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Effect of biocontrol insects on diffuse knapweed (*Centaurea diffusa*) in a Colorado grassland

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Four species of biocontrol insects (knapweed root weevil, lesser knapweed flower weevil, spotted knapweed seedhead moth, and bronze knapweed root borer) were released at a diffuse knapweed site located about 10 km east of the Colorado Front Range. Two other biocontrol agents (banded gall fly and knapweed seed head fly) were already present at this site. Densities of rosettes and flowering plants, seedhead production per plant, and seeds per seedhead on mowed and unmowed areas were studied for 5 yr, 1997–2001. Of the six biocontrols, five (*Urophora* spp., bronze knapweed root borer, knapweed root weevil, and lesser knapweed flower weevil) obtained sizable densities relative to weed abundance. Diffuse knapweed declined from 8.3% in absolute cover in June 2000 to 1.9% by September 2001. Vegetation transects closest to the insect release areas showed the largest declines, with diffuse knapweed disappearing entirely on one transect. In contrast, diffuse knapweed abundance at a nearby prairie increased during the same interval from an absolute cover value of 14.5 to 17%. Seed production of diffuse knapweed on the insect release site declined from nearly 5,000 seeds m^{-2} in 1997 to less than 100 seeds m^{-2} in 2001. Lesser knapweed flower weevil larvae appeared responsible for much of the seed reduction, whereas adults of this species were effective in damaging bolting plants. The extent to which grazing removal and individual insect species contributed to this reduction in diffuse knapweed abundance cannot be identified from this study. These results support the contention that a significant reduction in abundance of diffuse knapweed using insects is possible at least in some regions of the western United States.

Nomenclature: Diffuse knapweed, *Centaurea diffusa* Lam. CENDI; Alyssum, *Alyssum minus* (L.) Rothm. AYSMI; Japanese brome, *Bromus japonicus* Thunb. Ex. Murr. BROJA; field bindweed, *Convolvulus arvensis* L. CONAR; banded gall fly, *Urophora affinis* Frauenfeld; knapweed seed head fly, *Urophora quadrifasciata* Meigen; bronze knapweed root borer, *Sphenoptera jugoslavica* Obenb.; knapweed root weevil, *Cyphocleonus achates* Fahraeus; spotted knapweed seedhead moth, *Metzneria paucipunctella* Zell.; lesser knapweed flower weevil, *Larinus minutus* Gyllenhal.

Key words: Biological control, compensatory growth, population dynamics.

Diffuse knapweed is an opportunistic biennial forb in the Asteraceae that is native to Europe and Asia and has become an invasive weed in much of the western United States. The plant tends to be largely unpalatable to livestock and other wildlife, may enhance soil erosion rates, and is a potential threat to native plant species (DiTomaso 2000; Lacey et al. 1989; Sheley et al. 1998; Watson and Renny 1974). The weed is particularly a problem in the northwestern United States (Sheley et al. 1998) and Canada (Harris and Cranston 1979), and these regions have been the sites of most of the research on this weed. More recently, diffuse knapweed has become a serious concern in parts of Colorado. The remaining grasslands along the Front Range of Colorado are currently either infested or at risk of invasion. Concurrently, these lands are being fragmented by urban and suburban development. Local governments have purchased substantial areas to deter development as well as to maintain native ecosystems. By 1996, diffuse knapweed was viewed as a sufficiently large problem on these lands along the Front Range to warrant an aerial herbicide program (Woodall et al. 2000).

Insect herbivory may offer a potentially inexpensive, relatively low-risk, self-perpetuating mechanism for reducing the abundance of certain weeds that is particularly suited to the management of large areas (Mack et al. 2000), grasslands of low economic value (Maddox 1979), or areas containing desirable species sensitive to other management options. However, demonstrations of reductions of established populations of weeds such as diffuse knapweed remain few, either because the weed simply cannot be controlled by these insects or because the right combination of biocontrol insects has not been attempted (McEvoy and Coombs 1999).

Two flies introduced from Europe, the banded gall fly and the knapweed seed head fly (Diptera: Tephritidae) (Harris 1980a, 1980b), have been used in North America for several decades to reduce seed production in diffuse and spotted knapweed. The bronze knapweed root borer (Coleoptera: Buprestidae), a root feeder of knapweed rosettes, has also been used as a biocontrol in North America for over 20 yr. The knapweed root weevil (Coleoptera: Curculionidae), another root feeder of knapweed rosettes, was released in the United States in the late 1980s. The spotted knapweed (*Centaurea maculosa* Lam.) seedhead moth (Lepidoptera: Gelechiidae) was released in Montana in 1980 and has been established in three states (Lang et al. 2000b). A more recent introduction is the lesser knapweed flower weevil (Coleoptera: Curculionidae), a weevil that feeds on seedheads, which was released in the United States in 1991 and is now found in 10 states (Lang et al. 2000b). The effects of this weevil on spotted knapweed have been documented (Lang et al. 2000a), but few studies have documented the effect of this weevil or that of the knapweed root weevil and the spotted knapweed seedhead moth on diffuse knapweed.

These insect species collectively offer some hope for reduction of diffuse and spotted knapweed (Rees et al. 1996). However, knowledge of the effects of these biocontrols, if available at all, is limited to more northern prairies that differ substantially in biological and physical characteristics when compared with those found on the Front Range of Colorado. Further, the combined effects of several of these insects, particularly the lesser knapweed flower weevil and the knapweed root weevil, have yet to be reported in the literature.

