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Monitoring a Gall Midge Population on Russian Knapweed (*Acroptilon repens*)

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This paper describes postrelease monitoring of a population of *Jaapiella ivannikovi*, a gall-forming midge that was introduced for biological control of Russian knapweed. In 2011 to 2013, from late May to early June through August, we monitored 100 permanent plots at one of the first release sites of *J. ivannikovi* in central Wyoming. Based on the phenology of gall formation, an appropriate window for collection of galls to distribute to new sites is from early to mid-June through early August. Although *J. ivannikovi* established successfully, 4 yr after release, the percentage of ramets that were galled remained low (1 to 2%), indicating that *J. ivannikovi* is not yet having a significant effect on Russian knapweed at the site.

Nomenclature: Russian knapweed, *Acroptilon repens* (L.) DC., ACRE3.

Key words: Biological control, phenology, population dynamics.

After an agent has been permitted and released, the focus of weed biological control research shifts from host specificity testing to postrelease studies in the introduced range. Postrelease monitoring of the agent's population dynamics within a season (phenology) can yield information that is useful to distribution efforts. Knowing the timing of the first and peak occurrence of agents can inform land managers about the best time to collect insects for distribution (Hansen 2004a; Skinner et al. 2006).

Postrelease monitoring of population dynamics among seasons or years can provide information about whether a biological control program is successful. First, successful weed biological control requires establishment of the agent. Second, densities of the agent and the frequency of attack of target plants or plant parts should increase following release. Although an increase in agent density and frequency of attack does not guarantee an effect on weed density (McClay 1992), consistently low densities and limited rates of attack are likely to be associated with minimal effect. Ultimately, successful biological control should be characterized by a reduction in weed density, biomass, or both (McEvoy et al. 1991).

This paper describes postrelease monitoring of the population dynamics of the gall midge *Jaapiella ivannikovi* Fedotova, a relatively new biological control agent for Russian knapweed [*Acroptilon repens* (L.) DC]. Russian knapweed has been targeted for biological control because of its toxicity to horses (Young et al. 1970), propensity for negatively affecting desirable forage species (Grant et al. 2003; Mealor et al. 2004) and the short-term nature of herbicidal control (Benz et al. 1999; Bottoms and Whitson 1998; Laufenberg et al. 2005). The first biological control agent released against Russian knapweed was the gall-forming nematode *Subanguina picridis* (Kirjanova) Brzeski in the 1990s (Littlefield and Coombs 2004; Rosenthal and Piper 1995). *Subanguina picridis* has largely been viewed as a failure because of an apparent reliance on moist conditions, low incidence, and extremely slow spread (Littlefield and Coombs 2004).

Jaapiella ivannikovi was permitted and first released in 2009 from material originally collected in Uzbekistan. As of 2014, *J. ivannikovi* has been released at sites in 10 U.S. states: first in Montana and Wyoming and later in California, Colorado, Idaho, Nevada, New Mexico, Oregon, Utah, and Washington (R. Hansen, personal communication).

Adult female midges oviposit on the growing tips of Russian knapweed shoots, which leads to the formation of "rosette-type" galls (Djamankulova et al. 2008). Up to 15 galls per ramet have been observed in the native range (Djamankulova et al. 2008). In a field impact experiment conducted in Uzbekistan, galled ramets produced 92% fewer seeds and were 24% smaller than ungalled ramets

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Management Implications

A new biological control agent for Russian knapweed, the gall forming midge *Jaapiella ivannikovi* was permitted and first released in the U.S.A. in 2009. We addressed two questions of interest to weed managers utilizing *J. ivannikovi* for Russian knapweed management. What is the appropriate time to collect galls for release at new sites? Is *J. ivannikovi* having an impact on Russian knapweed four years after release? From 2011-2013, we monitored Russian knapweed ramets (annually) and *J. ivannikovi* galls (weekly). We also determined emergence times of adult midges by caging galls.

Gall formation occurred from late-May or early-June to mid-August and peaked in early July in 2011 and 2013; there was no clear peak in 2012. Adult midges emerged most frequently from galls between two and three weeks after a gall was first observed. Our results therefore suggest that an appropriate window for collection of galls for release at new sites is from early- to mid-June, about two weeks after galls first appear, through early-August.

