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SHORT COMMUNICATION

Revised methods for the mass-rearing of the spotted knapweed biological control agent, *Cyphocleonus achates* (Coleoptera: Curculionidae), in field corrals

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Cyphocleonus achates (Fahraeus), a root-feeding weevil introduced from Eurasia, is an effective biological control agent against spotted knapweed, *Centaurea stoebe* L. ssp. *micranthos*. Because *C. achates* is univoltine and does not fly, distribution of the weevil has been slow. To hasten the weevil's distribution, a rearing effort using field corrals was initiated at a facility in Corvallis, Montana. Procedures for mass-rearing the weevil in field corrals are described, with an emphasis on improvements over earlier methods. The described field-corral approach is effective and appropriate for producing *C. achates* for distribution in the western United States.

Keywords: *Cyphocleonus achates*; *Centaurea stoebe*; spotted knapweed; insect rearing; biological control; corral

Spotted knapweed, *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek (often reported as *C. maculosa* Lamarck) (Ochsmann 2001), is a perennial plant from Eurasia that has become a serious weed on rangelands of the northwestern United States. First reported in North America in 1893, the plant now occurs in all but four states in the US, and in seven Canadian provinces (United States Department of Agriculture 2010). The weed infests 1.6 million ha in Montana alone (Lacey, Lacey, Fay, Story, and Zamora 1992).

A Eurasian, root-feeding weevil, *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae), was introduced into North America for biological control of the plant, beginning in 1988 (Story, White, and Good 1996). The biology of the weevil was described by Story et al. (1996). The weevil reduces spotted knapweed biomass (Jacobs, Sing, and Martin 2006) and causes significant mortality to mature spotted knapweed plants (Corn, Story, and White 2006) which, when combined with stresses by other biocontrol agents, has led to significant reductions in spotted knapweed density in some areas of western Montana (Story, Callan, Corn, and White 2006; Story, Smith, Corn, and White 2008) and reproductive potential in Colorado (Seastedt, Knochel, Garmoe, and Shosky 2007).

Because the weevil has a slow reproductive rate, is univoltine, and does not fly, a rearing effort using field corrals was initiated in 1991. Early rearing procedures were

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described by Story et al. (1996). Considerable changes to these rearing procedures occurred as we learned more about the insect's behavior and production methods were improved. This paper describes current procedures used to mass-rear *C. achates*.

The rearing of *Cyphocleonus achates* using revised procedures was conducted at the Montana State University Western Agricultural Research Center at Corvallis, Montana. We report the procedures used to develop one field plot, although the rearing program involved the use of multiple plots. The rearing effort, a 5-year process from knapweed seeding through 3 years of *C. achates* collection, is described as conducted during 2005 through 2009.

During the first year (seeding year), the field plot, 13.7×21.3 m in size, was tilled and then seeded with spotted knapweed using a small-plot seed drill in October. A total of 30 rows (10.7 m long) were planted, with a between-row spacing of 61 cm and a seeding rate of about 16 seeds per m of row.

During the second year (stocking year), emerging knapweed seedlings were thinned to a 30 cm within-row spacing (i.e., about 36 plants per row), and between-row areas were weeded in May. Using a tractor-mounted sprayer, the herbicides isoxaben was then applied at the rate of 1.1 (AI) kg/ha for pre-emergent broadleaf weed control, and prodiamine was applied at the rate of 0.72 (AI) kg/ha for pre-emergent grass and broadleaf weed control in late May or early June, after the knapweed plants had become well established. Herbicide applications were immediately followed by 1.25 cm of water applied by overhead sprinklers. The plot was irrigated several additional times as needed during late May through early July. Spot-spray applications of glyphosate were made with a backpack sprayer for weed control throughout the summer and prior to introduction of the *C. achates*. Fertilizer was applied with a broadcast spreader at the rate of 134.4 kg/ha N, 67.2 kg/ha P_2O_5, 67.2 kg/ha K_2O, and 67.2 kg/ha S in June. In July, the knapweed plants were mowed to a 10 cm height using a riding lawnmower. The plot was then enclosed by a wall made of aluminum flashing (25 cm in height), forming a corral (Story et al. 1996). The flashing was installed 1.5 m from the knapweed rows and was held in place by 60-cm wooden stakes placed every 1.2 m around the outside edge of the corral. The lower 5 cm of the flashing was inserted below the soil surface. The upper 5 cm of the flashing was folded downward toward the inside of the corral to prevent *C. achates* escapes (Figure 1).

