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RELEASE AND MONITORING OF *LARINUS MINUTUS*
(COLEOPTERA: CURCULIONIDAE), A BIOLOGICAL CONTROL
AGENT OF SPOTTED KNAPWEED IN ARKANSAS

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ABSTRACT

Spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek) is an invasive plant from Eurasia that degrades pastures and rangelands in the western United States and Canada. A biological control program for spotted and diffuse (*C. diffusa* Lamarck) knapweeds was initiated in the 1960s, with 13 species of insects introduced and established in the United States and Canada. The program has largely been considered a success and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) is considered to be one of the key agents responsible for the reduction of knapweeds. Spotted knapweed expanded its range into the southeastern United States, but biological control was not used against the weed in this region until recently. *Larinus minutus* were released in 6 counties in Arkansas from 2008 through 2012. Weekly sweep-net sampling to monitor weevil activity showed establishment of the weevils in 5 counties. The numbers of *L. minutus* captured at each site increased from 2010 to 2012. *Larinus minutus* in Arkansas was univoltine and sex ratios were 1:1, except during flowering, when the proportion of females captured increased. Eggs were not present in females until 1 week after flowering of spotted knapweed.

Key Words: *Centaurea stoebe* ssp. *micranthos*, weed biological control, non-native weed, invasive weed

RESUMEN

'Spotted knapweed' (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek) es una maleza invasiva originaria de Eurasia que degrada los pastizales y praderas del oeste de los Estados Unidos y Canadá. Un programa de control biológico para 'spotted' and 'diffuse' (*C. diffusa* Lamarck) 'Knapweeds' fue iniciado durante la década de los sesenta, en donde trece especies de insectos fueron introducidos y establecidos en los Estados Unidos y Canadá. En su mayoría el programa ha sido considerado un éxito del cual *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) ha sido considerado el agente principal responsable de la reducción de ambas especies de 'knapweeds'. *Larinus minutus* fue liberado en seis condados en Arkansas desde el año 2008 hasta el año 2012. Muestras semanales con redes entomológicas fueron usados para el monitoreo de la actividad del picudo revelando su establecimiento en cinco condados. El número de *L. minutus* capturados en cada sitio incrementó entre los años 2010 y 2012. *Larinus minutus* en Arkansas fue univoltino y la proporción entre sexos fue de 1:1, excepto durante la época de floración, donde la proporción de hembras capturadas incrementó. Huevos adentro de las hembras comenzaron a ser hallados una semana después de la floración de la maleza.

Palabras Clave: *Centaurea stoebe* ssp. *micranthos*, control biológico de malezas

Spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek) is an invasive, perennial plant that is native to Eurasia. It was accidentally introduced into North America in the late 1800s in contaminated ship ballast and alfalfa seed, and spread throughout much of the United States and Canada (Mauer et al. 2001). Spotted knapweed invades disturbed fields and pastures, roadways, railroad rights-of-way, and other dry, open, disturbed habitats (Harris and Cranston 1979; Tyser and Key 1988; Lacey et al. 1990).

A biological control program for diffuse (*Centaurea diffusa* Lamarck) and spotted knapweeds was initiated in the 1960s, and 13 insect species were released and established in the United States and Canada to control these invaders (Story 2002). Although there have been declines in both diffuse and spotted knapweeds, the agent (or agents) responsible for the success is not known with certainty. *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) is suspected as the agent (or one of the agents) responsible for the decline

in knapweeds (Myers 2004, Myers et al. 2009). *Cyphocleonus achates* (Fabr.) (Coleoptera: Curculionidae) has also been shown to reduce spotted knapweed density (Corn 2006; Story et al. 2006).

Larinus minutus are native to Europe and Asia (Bulgaria, Israel, Greece, Kazakhstan, and Rumania) and was first released into the United States in 1991 (Groppe 1990; Story 2002). Larvae feed on the seeds of spotted and diffuse knapweeds and can destroy up to 100% of the seed in a capitulum (Kashefi & Sobhian 1998). In the western United States, *L. minutus* are univoltine, and become active on knapweed plants in Jun, typically several weeks before knapweed buds appear. Adult females must feed on knapweed flowers to complete ovarian development (Groppe 1990). Oviposition occurs in newly opened flower heads, and oviposition occurred for 11 weeks during a laboratory study (Groppe 1990). In that same laboratory study conducted at 25 °C, larvae hatched in 3 to 4 days and development through 3 instars required approximately 4 weeks (Groppe 1990). In field studies in Arkansas, larval development time was accelerated and completed in 2 instars, instead of 3, in approximately 3 weeks (Alford 2013). Larvae feed on knapweed seeds and pupate in the capitulum, making a cocoon out of the seed-head material (Kashefi & Sobhian 1998). In the western United States, adult *L. minutus* emerge in late Sep and feed. Adults overwinter in the soil and emerge in Jun (Jordan 1995). The lifespan of *L. minutus* males ranged from 48 to 97 days and females from 17 to 58 days (Kashefi & Sobhian 1998). Sex ratios are male biased before and during the onset of flowering and female biased during peak flowering (Kashefi & Sobhian 1998).