We measured diffuse knapweed densities and seed production over five growing seasons for a site infested with diffuse knapweed. Our study site was not grazed during this interval, and mowing on a portion of the site was used as a mechanical treatment designed to stress the diffuse knapweed and limit tumbling of the weed. We also were interested in how mowing, a common management practice for knapweeds along roadsides, might affect use of the plants by insects. We monitored the abundance of *Urophora* spp. and the fate of small releases of spotted knapweed seedhead moth, bronze knapweed root borer, knapweed root weevil, and lesser knapweed flower weevil. The extent to which insects were able to establish, use the diffuse knapweed resource, and affect diffuse knapweed population dynamics on mowed and unmowed areas were concurrently measured. The present study did not assess individual insect species effects but monitored the relative success of these populations and quantified the combined effect of grazing removal and insect release on the relative abundance of diffuse knapweed and other vegetation.

Site Description and Methods

Research was conducted primarily at a 30-ha site in Boulder County, Colorado (40°N, 105°W). Soils are clay over loamy-skeletal montmorillonitic, mesic Aridic Arigiustolls (D. C. Moreland and R. E. Moreland 1975). This site was a degraded pasture, and diffuse knapweed composed almost 30% of the plant cover in 1997. Native grasses were a modest component of the vegetation, whereas nonnative species such as annual peppergrass or Alyssum (*Alyssum parviflorum*), Japanese brome, and field bindweed were common. The vegetation composition and cover provided by native grasses, native forbs (nonwoody dicots), diffuse knapweed, and other introduced plant species were quantified along four, 50-m point-intercept transects. An additional transect was established at a nearby prairie site experiencing moderate grazing. Along each transect, 200 points were projected downward using an optical device with a cross-hair. The device was mounted on a tripod and was designed to direct the line of view 50 cm to the side of the transect to avoid vegetation affected by the measuring tape used to construct the transect. The identity of each species (or bare soil, rock, litter, or standing dead) was recorded at each point. From these cover data, for purposes of this study, the relative cover

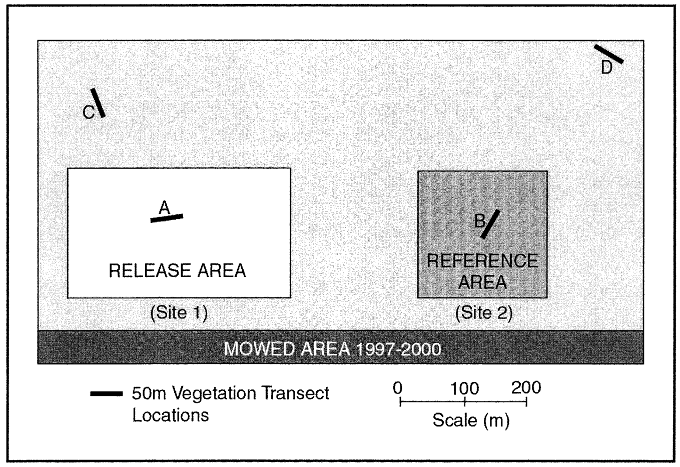

FIGURE 1. Main study site which consisted of an area that was used as the insect release area (Site 1) and a reference area (unmowed, without insect releases; Site 2), and a swath of prairie that was mowed once every summer during the 1997–2000 interval. Vegetation transects were placed within the release and reference areas and at two locations outside the diffuse knapweed (*Centaurea diffusa* Lam.) and insect measurements. An additional vegetation transect was located about 1 km from the release area.

of the four plant groups listed above as well as absolute cover values were summarized.

The main research site had been heavily grazed by cattle until initiation of the research in June 1997, but it was not grazed during the study. The area was divided into three areas that were monitored for diffuse knapweed response (Figure 1). A single mowing event per year was done on a strip of prairie with a 5.8-m "bat wing" rotary mower pulled by a tractor in the third or fourth week of June, just before or during initiation of flowering of diffuse knapweed. Plants were cut to a height of approximately 20 cm. Mowing is known to reduce seed production and alter the plant's phenological development (B. F. Roche and C. T. Roche 1998). Plants tend to sprout new growth from damaged stems, and flower later in the growing season. This treatment was terminated after mowing in 2000. An area adjacent to the mowed site was selected as the central release point for insects and is referred to as Site 1. This area included a vegetation transect (Transect A). A second unmowed site (Site 2) was selected about 300 m away from the main insect release site and included a second vegetation transect (Transect B). A third vegetation transect (C) was located about 200 m from Transect A and was adjacent to the insect release site. Transect D was located about 600 m from the release area (Figure 1). An additional transect (Transect E, not shown in Figure 1) was located about 1 km from the insect release area. Transect E was selected because of similarities in vegetative composition with the main study area. Vegetation inventories and cover estimates were conducted in the month of June in 1997, 2000, and 2001 and also in September 2001.

The banded gall fly and the knapweed seed head fly were released in the Front Range in 1989, but the knapweed seed head fly may have arrived previously on its own (Jerry Cochran, Colorado Department of Agriculture Biocontrol Division, personal communication). Although both species were assumed to be present on our sites from the initiation of research in 1997, only the knapweed seed head flies were observed as adults in the field or emerged from seedheads

TABLE 1. Releases of biocontrol insects, 1997–2000.

Year	Number of adults released			
	Larinus	*Sphenoptera*	*Cyphocleonus*	*Metzneria*
1997	200	400	50	2,000
1998	50	250	—	—
1999	300	850	100	—
2000	—	—	50	—

kept in the laboratory through 1998. Other species of biocontrols were obtained from insectaries and released at the site (Table 1). Lack of a local source of insects precluded larger releases of lesser knapweed flower weevil and knapweed root weevil. The bronze knapweed root borer was available from an insectary established on the Front Range by Colorado Department of Agriculture Biocontrol Division personnel. In addition to these insects, 2,000 adult spotted knapweed seedhead moths were obtained from a source in Montana and were released in the summer of 1997.