In mid-August, 500 adult *C. achates* were released into the plot, based on a rate of one pair of *C. achates* per four spotted knapweed plants, and with an assumed 50:50 sex ratio. The released weevils were scattered throughout the plot. In early November, after adult *C. achates* activity had ended, opposite ends of the corral wall were dismantled to enable mowing of the spotted knapweed plants with a tractor-drawn flail mower.

During the third through fifth years (collection years), maintenance of the plot was identical to the second year except that fertilizer was not applied, between-row tillage was conducted with a small tractor-drawn tiller in June and early July as needed, and the plants were mowed with a riding mower in both early June and in July. No herbicides were applied after the onset of *C. achates* emergence.

Collection of *C. achates* adults from the corral began in late July or early August, about 2 weeks after the first weevils were observed. Collections occurred on Monday through Friday and generally occurred in late-morning or afternoon when the weevils were active and more readily found. Although some adult *C. achates* were

Figure 1. Photo of *Cyphocleonus achates* rearing corrals showing aluminum flashing walls and support stakes.

collected with a sweep net, most were hand-collected by searching the ground along the corral walls and the basal foliage and top of knapweed plants in the corral. Collected *C. achates* were placed in 1-L paper cans (150 *C. achates* per can) containing fresh knapweed stems with mature flower buds removed, and held at 4°C for 1–3 days until shipment to cooperators.

Cyphocleonus achates collection was conducted from 31 July to 26 September in 2007, from 23 July to 25 September in 2008, and from 3 August to 23 September in 2009. Using the described procedures, we collected a mean of 6666.7 ± 3432.7 SD (range 2206–14,651) *C. achates* adults from each of 15 13.7×21.3-m corrals in 2007 for a total of 100,000 *C. achates*, 4325.4 ± 2151.7 SD (range 1247–7965) from each of 17 corrals in 2008 for a total of 73,532 *C. achates*, and 5817.4 ± 2121.1 SD (range 3776–9023) from each of 19 corrals in 2009 for a total of 110,530 *C. achates*. Mean number of *C. achates* collected per corral was highest in 2007 and lowest in 2008. A statistical comparison of the means among years was not conducted because the exact number of collection man-hours used per year was not recorded, and there was considerable variability in the number of corrals of each collection age in each year (i.e., the number of corrals being collected from for the first time, the number of corrals being collected from for the second time, etc.). The mean number of *C. achates* collected per corral did not necessarily reflect the total number of *C. achates* produced per corral because many weevils went uncollected, particularly upon termination of the collection effort in September.

The number of years that a corral was used for collection did not seem to affect *C. achates* collection numbers. A mean of 5621.7 ± 532.5 (SEM) *C. achates* was collected in corrals collected from in the first year, compared to 6431.3 ± 879.1 in corrals collected from for a second year, and 5037.7 ± 2001.2 in corrals collected from

for a third year. A comparison of these means was not made due to the man-hour variability mentioned above.

The procedures described here for mass-rearing *C. achates* include significant changes from the procedures described in 1996 (Story et al. 1996). Most notably, *C. achates* were collected from a corral for three consecutive years before the corral was replanted, in contrast to 1996 when collections were made for only one year before replanting the corral. We determined that, if the spotted knapweed plants in the corrals were provided with fertilizer and timely irrigation, the plants could support the production of *C. achates* for 3 years in a corral before the corral had to be replanted with new spotted knapweed plants. Collections from the corrals could possibly have been made for one or two more years (i.e., a total collection period of 4 or 5 years) but we chose to limit the use of a corral to three collection years because maximum production was our priority. This decision was driven by available space and the fact that the number and size of mature spotted knapweed plants in the third year-collection corrals appeared less than in first year-collection corrals, although no data were collected to confirm this observation.

