

1 Running head: Seastedt et al. Reduction in diffuse knapweed by insects

2

3 **Impact of Biocontrol Insects on Diffuse Knapweed in a Colorado Grassland**

4

5 T. R. Seastedt

6 corresponding author, email: timothy.seastedt@colorado.edu

7 Institute of Arctic and Alpine Research,

8 University of Colorado,

9 Boulder, CO 80309-0450

10

11 Nathan Gregory

12 Department of Environmental, Population, and Organismic Biology

13 University of Colorado,

14 Boulder, CO 80309-0334

15 and

16 David Buckner

17 ESCO Associates Inc.

18 P.O. Box 18775

19 Boulder Colorado 80308

20

21 _____

22

1 *Abstract*

2 Four species of biocontrol insects, (*Cyphocleonus achates*, *Larinus minutus*, *Metzneria*
3 *paucipunctella*, and *Sphenoptera jugoslavica*) were released at a diffuse knapweed (*Centaurea*
4 *diffusa*) site located about 10 kilometers east of the Colorado Front Range. Two other biocontrols
5 (*Urophora affinis*, *U. quadrifasciata*) were already present at this site. Densities of rosettes and
6 flowering plants, seedhead production per plant, and seeds per seedhead on mowed and
7 unmowed areas were studied for a five-year interval, 1997-2001. Of the six biocontrols, five
8 (*Urophora* spp., *S. jugoslavica*, *C. achates*, and *L. minutus*) obtained sizable densities relative to
9 weed abundance. Knapweed declined from 8.3% in absolute cover in June 2000 to 1.9% by
10 September 2001. Vegetation transects closest to the insect release areas showed the largest
11 declines, with knapweed disappearing entirely on one transect. In contrast, knapweed
12 abundance at a nearby prairie increased during the same interval from an absolute cover value of
13 14.5% to 17%. Seed production of knapweed on the insect release site declined from nearly
14 5000 seeds per m² produced in 1997 to less than 100 seeds per m² in 2001. *Larinus minutus*
15 larvae appeared responsible for much of the seed reduction, while adults of this species were
16 effective in damaging bolting plants. The extent to which grazing removal and individual insect
17 species contributed to this reduction in knapweed abundance cannot be identified from this study.
18 These results support the contention that a significant reduction in abundance of knapweed using
19 insects is possible at least in some regions of the western United States.

20

21 **Nomenclature:** diffuse knapweed; *Centaurea diffusa* Lam. CENDI; *Urophora affinis*, banded
22 gall fly; *U. quadrifasciata* knapweed seed head fly; *Sphenoptera jugoslavica*, bronze knapweed
23 root borer; *Cyphocleonus achates*, knapweed root weevil; *Metzneria paucipunctella*, spotted
24 knapweed seedhead moth; *Larinus minutus*, lesser knapweed flower weevil.

25

26 **Key words:** Biological control, compensatory growth, population dynamics

27

28

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

14 Insect herbivory may offer a potential inexpensive, relatively low-risk, self-perpetuating
15 mechanism for reducing the abundance of certain weeds that is particularly suited to the
16 management of large areas (Mack et al. 2000), grasslands of low economic value (Maddox,
17 1979), or in areas containing desirable species sensitive to other management options. However,
18 demonstrations of reductions of established populations of such weeds as diffuse knapweed
19 remain few, either because the weed simply cannot be controlled by these insects or the right
20 combination of biocontrol insects has not been attempted (McEvoy and Combs 1999).

21 Two flies introduced from Europe, *Urophora affinis* Frauenfeld, the banded gall fly, and
22 *U. quadrifasciata* Meigen, the knapweed seed head fly, (Diptera:Tephritidae), have been used in
23 North America for several decades to reduce seed production in diffuse and spotted knapweed.
24 *Sphenoptera jugoslavica* Obenb., (Coleoptera: Buprestidae), a root feeder of knapweed rosettes,
25 has also been used as a biocontrol in North America for over 20 years. *Cyphocleonus achates*
26 Fahraeus (Coleoptera: Curculionidae), another root feeder of knapweed rosettes, was released in
27 the U.S. in the late 1980s. The spotted knapweed seedhead moth, *Metzneria paucipunctella* Zell.
28 (Lepidoptera: Gelechiidae), was released in Montana in 1980 and has been established in three

1 states (Lang et al. 2000a). A more recent introduction is *Larinus minutus* Gyllenhal (Coleoptera:
2 Curculionidae), a weevil that feeds on seedheads, which was released in the U.S. in 1991 and is
3 now found in 10 states (Lang et al. 2000a). *Larinus minutus* effects on spotted knapweed have
4 been documented (Lang et al., 2000b), but studies documenting the impact of *L. minutus* or that
5 of *Cyphocleonus achates* and *Metzneria paucipunctella* on diffuse knapweed are few.