There have been no documented releases of *L. minutus* in the southern United States other than those reported from our laboratory (Minteer et al. 2011) and its seasonal dynamics have not been investigated. In Arkansas, spotted knapweed begins to flower in May and can continue flowering into October (personal observation), which is a longer period than for knapweed growing in the northwestern United States. Because knapweed has a longer growing season in Arkansas, we hypothesized that *L. minutus* may produce an additional generation, resulting in a greater decrease in spotted knapweed than that seen in the western United States and Canada. Additional generations of univoltine weevils (e.g., plum curculio) and other univoltine insects are sometimes found in the warmer areas of their range (Chapman 1938; Schoene 1936) or during warmer than normal summers (Saulich & Musolin 1996). The objective of this study was to describe the expanding of the distribution of *L. minutus* in Arkansas and to compare adult *L. minutus* activity to that reported for the western United States and Canada.

MATERIALS AND METHODS

Larinus minutus adults were collected in Colorado Springs, Colorado, USA during the summers of 2008 through 2011 and released into the Ozark Plateau and the Gulf Coastal Plain Regions of Arkansas. Over 29,000 *L. minutus* adults were released across 40 sites in 6 Arkansas counties (Washington, Madison, Benton, Howard, Carroll, and Boone) (Fig. 1). Releases ranged from 300 to 1,500 weevils per site, depending on the size of the knapweed infestation and the number of weevils collected the yr of release. On average, 750 weevils were released per site. One release was made per site. The first 2 releases in 2008 were made during the day (caged), whereas all subsequent releases were conducted at night (open field) to increase the probability that weevils would remain near the release site. Releases were monitored visually and via sweep net for the presence of *L. minutus* adults. Monitoring began a few months after release and was repeated in Jun of each subsequent yr until establishment was confirmed (after 3 yr of positive collections at a site), or until no *L. minutus* adults were found at the site for 2 consecutive yr.

Three release sites were surveyed more intensively to monitor for adult activity. Weekly sweep net samples were collected in 2010 through 2012 (4 sets of 25 sweeps at each site) starting in Apr of each yr, continuing until 2 weeks after no *L. minutus* adults were found. In 2012, only 2 sites were surveyed because of a drastic reduction of the number of spotted knapweed plants at the third site. *Larinus minutus* were released at each of these sites in 2009, so *L. minutus* had successfully overwintered at each site before surveying. Sweep samples were conducted between 10 am and 12 pm, when weevils are reported to be most active (Groppe 1990, Kashefi & Sobhian 1998). Adult *L. minutus* were preserved in 95% ethanol until sex could be determined, using the method described in Kashefi (1993). Females were dissected to determine the presence of eggs in the ovarioles in 2011 and 2012.

We used a chi-square test to determine deviations from an even sex ratio 3 times during the growing season; pre-flowering (of spotted knapweed), flowering, and post-flowering/senescence. Ten mature capitula (flowers senesced, but capitulum still closed) per site per week were collected as well and placed individually in plastic diet cups with cardboard lids and held in a screen house at ambient temperatures. These capitula were monitored weekly for emergence of adult *L. minutus* for the remainder of the season to determine when *L. minutus* emerged from spotted knapweed capitula at ambient temperatures.



Fig. 1. *Larinus minutus* releases in Arkansas. Release counties indicated by gray-shading. Releases were made in Arkansas from 2008 through 2011.

RESULTS

Releases of *Larinus minutus* Weevils

Of the 40 releases, 38 (or 95%) were successful and *L. minutus* were established or present during sweeps and/or visual assessments in 2011 and/or 2012 (release information available upon request). Two of these releases failed to establish. Both of these releases were made in 2010.