Diffuse knapweed flowering stem and rosette densities were sampled using 1-m^2 quadrats selected at random. Within each of the three areas, a marker was thrown in a general direction (i.e., "over the shoulder"), and the quadrat was placed along a fixed direction with the marker forming the center of the quadrat. Flowering stem densities were estimated by counting stems from 30 quadrats on one or more of the three sampling areas each year (Table 2). Rosette densities were counted using similar quadrats sampled in a similar way. Inventories were usually taken in October, when plants that had germinated earlier in the summer were large enough to be identified. These rosette estimates did not include small seedlings, if present, that had yet to form the characteristic rosette morphology. A count of rosettes was also made in June 2001 and April 2002 (Table 2). Seedhead numbers per plant were obtained by randomly selecting 30 individual plants within the three treatment areas and counting all seedheads on the selected plant. Again, we used a marker thrown in a general area, and the specific flowering plant counted was the one whose stem was closest to the marker. By using the closest stem rather than the closest branch, smaller plants were adequately represented. Seeds per seedhead and *Urophora* spp. per seedhead were sampled by removing at least six developed seedheads from each of the 30 plants selected at random from the three areas. A preliminary study conducted by one of us in 1997 (N. Gregory, unpublished data) indicated that neither seed number nor *Urophora* counts were affected by the location of the seedheads on plants. Until the 2000 field season, we attempted to sample only mature seedheads that retained intact petals. This minimized seed loss either before collection or during handling before counts. However, extensive weevil activity by 2000 required that a random sample of seedheads, irrespective of intact petals, be obtained. With one exception, collection of these seedheads occurred during plant senescence in September to ensure that the second generation of knapweed seed head fly was visible within the seedheads (Table 2). We dissected seed heads and counted the numbers of larvae, remains of pupae, and seeds with the aid of a dissecting scope. Evidence for individuals of the first generation of knapweed seed head fly were obtained by counting the remains of pupae. By 2000, we recognized that the lesser knapweed flower weevil was affecting seed counts in all our initial three sampling areas. We therefore expanded our seeds and insects per seedhead count to include diffuse knapweed from an adjacent pasture close to Transect E. These sites were not heavily affected by insects other than *Urophora* spp. and provided an index of seed production by plants not experiencing extensive herbivory by insects.

Statistical inferences from random samples obtained within each of the three diffuse knapweed sample areas are valid for comparisons within this site (Wester 1992) as well as for identifying numerical trends in insect numbers and weed

TABLE 2. Sampling schedule and sampling activities.

Year	Variable	Site 1 (release site)	Site 2 (reference)	Site 3 (mowed, 1997–2000)
1997	Stem densities	August	August	No data
	Seedhead counts	No data	September	September
	Seeds per seedhead[a]	No data	September	September
	Rosettes	No data	October	No data
1998	Stem densities	September	September	September
	Seedhead counts	September	September	September
	Seeds per seedhead[a]	No data	September	September
	Rosettes	No data	No data	No data
1999	Stem densities	September	September	September
	Seedhead counts	September	September	September
	Seeds per seedhead[a]	September	September	September
	Rosettes	No data	October	No data
2000	Stem densities	September	September	September
	Seedhead counts	September	September	September
	Seeds per seedhead[a]	September	Late July	September
	Rosettes	October	October	No data
2001	Stem densities	August	August	August
	Seedhead Counts	August	August	August
	Seeds per seedhead[a]	August	September	September
	Rosettes	June	June	No data
2002	Rosettes	April	April	No data

[a] *Urophora* spp. and *Larinus* abundance also were estimated from these data.

TABLE 3. Cover data from knapweed-monitoring transects and reference transect, June 1997, 2000, and 2001, and September 2001.

Vegetation	Transect A Relative cover	Transect A Absolute cover	Transect B Relative cover	Transect B Absolute cover	Transect C Relative cover	Transect C Absolute cover	Transect D Relative cover	Transect D Absolute cover	Average A–D Relative cover	Average A–D Absolute cover	Transect E[a] Relative cover	Transect E[a] Absolute cover
June 1997												
Native grasses	9.5	3.5	31.3	13.0	37.0	25.0	29.8	17.0	28.8	14.6	18.3	9.0
Native forbs	4.1	1.5	8.4	3.5	3.0	2.0	5.3	3.0	4.9	2.5	7.1	3.5
Diffuse knapweed[b]	51.4	19.0	19.3	8.0	28.9	19.5	17.5	10.0	27.8	14.1	60.2	29.5
Other nonnative plants	33.8	43.4	38.6	16.0	30.4	20.5	43.9	25.0	36.4	18.5	12.2	6.0
Total vegetation cover[c]	100	37.0	100	41.5	100	67.5	100	57.0	99.9	50.8	100	49.0
June 2000												
Native grasses	26.7	6.04	2.9	10.5	42.2	9.5	67.3	17.5	45.5	10.9	35.5	11.0
Native forbs	8.9	2.0	2.0	0.5	0.0	0.0	1.9	0.5	3.1	0.8	9.7	3.0
Diffuse knapweed	51.1	11.5	40.8	10.0	26.7	6.0	21.2	5.5	34.5	8.3	46.8	14.5
Other nonnative plants	8.9	2.0	10.2	2.5	26.7	6.0	5.8	1.5	12.6	3.0	8.1	2.5
Total vegetation cover[c]	100	22.5	100	24.5	100	22.5	100	26.0	99.9	23.9	100	31.0
June 2001												
Native grasses	14.1	5.0	45.2	19.0	35.4	20.0	21.1	11.5	29.5	13.9	48.7	28.5
Native forbs	7.0	2.5	7.1	3.0	6.2	3.5	10.1	5.5	7.7	3.6	4.3	2.5
Diffuse knapweed	12.7	4.5	1.2	0.5	0.0	0.0	6.4	3.5	4.5	2.1	18.0	10.5
Other nonnative plants	54.9	19.5	46.4	19.5	57.5	32.5	57.8	31.5	54.7	25.8	24.9	14.5
Total Vegetation cover[c]	100	35.5	100	42.0	100	56.5	100	54.5	100.1	47.1	100	58.5
September 2001												
Native grasses	30.1	11.0	57.5	23.0	59.6	29.5	46.7	25.0	48.5	22.1	56.3	31.5
Native forbs	4.1	1.5	6.3	2.5	2.0	1.0	1.9	1.0	3.6	1.5	2.7	1.5
Diffuse knapweed	2.7	1.0	5.0	2.0	0.0	0.0	8.4	4.5	4.0	1.9	30.4	17.0
Other nonnative plants	41.1	15.0	27.5	11.0	36.4	18.0	42.1	22.5	36.8	16.6	7.2	4.0
Total vegetation cover[c]	100	36.5	100	40.0	100	49.5	100	53.5	100	44.9	100	56.0

