas. The wider distribution of the host plant, the greater the potential ranging distance for individuals within the population to find unoccupied host plants (Kim and Sappington 2013). In Wisconsin, spotted knapweed was first reported in the early 1900's (Fassett 1927), and has since been reported in 69 of the 72 counties (WDNR 2015) with the potential of spread to over 24.2 million acres (Fig. 2). In Arkansas, the first reported spotted knapweed plant was in the mid 1940's and in 2007 only 20 of the 75 counties had reported spotted knapweed populations; those occurring almost exclusively in the northwest counties of Arkansas (Kring *et al.,* 2012). In British Columbia, over 100,000 acres are knapweed infested with populations, predominantly found in the southern and central areas, with a potential for spread to 2.7 million acres. These populations include both spotted and diffuse knapweed, with diffuse knapweed being the more common of the two varieties (Ministry of Agriculture, 2015). Michigan spotted knapweed population distribution is very similar to Wisconsin having populations reported in 74 or the 83 counties (USDA, 2015), but *Larinus* spp. have only been released in 7 of those counties (Carson and Landis, 2014). This wide distribution of spotted knapweed, but low *Larinus* spp., population might explain the lower reported dispersal rate by Carson and Landis, (2014) than our study in Wisconsin, because of the close proximity of large areas of unoccupied host plants. This also might explain why Carson and Landis (2014) observed a smaller *Larinus* spp. dispersal rate than Alford's (2013) study in Arkansas, and the Nelson Forestry Region study in British Columbia, both of which had larger *Larinus* spp. releases and smaller knapweed distribution and why our study in Wisconsin observed a larger *Larinus* spp. dispersal rate than all of the studies due to the a larger *Larinus* spp. population and a wide distribution of knapweed.

Suppression of spotted knapweed populations will not come from one method of control, such as *Larinus* spp. releases, but from a wide scale, integrated approach with biological and chemical control efforts (Muller-Scharer, 1991). Having multiple species of spotted knapweed biological control agents has been shown to increase control of knapweed (Story *et al*., 1999, Story *et al.,* 2006). To display the spatial extent of the potential distribution of *Larinus* spp. in Wisconsin the calculated average annual dispersal rate (Fig. 3) was applied to all existing 326 *Larinus* spp. releases as a buffer using ESRI ArcGIS™10.3.1 ESRI (Redlands, CA). The calculated average annual dispersal rate (Fig. 3) only included sites 2 – 7 years old, and since our data can not predict outside of this timescale, sites older than 7 years were conservatively, also given the 7 year dispersal rate. This map (Fig. 4) shows the potential distribution of *Larinus* spp. for the year 2014, covering approximatly 96.3% of the potential spotted knapweed distriubtion. If no releases of *Larinus* spp. were made between 2014 to 2017 using the same calculated annual dispersal rates, and using the 7 year dispersal rate for all sites over 7 years old or older, the *Larinus* spp. potential distribution would be 100% of Wisconsin.

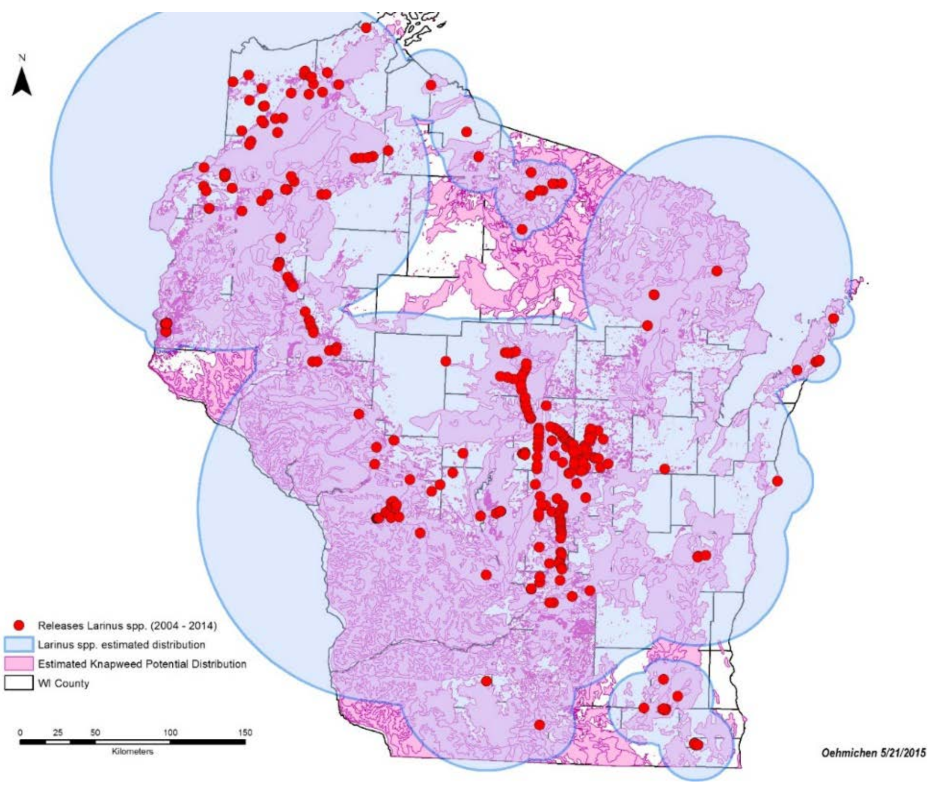

Fig. 4. The potential distribution of *Larinus* spp. in Wisconsin based on calculated dispersal rate applied to all 326 *Larinus* spp. releases with spotted knapweed potential distribution. The dispersal rate of *Larinus* spp. based on data collected in 2014 from spotted knapweed biological control releases made in 2007 (n=1), 2008 (n=3), 2009 (n=3), 2010 (n=4) and 2012 (n=1). *L.* spp. potential distribution covers 96.3% of the potential spotted knapweed distribution. Sites older than 7 years old received the buffer rate of 7 year sites. Data was mapped using ESRI ArcGIS™10.3.1 ESRI (Redlands, CA).

This study adds *Larinus* spp. to the list of knapweed biocontrol agents that are likely already state-wide in their distribution, including *Urophora affinis* and *U. quadrifasicata* (WDA 2013). The scale of distribution of *Larinus* spp. eliminates the need to further collect and redistribute *Larinus* spp. within the state, allowing future efforts to focus on expanding populations of two other knapweed biological control agents, *Cyhpocleonus achates* (root-mining weevil) and *Agapeta zoegana* (root-mining moth), both of which currently have limited distribution within the state, but have been shown to be effective agents in controlling knapweed (Story *et al.,* 1999, Story *et al.,* 2006).

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