6 These insect species collectively offer some hope for reduction of diffuse and spotted
7 knapweed (Rees et al. 1996). However, knowledge of the impacts of these biocontrols, if
8 available at all, is limited to more northerly prairies that differ substantially in biological and
9 physical characteristics than those found in the Front Range of Colorado. Further, the combined
10 effects of several of these insects, particularly *L. minutus* and *C. achates*, have yet to be reported
11 in the literature.

12 We measured diffuse knapweed densities and seed production over five growing
13 seasons for a prairie site invested with diffuse knapweed. Our study site was not grazed during
14 this interval, and mowing on a portion of the site was used as a mechanical treatment designed to
15 stress the knapweed and limit tumbling of the weed. We also were interested in how mowing, a
16 common management practice for knapweed along roadsides, might impact use of the plants by
17 insects. We monitored the abundance of *Urophora* spp., and the fate of small releases of *M.*
18 *paucipunctella*, *S. jugoslavica*, *C. achates*, and *L. minutus*. The extent to which insects were able
19 to establish, use the knapweed resource, and impact knapweed population dynamics on mowed
20 and unmowed areas were concurrently measured. The present study did not assess individual
21 insect species impacts, but monitored the relative success of these populations and quantified the
22 combined effect of grazing removal and insect release on the relative abundance of knapweed
23 and other vegetation.

24

25

Site Description and Methods

26

27

28

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 (Moreland
and Moreland 1975). This site was a degraded pasture, and diffuse knapweed composed almost

1 30% of the plant cover in 1997. Native grasses composed a modest component of the vegetation,
2 while nonnative species such as annual peppergrass or Alyssum (*Alyssum parviflorum*),
3 Japanese brome (*Bromus japonicus*), and bindweed (*Convolvulus arvensis*) were common. The
4 vegetation composition and cover provided by native grasses, native forbs (non-woody dicots),
5 diffuse knapweed, and other introduced plant species were quantified along four, 50 m point-
6 intercept transects. An additional transect was established at a nearby prairie site experiencing
7 moderate grazing. Along each transect, two hundred points were projected downward using an
8 optical device with a cross-hair. The device was mounted on a tripod and was designed to direct
9 the line of view 50 cm to the side of the transect to avoid vegetation affected by the measuring
10 tape used to construct the transect. The identity of each species (or bare soil, rock, litter or
11 standing dead) was recorded at each point. From these cover data, for purposes of this study,
12 the relative cover of the four plant groups listed above as well as absolute cover values were
13 summarized.

14 The main research site had been heavily grazed by cattle up until the initiation of the
15 research in June, 1997, but was not grazed during the study. The area was divided into three
16 areas that were monitored for knapweed response (Figure 1). A single mowing event per year
17 was done on a strip of prairie with a tractor in the third or fourth week of June, just prior to or
18 during the initiation of flowering of the knapweed. Mowing is known to reduce seed production
19 and alter the phenological development of the plant (Roche and Roche 1998). Plants tend to
20 sprout new growth from damaged stems, and flower later in the growing season. This treatment
21 was terminated after mowing in 2000. An area adjacent to the mowed site was selected as the
22 central release point for insects, and is referred to as site 1. This area included a vegetation
23 transect (transect #1). A second unmowed site (site 2) was selected about 300 m away from
24 the main insect release site, and included a second vegetation transect (transect #2). A third
25 vegetation transect was located about 200 m from transect 1 and was adjacent to the insect
26 release site. Transect 4 was located about 600 meters from the release area (Figure 1). An
27 additional transect (not shown in Figure 1) was located about 1 km from the insect release area.
28 This reference site was selected because of similarities in vegetation composition to the main

1 study area. Surveys were conducted in June of 1997, 2000, and 2001 and also in September of
2 2001.

3 *Urophora affinis* and *U. quadrifasciata* were released in the Front Range in 1989, but *U.*
4 *quadrifasciata* may have arrived previously on its own (Jerry Cochran, Colorado Department of
5 Agriculture Biocontrol Division, personal communication). While both species were assumed to
6 be present on our sites from the initiation of research in 1997, only *U. quadrifasciata* was
7 observed as adults in the field or emerged from seedheads kept in the laboratory through 1998.
8 Other species of biocontrols were obtained from insectaries and released at the site (Table 1). A
9 lack of a local source of insects precluded larger releases of *L. minutus* and *C. achates*.
10 *Sphenoptera jugoslavica* were available from an insectary established on the Front Range by
11 Colorado Department of Agriculture Biocontrol Division personnel. In addition to these insects,
12 2000 adult *Metzneria paucipunctella*, the spotted knapweed seed head moth, were obtained from
13 a source in Montana and released in the summer of 1997.