[a] Transect located about 1 km from the main research area.
[b] *Centaura diffusa* Lam.
[c] Total vegetation cover includes the succulents and shrubs not shown.

densities through time. Statistical procedures used an analysis of variance or *t* test after first checking for homogeneity of variances. To test for variation in seed production as a function of treatment, data obtained from individual seedheads were first averaged on a per-plant basis before statistical analyses. This averaging was also done on estimates of *Urophora* spp. abundance in seedheads. *Urophora* spp. exhibited aggregated distributions in seedheads, so data on fly abundance per seed head were transformed using a square-root transformation before statistical analyses were used to evaluate changes through time or among treatments. If statistical significance was reported for the entire model containing more than two treatments, tests for individual differences were conducted using the Student–Newman–Keuls (SNK) test (SAS 1988).

Results and Discussion

Plant Responses to Insect Release

The Boulder, CO area receives an average of 26.4 cm of precipitation from January through June (NOAA 2002). As of the end of June 1997, the precipitation recorded for Boulder was 46% above average. In 1998 this value was 24% above average, and in 1999 the value was 16% above average. As of the end of June 2000, precipitation was 24% below average for the calendar year. The first six months of 2001 saw precipitation 10% above average. Annual precipitation during the 1997–1999 interval was above average for all 3 yr. Precipitation in 2000 was 86% of average, and that of 2001 was 98% of average (NOAA 2002). Hence, conditions for spring and early-summer plant growth in 1997–1999 were very favorable, 2000 conditions represented a mild drought, and 2001 conditions were close to average. The 2000 drought accompanied a decline in total vegetation cover of over 50% relative to the 1997 values (Table 3). Diffuse knapweed cover for Transects A to D declined 41%, whereas cover by other nonnative species dropped 84%. Because cover by diffuse knapweed declined in 2000 less than did total vegetation cover, its relative cover actually increased. From 1997 to 2000, average native perennial grass cover declined only slightly and to a lesser degree than did total vegetation cover, hence it actually increased in relative cover.

With improved moisture conditions in 2001, total vegetation cover and native perennial grass cover rebounded to levels nearly as high as those in 1997 (Table 3). Cover by nonnative species other than diffuse knapweed jumped in 2001 to a level 40% greater than that in 1997. An example of the latter pattern, intermediate wheatgrass (*Thinopyrum intermedium*), an introduced grass notable for its weed-like aggressiveness in the Front Range, averaged 1.0% cover in 1997, dropped to 0.1% cover in 2000, and took advantage of the recovery year of 2001 with an increase to 5.9%. However, diffuse knapweed cover exhibited a trend opposite to that of the cover of other introduced species and declined at our main research site between 2000 and 2001. It even declined below measurable abundance in one transect. A comparison of the vegetation cover in June and again in

September showed diffuse knapweed further declining on all transects of the main study. Those transects nearest the insect release area (e.g., transects A and C) exhibited the largest declines in knapweed abundance and in September 2001 averaged 1.8% relative cover vs. 6.7% cover on the more distant transects. In terms of absolute cover, the site was 8.3% diffuse knapweed in June 2000 and 1.9% diffuse knapweed in September 2001.

Data obtained from the lightly grazed pasture about 1 km northwest from this site (Transect E) showed a somewhat different pattern (Table 3). Diffuse knapweed cover on this remote site declined from 60.2% relative cover (29.5% absolute) in 1997 to 46.8% cover (14.5% absolute) in 2000 and further downward to 24.9% relative cover (10.5% absolute) by June 2001. This decline probably related primarily to increasingly dry conditions. With the return of near-average moisture conditions in 2001, by late summer the cover by diffuse knapweed in this remote area had increased to 30.4% relative cover (17.0% absolute). In contrast, in the main (i.e., insect treated) study area, diffuse knapweed cover defied this trend of rebound and showed an overall decline. This average decline included a steep decline in diffuse knapweed cover at Transect A within the release site (12.7% relative cover, 4.5% absolute) in June 2001, dropping to 2.7% relative cover (1.0% absolute) in September 2001. Diffuse knapweed cover in the next nearest transect (C) continued to be below measurable abundance in September 2001, as it had been in June 2001. Transects further away from the insect release site (B, D) showed a much muted version of the overall rebound of diffuse knapweed in the more distant site, Transect E.

Mowing that was initiated in 1997 apparently increased diffuse knapweed flowering stem densities in 1998 through 2000 (Figure 2). Mowing was terminated in 2000, and flowering stem densities in this area (produced from plants that germinated under the mowing regime) declined in 2001. Flowering stem densities at the insect release site, Site 1, remained fairly constant from 1997 through 1999. However, in 2000, flowering plants at Site 1 increased to densities three times the number observed in 1997, and stem densities in both the mowed site and Site 1 in 2000 were significantly greater than those in Site 2 (SNK; $P < 0.05$; Figure 2A). The peak densities observed in 2000 were followed by low densities in 2001.