Another change implemented in the current approach was the planting of spotted knapweed using seeds rather than transplants. Planting spotted knapweed by seed may have helped reduce the problem with the plant pathogen, *Sclerotinia sclerotiorum* (Lib.) de Bary, reported in 1996 (Story et al. 1996); much of the disease problem reported by Story et al. (1996) was likely due to the use of transplants from heavily *S. sclerotiorum*-infected field sites. Other changes in the current approach include a *C. achates* stocking rate of 1:4 (one *C. achates* pair to four spotted knapweed plants) compared to a 1:12 rate in 1996, the use of herbicides when appropriate, and the expanded use of machinery such as a seed drill, mower, and tiller to plant and maintain the spotted knapweed in the corrals instead of relying solely on hand labor as in 1996. Also, in contrast to the 1996 procedures, we did not use a shade cloth over the corral walls to reduce solar heating of the walls and surrounding soil. Adult *C. achates* mortality due to heat was not enough to justify the added time and effort involved in maintaining the shade cloth in our large (19-corral) operation. The use of the shade cloth for small operations (one or two corrals) is helpful, but not essential, especially if *C. achates* adults are collected daily.

The herbicides used in the study caused no observed harmful effects to either the spotted knapweed or *C. achates* in the corrals. Herbicides probably affect *C. achates* only when the herbicides kill the knapweed plants while the larvae are still feeding in the root (Story and Stougaard 2006). Isoxaben and prodiamine, applied as preemergent herbicides, had no impact on established spotted knapweed plants. Glyphosate is capable of killing spotted knapweed, but it was applied very carefully and caused no injury to the spotted knapweed in the corrals.

Mowing knapweed plants in the corrals was very important to the rearing effort. Mowing reduced the plant cover which aided collecting efforts, and increased soil temperature, permitting earlier adult emergence. Story et al. (1996) reported that cooler soil temperatures under dense knapweed canopies could delay *C. achates* emergence by up to 3 months. The need to minimize canopy cover was also the reason for the 61-cm between-row spacing.

The collection methods used efficiently exploited *C. achates*' behavior and life history. Adult *C. achates* climbed atop vertical structures during the first 2 weeks after emergence. After those first 2 weeks, the adults ended their climbing tendency,

returned to the ground, and assumed a roaming behavior characterized by determined walking. The roaming *C. achates* wandered in the corrals until they confronted a corral wall, after which they congregated on the ground along the walls during the afternoon. As a result of these differing behaviors and the fact that the adult *C. achates* were of different ages due to protracted emergence, all collection methods were used throughout the summer (i.e., sweeping and hand-collecting of *C. achates* from the tops of spotted knapweed plants, and hand-collecting weevils from the foliage and along the walls). Hand collection was found to be the most efficient means of collecting *C. achates* on or near the ground; hand vacuums were not used due to the tenacity with which the weevils clung to substrate. *C. achates* were also collected from the vegetation in the corrals but that method was the least efficient due to the weevil's cryptic coloration which made them very difficult to locate. Collections were most successful in the afternoon, during the warmest parts of the day when the weevils were the most active and noticeable.

Predation of *C. achates* in the corrals occurred, and was occasionally significant. Skunks and birds (including magpies, crows and ravens) would uproot up to 30% of the spotted knapweed plants in a corral and remove the exposed *C. achates* larvae. Adult *C. achates* were often eaten by birds, skunks, and rodents (such as shrews and deer mice), but the predation was not significant.

The current rearing protocol was based on the assumption that *C. achates* does not fly (Stinson, Schroeder, and Marquardt 1994), but we observed one adult flying approximately 0.5 m in 2009. However, since only one *C. achates* flight was observed despite extensive observations by many workers over the years, it is unlikely that flight is an important means of dispersal.

The primary cost of the rearing effort was labor, as it was in 1996 (Story et al. 1996). However, because of the increased use of machinery in the current approach, the labor costs involved with initiating and maintaining a 13.7×21.3-m rearing corral were considerably less than in 1996. Current procedures required 7 man hours per corral per year over a 5-year period, which was an 89% reduction from 1996 levels. Collection of *C. achates* required 30 man hours per corral in 2009. Material costs included aluminum flashing, support stakes, hardware, herbicides, paper cans, fuel, and machinery use. A cost per insect was not calculated but, because of the reduced labor needs and the greater overall production numbers in the current approach, the cost per insect would certainly be less than that reported in the 1996 report ($1.63 per insect; Story et al. 1996).

In view of the serious spotted knapweed threat to western North America and the proven effectiveness of *C. achates*, it is essential that efforts to establish and distribute this agent be continued. The field-corral rearing approach, with the improvements described herein, is effective, appropriate and justified for the distribution of *C. achates* in Montana and other spotted knapweed-infested areas of western North America.

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