14 Knapweed flowering stem and rosette densities were sampled using 1 m² quadrats
15 selected at random. Within each of the three areas, a marker was thrown in a general direction
16 (i.e. "over the shoulder") and the quadrat was placed along a fixed direction with the marker
17 forming the center of the quadrat. Flowering stem densities were estimated by counting stems
18 from 30 quadrats on one or more of the three sampling areas of each year (Table 2). Rosette
19 densities were counted using similar quadrats sampled in a similar way. Inventories were usually
20 taken in October, when plants that had germinated earlier in the summer were large enough to
21 identify. These rosette estimates did not include small seedlings, if present, that had yet to form
22 the characteristic rosette morphology. A counts of rosettes were also made in June of 2001 and
23 April, 2002 (Table 2). Seedhead numbers per plant were obtained by randomly selecting 30
24 individual plants within the three treatment areas and counting all seedheads on the selected
25 plant. Again, we used a marker thrown in a general area, and the specific flowering plant counted
26 was the one whose stem was closest to the marker. By using the closest stem rather than the
27 closest branch, smaller plants were adequately represented. Seeds per seedhead and *Urophora*
28 spp. per seedhead were sampled by removing at least six developed seedheads from each of 30

1 plants selected at random from the three areas. A preliminary study conducted by one of us in
2 1997 (N. Gregory, unpublished results) indicated that neither seed number nor *Urophora* counts
3 were affected by the location of the seedheads on plants. Up until the 2000 field season, we
4 attempted to sample only mature seedheads that retained intact petals. This minimized seed loss
5 either before collection or during handling prior to counts. However, extensive weevil activity by
6 2000 required that a random sample of seedheads, irrespective of intact petals, be obtained.
7 With one exception, collection of these seedheads occurred during plant senescence in
8 September to ensure that the second generation of *U. quadrifasciata* was visible within the
9 seedheads (Table 2). We dissected seed heads and counted the number of larvae, remains of
10 pupae, and seeds with the aid of a dissecting scope. Evidence for individuals of the first
11 generation of *U. quadrifasciata* were obtained by counting remains of pupae. By 2000, we
12 recognized that *L. minutus* was impacting all of our initial three sampling areas, so we expanded
13 our seedhead counts to include knapweed from adjacent pastures close to the reference
14 vegetation transects that were grazed by cattle but not heavily impacted by insects other than
15 *Urophora* spp.

16 Statistical inferences from random samples obtained within each of the three knapweed
17 sample areas are valid for comparisons within this site (Wester 1992) as well as for identifying
18 numerical trends in insect numbers and weed densities through time. Statistical procedures
19 employed an ANOVA or t-test after first checking for homogeneity of variances. To test for
20 variation in seed production as a function of treatment, data obtained from individual seedheads
21 were first averaged on a per-plant basis before statistical analyses. This averaging was also
22 done on estimates of *Urophora* spp. abundance in seedheads. *Urophora* spp. exhibited
23 aggregated distributions in seedheads, so data on fly abundance per seed head were
24 transformed using a square-root transformation before statistical analyses were employed to
25 evaluate changes through time or among treatments. If statistical significance was reported for
26 the entire model containing more than two treatments, tests for individual differences were
27 conducted using the Student-Newman-Keuls (SNK) test (SAS 1988).

28

Results and Discussion

Plant Responses to Insect Release

As of the end of June 1997, the precipitation for the calendar year recorded for Boulder, Colorado, was 46% 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. Hence, conditions for plant growth in 1997 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 1997 values (Table 3). Diffuse knapweed cover declined by 41% while cover by other nonnative species dropped by 84%. Since cover by diffuse knapweed declined in 2000 less than the decline in 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 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 knapweed jumped in 2001 to a level 40% greater than 1997. An example of the latter pattern, intermediate wheatgrass (*Thinopyrum intermedium*), and introduced grass notable for its weed-like aggressiveness in the Front Range, averaged 1.0 percent 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% in cover. However, diffuse knapweed cover exhibited an opposite trend to the 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 knapweed further declining at all transects on the main study. Those transects nearest the insect release area (e.g., transects 1 & 3) exhibited the largest declines in knapweed abundance and in September of 2001 averaged 1.8% relative cover versus 6.7% cover at the more distant transects. In terms of absolute cover, the site was 8.3% knapweed in June of 2000 and 1.9% knapweed in September of 2001.

1 Data obtained from a lightly grazed pasture about 1 km from this site showed a
2 somewhat different pattern (Table 3). That site had 60.2% relative cover by knapweed in 1997,
3 which declined to 46.8% cover in 2000 and to 24.9% relative cover in June, 2001. In contrast to
4 the main study area, knapweed showed a recovery to 30.4% of relative cover by September. In
5 terms of absolute cover, the reference site was 14.5% knapweed in 2000 and 17.0% knapweed
6 in September, 2001.