Diffuse knapweed plants at our main study area, while dominating plant cover through 2000, were relatively small compared with those observed on adjacent disturbed areas and roadside sites. Diffuse knapweed seedhead numbers per plant were low, and average seedhead numbers per plant declined at Sites 1 and 2 throughout the study interval (Figure 2B). Plants mowed once per season, during the initiation of flowering, were able to regenerate about half the seedheads observed in unmowed plants. Numbers of seedheads per plant measured at Site 1 in 2000 were similar to those averages found in the mowed site and were significantly reduced when compared with those for Site 2 (SNK; $P < 0.05$; Figure 2B). By 2001, seedheads per plant at Site 2 declined to levels matching those of the other sites.

Seed production per seedhead declined from an average of about four seeds per seedhead in 1997 to about 0.6 seeds per seedhead in 2001 (Figure 2C). Seeds per seedhead of diffuse knapweed were substantially reduced at Site 1 relative to the other sites in 2000, and seeds per seedhead did not show a significant decline at Site 2 until 2001 (Figure 2C). Mowing significantly reduced the average number of seeds per seedhead to about 60% of the Site 2 values. The 2000 results indicated that all treatments were significantly different from one another (SNK of log-transformed data; $P < 0.05$). By 2001, however, seed production per seedhead was again similar among all sites within the study area.

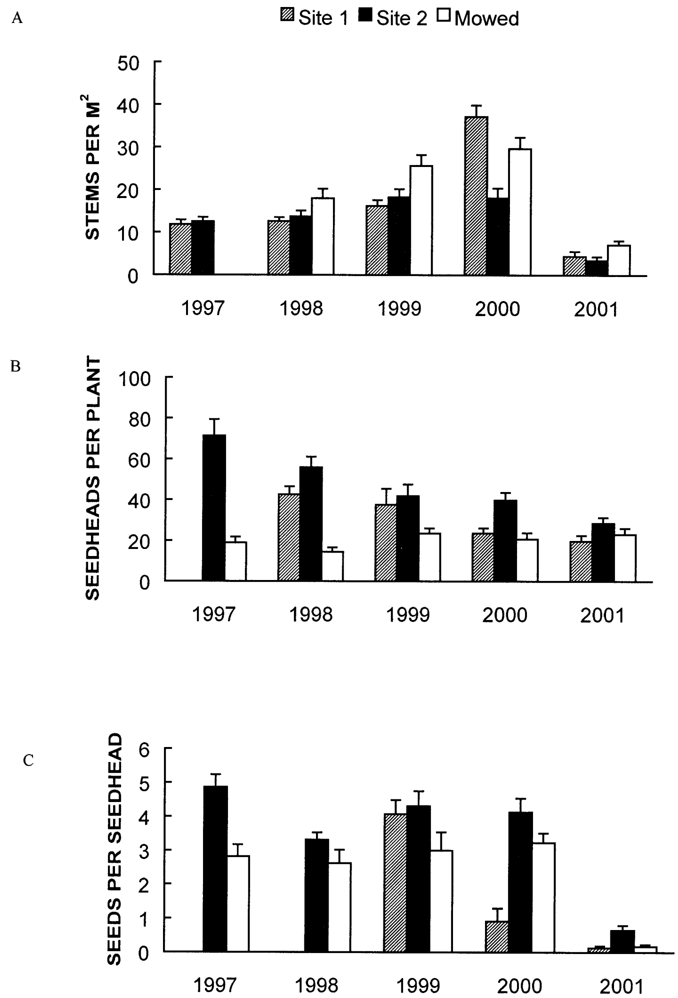

FIGURE 2. Stem densities (A), seedhead production per plant (B), and seeds per seedhead (C) from diffuse knapweed (*Centaurea diffusa* Lam.) in 1997–2001. Values shown are means ± one SE of 30 samples per site per year.

Seeds per square meter were calculated by using the above data, i.e., stems per square meter times seedheads per stem times seeds per seedhead (Figure 3). The uncertainty associated with this estimate is confounded by collection procedures, and no estimates of the error associated with these values were attempted. Nonetheless, the data show trends expected from the dominant patterns seen in Figure 2. Seeds per square meter at Site 2 declined after the first year of study from about 5,000 to 3,000 seeds m^{-2} between 1998 and 2000 and then to less than 100 seeds m^{-2} in 2001. This result was initially caused by the large decline in seedheads per plant and then by the large reduction in seeds per seedhead observed in 2001. Seed production in the mowed area increased through time, consistent with increases in stem densities and the relatively constant seedhead per plant average observed in this area. Seed production at Site 1 was

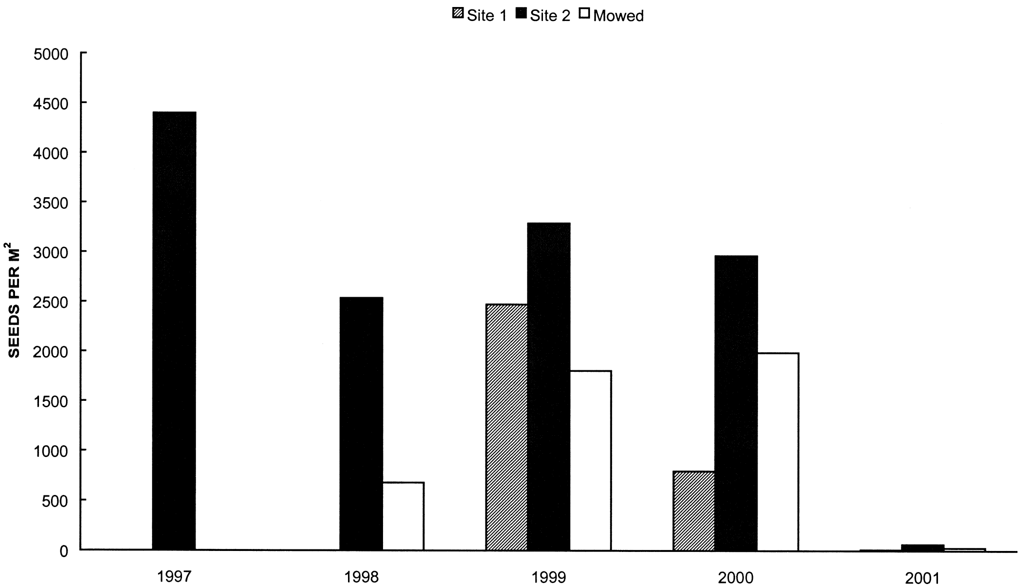

FIGURE 3. Estimates of seed density (seeds m^{-2}) in 1997–2001. Values shown are calculated from data shown in Figure 2.