Adult Collections of *Larinus minutus* Weevils

The numbers of weevils collected at the intensely monitored sites increased from 2010 through 2012 and adult *L. minutus* collections indicated that only 1 generation occurred per yr (Fig. 2). Each yr there was an initial increase in the number of overwintering adult weevils col-

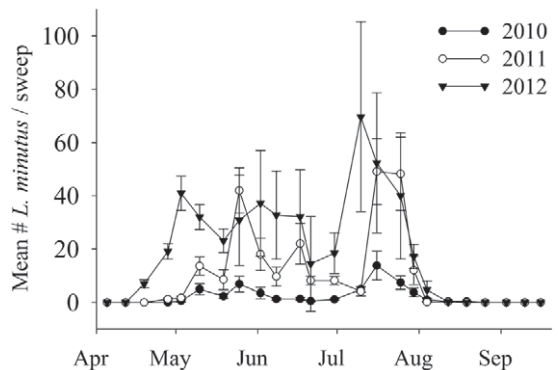


Fig. 2. Mean numbers of adult *Larinus minutus* collected per sweep during 2010 through 2012 at 3 release sites in Arkansas. Weevils were monitored weekly with sweep nets (4 sets of 25 sweeps per site).

lected in mid to late Apr, followed by a slight dip in numbers collected, then followed by another increase from the F1 generation emergence, which aligned with the emergence of adult weevils from the capitula kept in the screen house (Fig. 3). Adult weevils were observed in collections until mid-Sep (Fig. 2).

The F1 generation emerged in early to mid-Jul, as seen by the apparent third peak of adults collected (Fig. 2) and the presence of emergence holes on plants (personal observation). In 2011 adult weevils from the capitula held in the screen house also started to emerge in early Jul (Fig. 3). Data for screen house emergence in 2010 are not shown due to the extremely low numbers of *L. minutus* that emerged during this time.

Sex Ratio

Males and females became active at the same time of the yr (data not shown). Sex ratios were 50:50 before spotted knapweed flowering and after flowering during all 3 yr of the study. Sex ratios during flowering (Jun-Jul) were female biased in 2011 and 2012, 0.46 ($\chi^2 = 49.7$, $df = 22$, $P = 0.003$) and 0.47 ($\chi^2 = 115.3$, $df = 17$, $P < 0.0001$) proportion males, respectively. No deviation from an even sex ratio during flowering was recorded in 2010 ($\chi^2 = 38.4$, $df = 31$, $P = 0.17$).

Female *L. minutus* were dissected to determine the presence of eggs. Eggs were found in females starting the week after the first flowers were seen during all yr of the study (Fig. 4).

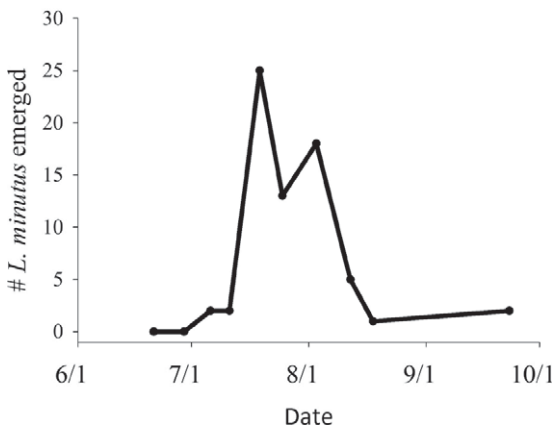


Fig. 3. *Larinus minutus* emergence from capitula collected weekly from 3 release sites in the Ozark Plateau region of Arkansas in 2011. Capitula were collected weekly and held at ambient temperatures in an outdoor screen house.

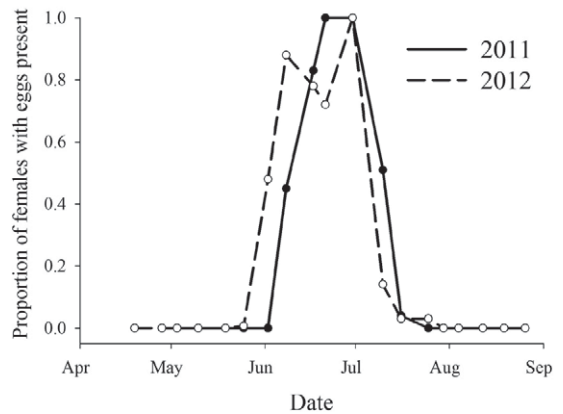


Fig. 4. Proportion of females with eggs present upon dissection from weekly sampling in Arkansas in 2011 and 2012. Flowering of spotted knapweed began on Jun 8 in 2011 and May 17 in 2012.