Across the three years of monitoring, *J. ivannikovi* populations were relatively low. Russian knapweed ramet (main shoot) densities were relatively constant across years, and most (98%) Russian knapweed ramets escaped attack by *J. ivannikovi*. Our results suggest that *J. ivannikovi*'s impacts, if they do ultimately occur, are likely to take longer than the four years that *J. ivannikovi* has been present at our site.

(Djamankulova et al. 2008). Note that because *A. repens* is a clonal plant species, aboveground main shoots are considered "ramets."

We conducted 3 yr of monitoring (2011 to 2013) of a *J. ivannikovi* population at one of the first release sites in North America, a Russian knapweed infestation in central Wyoming. Ideally, we would have established additional monitoring sites. During 2010 to 2012, we released *J. ivannikovi* at five additional sites in Wyoming; however, none of these releases produced an established population.

Our first objective was to characterize within-season patterns in gall formation (i.e., when first- and peak-gall formation occurred). Second, we examined the time required for adult midges to emerge after a gall was first observed—useful information for collection and distribution efforts. Our third objective was to document among-season changes in the density of galls, the frequency of attack of knapweed ramets, and the density of Russian knapweed ramets. We sought to gain an indication of the initial impact of *J. ivannikovi*. Information about the timescale of agent population growth and effect on target weed densities is important for weed managers implementing biological control.

Materials and Methods

Study Site. The study site was located on the Wind River Indian Reservation, approximately 3 km northeast of the city of Riverton, in Fremont County, Wyoming. The elevation of the site is 1,490 m. The closest weather station (in Riverton) reports an average high of 29 C in summer and an average low of −14 C in winter (NOAA 2014a). In 2011, 2012, and 2013, annual precipitation in Riverton was 231, 92, and 271 mm, respectively (NOAA 2014b).

Dominant vegetation at the site consisted of Russian knapweed mixed with crested wheat grass [*Agropyron cristatum* (L.) Gaertn.] and smooth brome grass (*Bromus inermis* Leyss.). A wooded area containing cottonwood (*Populus deltoides* W. Bartram ex Marshall) and willow (*Salix amygdaloides* Andersson) occurred on the immediate northern edge of the site. Scattered individuals of big sagebrush (*Artemisia tridentata* Nutt.) and yellow rabbitbrush [*Chrysothamnus viscidiflorus* (Hook.) Nutt.] occurred to the south and east.

The site had not been treated with herbicides or grazed by livestock since 1990, although trespassing cattle have occasionally had access to the area. The area containing the monitoring plots has not been irrigated since 1990, although overflow from an adjacent agricultural field has occasionally flooded the wooded area ca. 20 m from the plots.

Jaapiella ivannikovi was released on May 19 and July 8, 2009. Approximately 40 galls were placed in the center of the site, with less than 10 additional galls being placed about 300 m to the west and 200 m northeast of the main release point. Galls were obtained from the Montana State University and were reared from material originally collected between the Fergana Valley and Samarkand, Uzbekistan (J. Littlefield, personal communication).

Monitoring Protocol. Forty permanent ¼-m^2 plots were established in June 2010 along two perpendicular transects, each about 100 m long. The east to west transect ran parallel to the edge of the area containing cottonwood and willow trees on the north side of the site. The two transects met at their midpoints at the location where the greatest number of galls was released in 2009. Eighty additional plots were established in May 2011. These new plots occurred in four additional east to west transects running parallel to and to the north of the 100-m east to west transect established the previous year. The new transects also were 100 m long and consisted of 20 plots. All transects were 10 m apart. Plots within a transect were 5 m apart and delineated by wooden or metal stakes in at least two corners.

Monitoring was conducted biweekly in 2010 and weekly in 2011 to 2013 over a 15-wk period from the third week of May until the end of August. In early June 2013, data were inadvertently not gathered from 20 plots in one of the rows, and many of these plots were damaged by an off-road vehicle later that summer. We therefore present only the weekly data for 2011 to 2013 from the 100 plots for which complete data is available and that remained "undisturbed."