measured only for the last 3 yr of the study and indicated a decline from about 2,500 seeds m^{-2} in 1999 to under 100 seeds m^{-2} in 2001. These numbers initially reflected the small size of the plants and the low average of seeds per seedhead found in that area. By 2001, the low number of flowering plants per square meter combined with the other characteristics to greatly reduce the reproductive potential of this weed.

Rosette densities declined from about 50 rosettes m^{-2} observed in the autumn of 1997 in Site 2 to only about 15 plants m^{-2} in that area in autumn 2000 and to less than 3 rosettes m^{-2} in the summer of 2001 (Figure 4). Site 1, monitored only after 1999, contained an average of 11 rosettes m^{-2} in the autumn of 2000, which produced less than three bolting plants and three rosettes by July 2001. Rosette counts in the spring of 2002 indicated only slightly more than two rosettes on the release site and about nine rosettes on the reference site. These findings suggest little change in the density of flowering diffuse knapweed in 2002.

Insect Abundance

Urophora spp. numbers in seedheads were more abundant in mowed plots during several of the years (Figure 5). These data reflect the combined numbers of knapweed seed head flies and banded gall flies, although adults of the latter species were not observed on the site until 1999. *Urophora* spp. densities in seedheads on the mowed site through 2000 were significantly larger than those found on the other sites (SNK test; $P < 0.05$). Presumably, the relatively late flowering of plants in the mowed area was particularly attractive to a

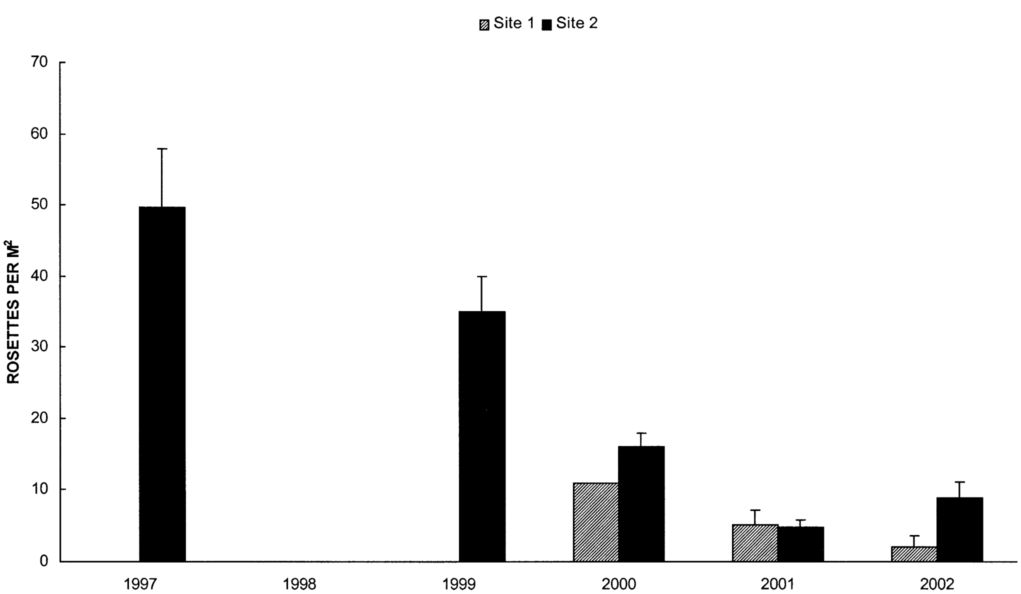

FIGURE 4. Rosette densities (number m^{-2}) in 1997–2001. Values shown are means ± one SE (n = 30 per date, except in 1999 when n = 8). Data for 1997–2000 were collected in autumn and for 2001–2002 in late spring and include bolting rosettes.

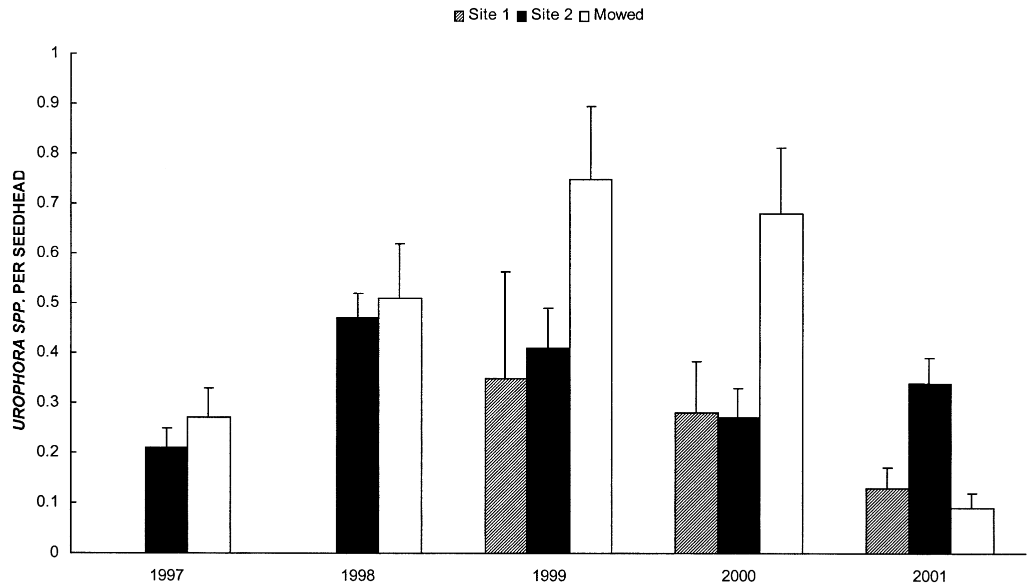

FIGURE 5. *Urophora* spp. abundance in seedheads of diffuse knapweed (*Centaurea diffusa* Lam.) in 1997–2001. Data shown are means ± one SE of 30 samples per site per date.