DISCUSSION

Releases of *Larinus minutus* Weevils

Weevil survival was unsuccessful at 2 sites. One of these releases was in an area of a small, isolated population of spotted knapweed in Howard County, 161 km from the closest known infestation area (Fig. 1). This population of spotted knapweed was not present the yr following release. The reason for the disappearance is not known. The other unsuccessful release site was sprayed with herbicide, killing all of the spotted knapweed shortly after *L. minutus* were released. *Larinus minutus* adults were detected at all other release sites at the end of the growing season during the yr of release except one. *Larinus minutus* adults were not found at this site until one yr after release, likely due to the low number of weevils released (300) and the scattered nature of the spotted knapweed plants in this location. Weevil numbers in this location, where the plants were very scattered, were likely too low to be detected easily during the months following release. Weevil numbers had likely increased by the following yr, leading to successful detection. Although we successfully established *L. minutus* at 2 sites where we released the smallest number of *L. minutus* (300 weevils), larger releases (> 600 weevils) may be more effective.

Adult Activity

As expected, the numbers of weevils recovered at release sites increased across the yr of this study. We anticipated a possible second generation per yr, because of the longer grow-

ing season in the southeastern United States. Spotted knapweed flowers earlier (late Apr to early May), and *L. minutus* are active much earlier in the yr in Arkansas than in its native range (late May to early Jun) (Groppe 1990). Knapweed flowers also persist much longer in Arkansas, as they are readily evident into mid Oct (personal observation). *Larinus minutus* are not known to be bivoltine elsewhere, but Arkansas is at lower latitudes than most of the native range of the weevil and that of the rest of the introduced range in North America (Groppe 1990; Story 2002). We are unaware of published records of the lifecycle of *L. minutus* in the most southern portions of its native range (Israel), where the weevil is at latitudinal ranges lower than Arkansas. Adult *L. minutus* collections in Arkansas indicated that only 1 generation per yr occurred. Explanations for a lack of increase in the number of generations include, the growing season may not be long enough to facilitate another generation, the environmental cues to facilitate another generation may not be present, or phenotypic plasticity could have been reduced, perhaps because of the bottle neck experienced during the importation of the weevil into North America.

In each of the 3 yr, there was a peak of weevils collected in early May, followed by a slight decrease in numbers collected, then followed by another increase (mid May to early Jun). It is likely that these first 2 peaks in adults collected are likely due to the adults emerging from their overwintering sites and becoming active, not the emergence of the F1 generation. Spotted knapweed flowers were available during this time period; however, there was not enough time since the onset of spotted knapweed flowering (early May) for egg laying, hatch, and full larval development, which takes approximately 3-4 weeks (Groppe 1990; Alford 2013). Emergence of adults from the capitula collected and held in the screen house in 2011 further supported this as the F1 emergence from the capitula began in early Jul (Fig. 3).

The 2 peaks in numbers collected each yr at the beginning of monitoring likely were a result of the sampling method used. Sweeping collected the active adult weevils feeding in the plant terminals, but was not appropriate for monitoring the weevils that were at the base of the plants. *Larinus minutus* feed on the terminal leaves of the plant, but prefer to feed on flowers (Groppe 1990). When flowers are present more weevils move to the top of the plants to feed and are more easily collected via sweep net.

Sex Ratio

Male and female weevils became active at the same time of the yr. Females were collected more

frequently than males at the start of weevil emergence from overwintering sites in 2010, but not in 2011 or 2012. This trend in 2010 may have been a product of experimental error combined with the lower number of active weevils (compared with 2011 and 2012). Females may be more active at the tops of the plants earlier in the yr than the males and therefore showed an earlier activity based on sweeps, especially when the numbers of weevils active are lower.

Sex ratios were even during the time before spotted knapweed flowered (Apr-May) and post-peak flowering/senescence (Aug-Oct). During flowering, there were more females than males. This skew in sex ratio during flowering is consistent with previous studies done in the native range of the weevil (Kashefi & Sobhian 1998).

Egg Production

No eggs were found in females until flowers were present during all yr of the study. This result agrees with the published literature, which indicates that feeding on flowers is required for ovary development (Groppe 1990; Kashefi & Sobhian 1998). However, no other studies have been conducted to verify this correlation and sample size in the original study was low ($n = 15$).

During this study *L. minutus* were further expanded in Arkansas. One generation/yr occurred, similar to that observed in the native range of the insect and Colorado, where the weevils were collected. Sex ratios were skewed towards more females during peak flowering, which is similar to that seen in the native range. With the differences in the length of the growing season and the phenology of the target plant between Arkansas and Colorado (where the weevils were collected), and despite a reduction in the number of instars from 3 to 2, the univoltinism exhibited by *L. minutus* across its range suggests the phenology of the insect is genetically programmed. While, in the strict sense, this lack of phenological plasticity is a goal for biological control of weeds, an additional generation/yr of *L. minutus* might have provided for an increased level of control of spotted knapweed in the state. Long-term monitoring studies are currently being conducted to determine the effectiveness of *L. minutus* in the region.

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