On the first monitoring date of each year, the Russian knapweed ramets in each plot were counted. This was done in late May or early June, depending on when galls

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were first observed at the site as a whole. On each monitoring date thereafter, new galls were counted and their location on the plant was noted as "apical" (on the main shoot) or "lateral" (on a side shoot).

To investigate the timing and numbers of adult midges emerging per gall, we caged galls either on the day the gall was observed or 1 wk later. Galls that appeared to have been damaged, presumably from feeding by mice or birds, were not caged. Because of the large number of galls observed in 2013 ($n = 58$), only apical galls were caged. Both apical and lateral galls were caged in 2011 and 2012.

Each emergence cage consisted of a 270-ml waxed paper cup held in place over the gall with a partially cut, cylindrical foam plug, 76 mm diam and 48 mm tall, inserted into the open end of the cup and encircling the stem. Emergence cages had two 40 by 40-mm mesh areas ("No-see-um" Mesh, Rockywoods Outdoor Fabrics, Loveland, CO) for ventilation and to allow inspection for emerged adult midges. In 2012 and 2013, an additional 15 by 15-mm mesh area was added to the top of the cages to allow greater ventilation. On the first monitoring date that adult midges were observed inside a cage, the gall was collected, or if the midges were alive inside the cage, the cage was collected the following week. Galls were returned to the laboratory, where they were allowed to dry under ambient conditions for at least 1 mo. The number of adult midges that had emerged in the cage was counted and the gall was weighed.

Statistical Analyses. To evaluate seasonal patterns in gall formation, the 15-wk monitoring period was divided into three, 5-wk categories or classifications that maintained sufficiently large expected frequencies per classification for analysis (Zar 2010). Differences in gall mass between apical and lateral galls was evaluated using two-tailed t tests. Seasonal variation in average gall mass was analyzed using one-way ANOVA and using three seasonal categories. The relationship between gall mass and numbers of adults emerging per gall was analyzed using Spearman rank correlation. Finally, among-season changes in the numbers of knapweed ramets and total galls were analyzed using repeated measures ANOVA, where number per plot was square root–transformed (sqrt[$x + 3/8$]) (Zar 2010).

Results and Discussion

Time to Adult Emergence. Some caged galls failed to produce adult midges, possibly because midges emerged before the gall was caged or because the immature midges died within the gall. This phenomenon may have affected estimates of emergence times. In 2011, 40% (8 of 21 galls) produced no midges. However, all galls produced midges in both 2012 and 2013. Overall, galls produced adult midges between 0 and 5 wk after first observation of the gall (Figure 1). Fifty three percent of galls produced adult midges between 2 and 3 wk after a gall was observed. Thirty one percent of galls produced midges between 1 and 2 wk. The expected minimum time to emergence (SEM) was 1.9 (0.15) wk for all years combined and 1.4 (0.18), 2.6 (0.25), and 1.7 (0.13) wk for 2011, 2012, and 2013, respectively.

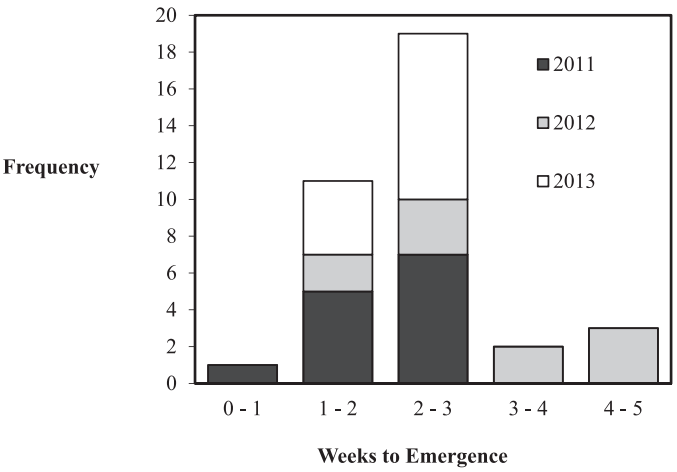

Figure 1. Frequency distribution for the time in weeks between the first observation of a gall and the emergence of adult midges from the gall; data are for caged galls that produced midges in 2011, 2012, and 2013.