second generation of knapweed seed head flies, which was not the case for the banded gall fly (Rees et al. 1996).

We never observed any *Urophora* spp. in seedheads occupied by full-sized larvae of the lesser knapweed flower weevil. Average seeds per seedhead values are also strongly influenced by the presence of the lesser knapweed flower weevil (Table 4). Large numbers of lesser knapweed flower weevils had invaded Site 2 from Site 1 by 2000. Seedheads from the mowed area in 2000 exhibited the lowest lesser knapweed flower weevil numbers, and we assume that the flowering of these plants occurred after the lesser knapweed flower weevil had largely finished egg laying. This same area in 2001 was not mowed and exhibited similar values for seeds and weevils as did the other sites.

In midsummer of 2000, 71% of the 180 seedheads examined at Site 1 contained lesser knapweed flower weevils. Additional flowering by diffuse knapweed after the period of egg laying by the weevil reduced this percentage to some extent. Using our autumn seedhead data, we estimated that 54% of seedheads at Site 1 contained lesser knapweed flower weevils. This percentage declined in samples obtained away from this area. Seedheads obtained about 1 km from the release site ("reference" sites in Table 4) showed continued high numbers of seeds per seedhead in the absence of weevils. Adult lesser knapweed flower weevils feed on bolting diffuse knapweed and can do extensive damage to adult plants (Lang et al. 2000a). We observed this at several sites in Colorado, and this activity was particularly evident at our site in 2001. We believe that this herbivory on the foliage and stems of diffuse knapweed by the adults, in addition to seed predation by the weevil larvae, contributed to the very low levels of seed production observed in 2001.

Bronze knapweed root borer and knapweed root weevil larvae were estimated by inspection of roots of rosettes or flowering plants. In midsummer of 2000, all adult flowering plants in six 1-m^2 quadrats at Site 1 were censused for root damage. We found 83% (126 of the 155 plants examined) showed root damage from beetle activity, almost all of which

TABLE 4. Impact of the lesser knapweed flower weevil (*Larinus minutus* Gyllenhal) weevils on seed production and *Urophora* spp. in seedheads of diffuse (*Centaurea diffusa* knapweed Lam.) in 2000 and 2001.[a]

	All data (weevils present)			Seedheads lacking weevils	
Site	% Seedheads with weevils	Seeds per seedhead	*Urophora* per seedhead	Seeds per seedhead	*Urophora* per seedhead
2000					
Site 1	53.9	0.9 ± 0.22	0.28 ± 0.04	1.8 ± 0.29	0.59 ± 0.08
Site 2	35.5	4.1 ± 0.41	0.28 ± 0.09	6.5 ± 0.60	0.41 ± 0.08
Mowed	14.6	2.9 ± 0.22	0.59 ± 0.06	3.1 ± 0.24	0.65 ± 0.10
Reference	9.6	6.4 ± 0.38	0.74 ± 0.08	7.10 ± 0.39	0.82 ± 0.10
2001					
Site 1	56.1	0.68 ± 0.13	0.16 ± 0.03	1.53 ± 0.27	0.35 ± 0.06
Site 2	50.5	0.65 ± 0.14	0.34 ± 0.05	1.30 ± 0.27	0.69 ± 0.09
Previously mowed	60.0	0.52 ± 0.13	0.11 ± 0.04	0.42 ± 0.15	0.24 ± 0.06
Reference	18.3	5.95 ± 0.40	0.40 ± 0.06	7.29 ± 0.42	0.50 ± 0.07

[a] Values are means ± SEs. Sample size = 180 seedheads for all data, with 83, 116, 153, and 163 seedheads lacking weevils in site 1, site 2, mowed, site and reference site, respectively, in 2000 and 79, 89, 72, and 147 seedheads lacking weevils in these same sites in 2001.

TABLE 5. Knapweed root weevil (*Cyphocleonus achates* Fahraeus) and bronze knapweed root bover (*Sphenoptera jugoslavica* Obenb.) larvae in roots of bolting diffuse knapweed (*Centaura diffusa* Lam.), May 31, 2001.

Site	Sample size	Plants with larvae	*Cyphocleonus*	*Sphenoptera*
Site 1	30	28 (93%)	15 (50%)	13 (43%)
Site 2	60	27 (45%)	14 (23%)	13 (22%)
Total	90	55 (61%)	29 (32%)	26 (29%)

appeared to be the result of bronze knapweed root borer activity. In late fall of 2000, 31 of the 50 rosettes sampled at random at Site 2 were found to contain insect damage. Before the spring of 2001, less than a dozen knapweed root weevil larvae were found in diffuse knapweed rosettes or bolting plants, and only low numbers of adults of this species had been found at or near release sites. This insect is very large relative to the size of the diffuse knapweed found at our site, and we initially believed that most plants were unable to provide enough resources to complete the development of this insect. However, by late spring of 2001, these larvae were as abundant as those of the bronze knapweed root borer (Table 5). Although we have no data on the actual effect of the root-feeding insects, the decline in rosette numbers observed at the release site is correlated with the abundance of these insects. Previous studies (e.g., Powell and Myers 1988) suggest that seed production is negatively affected by root feeders, and the seed reduction in seedheads not affected by lesser knapweed flower weevil larvae at Site 1 in 2000 (Table 4) supports this contention.