Gall "Quality." Measures of gall quality did not appear to vary much seasonally. Formation of both apical and lateral galls occurred over the entire season in all years (Figure 2). In 2013, the only year with a sufficient number of galls for analysis, there was statistically significant heterogeneity in the formation of apical vs. lateral galls in early, mid-, and late season ($\chi^2 = 6.58$; df = 2, $P < 0.05$). This reflected disproportionately greater formation of apical galls in the first third of the season. Excluding early-season galls, heterogeneity in the proportion of apical vs. lateral galls was not statistically significant ($\chi^2 = 0.76$; df = 1, $P = 0.31$).

Data from 2011 and 2012 indicated that apical and lateral galls were similar in weight (two-tailed t test, 2011: $t = -1.2$; df = 19, $P = 0.25$; 2012: $t = 0.66$; df = 17, $P = 0.52$; mean (mg, SEM) for apical and lateral galls in 2011: 66.6 (10.4) vs. 93.2; 2012: 57.4 (8.46) vs. 48.0 (10.0). Gall weight was also not related to time of season; galls that formed early or mid-season vs. late season were of similar weight in all years (one-way ANOVA; 2011: $F = 0.32$; df = 2, 18, NS; 2012: $F = 1.59$; df = 2, 17, NS; 2013: $F = 5.71$; df = 2, 9, NS).

Adult emergence from galls was quite variable. Galls produced anywhere from 0 to 112 adult midges (mean, 11.9; SEM, 3.2). The number of midges emerging per gall was correlated with gall weight in 2011, but not in 2012 or 2013 (Spearman rank correlation; 2011: $r = 0.528$, $n = 21$, $P = 0.02$; 2012: $r = -0.0083$, $n = 9$, NS; 2013: $r = 0.389$, $n = 13$, NS). The source of the high variability

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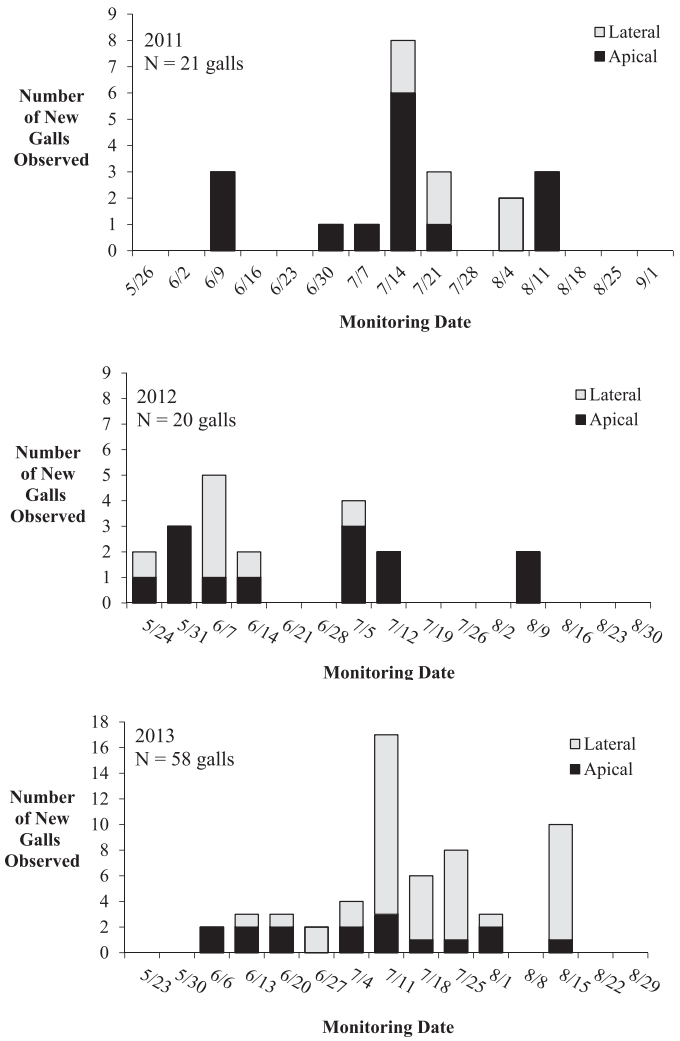

Figure 2. Within-season patterns in the appearance of apical and lateral *Jaapiella ivannikovi* galls on Russian knapweed in 2011, 2012, and 2013.

is unclear; however, one possibility is that caging the galls at variable times in their development might have affected the numbers of adults that emerged.

Phenology. The timing of gall formation was temporally heterogeneous within a year in all 3 yr (three classification goodness-of-fit test; 2011: $\chi^2 = 8.0$; df = 2, $P < 0.05$; 2012: $\chi^2 = 7.6$; df = 2, $P < 0.05$; 2013: $\chi^2 = 24.9$; df = 2, $P < 0.001$). The first galls of the season were observed from late May to early June (Figure 2). In 2011 and 2013, the formation of new galls appeared to peak in mid-July, but there was no clear peak in 2012. In all years, gall formation was reduced in late summer; no new galls were observed in the plots after August 15 in any year. However, newly formed galls were observed in low numbers outside of the plots in August and early September (K. Meyers, N. Pieropan, and T. Collier, personal observation).

On any given monitoring date, there frequently were "new" galls (0 to 7 d old), unemerged galls from the previous week (8 to 14 d old), unemerged galls from monitoring 2 weeks earlier (15 to 21 d old), and galls from which adults had recently emerged. This suggests that *J. ivannikovi* had multiple, somewhat overlapping generations at the site. Because galls usually took 2 to 3 wk to emerge, we estimate that three to six generations were produced during the 10- to 12-wk period that galls were observed. The presence of galls in September outside of the monitoring plots, albeit at reduced densities, suggests the possibility of an additional generation. In Uzbekistan, *J. ivannikovi* produces approximately four generations per year (Djamankulova et al. 2008), which is reasonably close to what we observed in Wyoming.

Implications for Distribution Efforts. Given that most galls produced adult midges between 2 and 3 wk after the gall was first observed, an appropriate time to start collecting galls for distribution to new sites would be the second or third week of June, about 2 wk after galls typically first appeared during the season. An alternative approach for determining when galls are ready to collect would be to dissect a few galls periodically starting in early June to determine when midges have pupated and galls are ready for collection. This is the approach recommended for another cecidomyiid biological control agent, *Spurgia esulae* Gagné (Hansen 2004b), which, like *J. ivannikovi*, has multiple generations per year. The latter approach has the advantage of accounting for among-year variation at the start of gall formation and emergence times, as we observed but which may be difficult for some weed managers. Our results further suggest that mid-August is the appropriate time during the season to stop collecting galls for distribution. Gall formation declined dramatically after mid-August in all 3 yr of monitoring. Adult midges emerging from galls collected later than mid-August would be unlikely to produce galls at new sites. Based on the data, an appropriate window of collection of *J. ivannikovi* for distribution is from early to mid-June, about 2 wk after galls first appear, through early August.

Although our results are based on *J. ivannikovi* phenology at a single site, we believe that the broad recommendations for the timing of collection efforts will be useful for other areas. The 7-wk window for collection that we propose should be most applicable to areas that have climatic conditions similar to those in central Wyoming—semiarid with cold winters and hot summers. In addition to the amount of precipitation, however, the timing of precipitation may be important. Our site receives most of its moisture, 50% or 107 mm, in the spring (March to June), with only 35 mm or 15% of rain falling on average in July and August. In areas with considerable monsoonal moisture, gall formation by *J. ivannikovi* is likely to extend later into the summer

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Table 1. Mean numbers per plot of Russian knapweed ramets, *Jaapiella ivannikovi* galls, and galled ramets as a function of monitoring year, with standard error of the mean (SEM; $n = 100$ plots).