The spotted knapweed seedhead moth did not establish at our site. After the release of adults in the summer of 1997, we found no indication that seedheads were used by the moth larvae, and no adults were ever recovered.

Combined Biocontrol Effects on Diffuse Knapweed

Our reference transect vegetation data from a moderately grazed prairie (Transect E; Table 3) indicated that absolute diffuse knapweed cover in September 2001 was about 121% of the June 2000 values. In contrast, diffuse knapweed cover in the insect study during the same period declined by 77%, from 8.3 to 1.9% of absolute cover. The reduction was larger in transects nearest the insect release areas.

Monitoring data suggest that insect population densities of *Larinus*, *Sphenoptera*, and *Cyphocleonus* increased in an exponential manner, and effects from these species were likely apparent in our seed production data measured at the release site by 2000 (Table 4). The following year, effects were believed to be evident for all the parameters measured here (Figure 2). Rosette numbers may have declined because of climatic factors as well as insect activities, as suggested by the decline in absolute cover at our reference transect site between 1997 and 2000. However, rosette densities observed at the release site in 2001 and 2002 do appear to be influenced by insect numbers.

The rather interesting and unexpected increase in diffuse knapweed stem densities on the insect release area appeared to be a transient response to insect release (Figure 2). Increased mortality in one part of a weed's life cycle due to management or biocontrol activities can result in increased survivorship at other stages of the life cycle (Myers et al. 1990). Mowing apparently caused a similar response, with this management activity producing increased densities of flowering plants. Mowing also has been reported to enhance the survivorship of flowering plants through a second growing season, but this response did not appear common at our site.

The success of diffuse knapweed is attributed in part to its high reproductive output and relatively high seedling survival. The plant has been reported to produce an average of about 13 seeds per seedhead (Watson and Renney 1974) and can produce anywhere from 1,000 (Strang et al. 1979) to up to 18,000 seeds per plant (Harris and Cranston 1979). Compared with these reports, plants at our site were small, even in 1997, and were likely constrained in their growth by some combination of water and nutrient limitations in addition to the damage inflicted by *Urophora* spp. Following the conceptual model of McEvoy and Coombs (1999), we envision that, in addition to the potential of enhanced mortality of seeds, rosettes, and even bolting plants, biocontrol insects limit the ability of knapweeds to exploit available water and nutrients, thereby allowing other plants to be better competitors for limiting resources. In theory, this increased competition from other plant species further reduces the survivorship and reproduction of the target species. However, not all biocontrols function in this manner (Callaway 1999), and compensatory responses by diffuse knapweed to moderate herbivory might be expected. Here, however, the decline in cover, stem densities, and seed production observed on the study area relative to the nearby reference site suggests a strong negative cumulative effect of these biocontrol insects.

The decline in the viability of diffuse knapweed appeared to be particularly enhanced by activities of the lesser knapweed flower weevil. Management activities to enhance their numbers and effects should perhaps receive more emphasis. Diffuse knapweed that develops later in the growing season and flowers after the egg-laying interval of lesser knapweed flower weevil can potentially escape seed predation by this insect. Mowing of diffuse knapweed just before flowering allowed plants to regrow and flower after the period of egg laying by lesser knapweed flower weevil had largely terminated (Table 4). Hence, mowing may not be appropriate if control by lesser knapweed flower weevil is desired. Mowing, however, does offer a refugium for *Urophora* (probably knapweed seed head fly; Table 4), and mowing adjacent to insect release sites, while waiting for insects to become established in high numbers, will also keep the plant from tumbling off-site. Grazing of bolting plants by cattle, sheep, or goats or even clipping by prairie dogs may cause a similar phenological shift in flowering of diffuse knapweed. Early-summer grazing may therefore be undesirable if lesser knapweed flower weevil is identified as a key biocontrol for diffuse knapweed. Damage by bronze knapweed root borer and knapweed root weevil to rosette roots also is believed to affect the phenology of flowering in diffuse knapweed. In particular, the time that the plant remains as a rosette may be extended. At present, we cannot state if bronze knapweed root borer or knapweed root weevil enhanced or diminished the biocontrol effect of lesser knapweed flower weevil in terms of seed destruction.

Multiple species of insects are believed to be necessary to reduce the reproduction output of diffuse knapweed below replacement levels (Muller-Scharer and Schroeder 1993). The extensive literature on biocontrol effects on diffuse knapweed and our own observations support this contention. At the same time, the number of biocontrol species used against a weed species should be minimized to reduce interference effects among biocontrols or risks of nonindigenous species additions to nontarget species (McEvoy and Coombs 1999). From our results we speculate that lesser knapweed flower weevil or the combination of lesser knapweed flower weevil and knapweed root weevil might be sufficient to reduce diffuse knapweed as effectively as the five species observed here.

After 4 yr, and using small releases of biocontrols, five species of knapweed-feeding insects (banded gall fly, knapweed seed head fly, lesser knapweed flower weevil, bronze knapweed root borer, and knapweed root weevil) were believed to have substantially affected the invasive characteristics of diffuse knapweed at a single site along the Colorado Front Range. The extent to which the exclusion of cattle contributed to this reduction remains unknown. Other local studies (Beck and Rittenhouse 2001) and our reference transect data on a grazed site suggest, however, that effects of light grazing by cattle on diffuse knapweed abundance, if present, are at most modest. Our study has shown that—for one ungrazed grassland site east of the Colorado Front Range—biocontrol insects reduced densities of established populations of diffuse knapweed. Whether this response can be sustained and replicated elsewhere remains a focus of ongoing and future research.

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