Year	Ramets (SEM)	Galls (SEM)	Galled ramets (SEM)
2011	9.1 (0.49)	0.21 (0.050)	0.18 (0.041)
2012	10.5 (0.61)	0.20 (0.065)	0.14 (0.038)
2013	11.0 (0.66)	0.58 (0.217)	0.23 (0.057)

than we observed at our site. At release sites in western Colorado, for instance, which receive significant rainfall in July and August, *J. ivannikovi* produces galls in late August and well into September (D. Bean, personal communication). Weed managers implementing biological control at sites that receive late-summer rain should consider collecting and releasing *J. ivannikovi* later in the season than mid-August.

Among-Season Dynamics. The average number of Russian knapweed ramets was relatively constant across monitoring years (Table 1; repeated measures ANOVA, $F = 1.46$; df = 2, 198, P = 0.23), as was the average number of galled ramets per plot (Table 1; repeated measures ANOVA, $F = 1.34$; df = 2, 198, P = 0.26). The number of *J. ivannikovi* galls in the plots was approximately three times higher in 2013 than in 2012 and 2011, but the relationship between monitoring year and average gall number per plot was not statistically significant (Table 1; repeated measures ANOVA, $F = 1.69$; df = 2, 198, P = 0.18). Finally, the percentage of ramets with galls was consistently low: 2.0, 1.3, and 2.1% in 2011, 2012, and 2013, respectively.

Results indicate that *J. ivannikovi* is established at the site, but 4 yr after release, the frequency of attack of Russian knapweed ramets is low and ramet density remains high. The lack of an effect of *J. ivannikovi* on ramet density at the site is not surprising. *Jaapiella ivannikovi*'s greatest effect at the level of a ramet is on seed production: galled ramets produced 98% fewer seeds than ungalled ramets (Djamankulova et al. 2008). Because recruitment of Russian knapweed from seed within an infestation is thought to be negligible (Watson 1980), even a large reduction in seed production would be unlikely to translate into a reduction in ramet density. If *J. ivannikovi* is to have an effect on ramet density within infestations, the mechanism will have to be a reduction in belowground shoot production or in interspecific competitive ability at high gall densities.

A reduction in seed production by *J. ivannikovi* might, however, affect Russian knapweed populations at larger spatial scales via a reduction in the establishment of new infestations. We did not directly assess seed production within the monitoring plots; however, given that 2% or fewer Russian knapweed ramets were attacked in a given year, 98% of the ramets produced seed, unaffected by the presence of *J. ivannikovi* galls. Because of the low frequency of attack, it seems clear that *J. ivannikovi* is not yet having a significant effect on seed production at the site. In general, high frequency of attack does not guarantee an effect on the weed at the population level (McClay 1992), but consistently low rates of attack are likely to be associated with minimal effect.

Reasons for the low densities and frequency of attack are not clear from our data or observations. High mortality was not evident; no parasitoids emerged in our cages (although they may have been in diapause within the gall), and damage by rodents or birds was rare—only 3% of galls overall. It is possible that the *J. ivannikovi* population has required time to adapt to the potentially novel environmental conditions at the site. Alternatively, the low densities that we observed may reflect the small population increases that initially characterize logistic population growth. In fact, the latter possibility is supported by monitoring conducted since 2013. In 2014 and 2015, we modified our monitoring protocol to include 60 of the 100 permanent plots. We also counted galls every 3 wk rather than weekly. The 2014 to 2015 data are therefore not directly comparable to the 2011 to 2013 data, but they suggest rapidly increasing gall densities in the fifth and sixth years after release.

In conclusion, demonstrating the effects of weed biological control agents is important for justifying the expense, effort, and potential for environmental risks associated with their release (Balcuinas and Coombs 2004). Our results represent some of the first information about the effects of *J. ivannikovi* as a biological control agent for Russian knapweed. We found no evidence of an effect 4 yr after release. In our opinion, however, it is too soon to conclude that *J. ivannikovi* is an ineffective biological control agent. Information about *J. ivannikovi*'s effects will need to be updated through continued monitoring, as well as information from other sites where it has established. Nevertheless, our results do suggest that the timescale of effects of *J. ivannikovi*, if they ultimately occur, may be relatively long—longer than the 4 yr that *J. ivannikovi* has been present at our site.

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