7 Mowing that was initiated in 1997 apparently increased knapweed flowering stem
8 densities in 1998 through 2000 (Figure 2). Mowing was terminated in 2000, and flowering stem
9 densities in this area (produced from plants that germinated under the mowing regime) declined
10 in 2001. Flowering stem densities at the insect release site, site 1, remained fairly constant from
11 1997 through 1999. However, in 2000, flowering plants at site 1 increased to densities three
12 times the number observed in 1997, and stem densities in both the mowed and site 1 in 2000
13 were significantly greater than site 2 (SNK, $P < .05$, Figure 2A). The peak densities observed in
14 2000 were followed by low densities in 2001.

15 Knapweed plants at this site, while dominating plant cover through 2000, were relatively
16 small compared to diffuse knapweed observed on adjacent roadside sites. Knapweed seedhead
17 numbers per plant were low, and average seedhead numbers per plant declined at sites 1 and 2
18 throughout the study interval (Figure 2B). Plants mowed once per season, during the initiation of
19 flowering, were able to regenerate about half of the seedheads observed in unmowed plants.
20 Numbers of seedheads per plant measured at site 1 in 2000 were similar to those averages found
21 in the mowed site and were significantly reduced from site 2 (SNK, $P < .05$, Figure 2B). By 2001,
22 seedheads per plant at site 2 declined to levels matching those of the other sites.

23 Seed production per seedhead declined from an average of about 4 seeds per seedhead
24 in 1997 to about 0.6 seeds per seedhead in 2001 (Figure 2C). Seeds per seedhead of knapweed
25 were substantially reduced at site 1 relative to the other sites in 2000. Seeds per seedhead did
26 not show a significant decline at site 2 until 2001 (Figure 2C). Mowing significantly reduced the
27 average number of seeds per seedhead to about 60% of site 2 values. The 2000 results indicated
28 that all treatments were significantly different from one another (SNK of log-transformed data, $P <$

1 .05). By 2001, however, seed production per seedhead was again similar among all sites within
2 the study area.

3 Seeds per m² were calculated by using the above data, i.e., stems per m² times
4 seedheads per stem times seeds per seedhead (Figure 3). The uncertainty associated with this
5 estimate is confounded by collection procedures, and no estimates of the error associated with
6 these values were attempted. Nonetheless, the data show trends expected from the dominant
7 patterns seen in Figure 2. Seeds per m² at site 2 declined after the first year of study from about
8 5000 to 3000 seeds per m² during the 1998-2000 interval, and then to less than 100 seeds per m²
9 in 2001. This result was initially caused by the large decline in seedheads per plant and then by
10 the large reduction in seeds per seedhead observed in 2001. Seed production in the mowed area
11 increased through time, consistent with increases in stem densities and relatively constant
12 seedhead per plant average observed in this area. Seed production at site 1 was measured only
13 for the last three years of the study and indicated a decline from about 2,500 seeds per m² in
14 1999 to under 100 seeds per m² in 2001. These numbers initially reflect the small size of plants
15 and the low average of seeds per seedhead found in that area. By 2001, the low number of
16 flowering plants per m² combined with the other characteristics to greatly reduce the reproductive
17 potential of this weed.

18 Rosette densities declined from about 50 rosettes per m² observed in the autumn of 1997
19 in site 2 to only about 15 plants per m² in that area in autumn 2000, and to less than 3 rosettes
20 per m² in the summer of 2001 (Figure 4). Site 1, monitored only in 2000 and 2001, contained an
21 average of 11 rosettes per m² in autumn 2000, which produced less than 3 bolting plants and
22 three rosettes by July 2001. Rosette counts in the spring of 2002 indicated only slightly more
23 than 2 rosettes on the release site and about 9 rosettes on the reference site. These findings
24 suggest little change in the density of the flowering knapweed for 2002.

25 **Insect Abundance**

26 *Urophora* spp. numbers in seedheads were more abundant in mowed plots during
27 several of the years (Figure 4). These data reflect the combined numbers of *U. quadrifasciata* and
28 *U. affinis*, although adults of the latter species were not observed on the site until 1999. *Urophora*

1 spp. densities in seedheads in the mowed site through 2000 were significantly larger than those
2 found on the other sites (SNK test, $P < .05$). Presumably, the relatively late flowering of plants in
3 mowed area was particularly attractive to a second generation of *U. quadrifasciata*, which is
4 lacking in *U. affinis*. (Rees et al. 1996).

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

12 In midsummer of 2000, 71% of 180 seedheads examined at site 1 contained *L. minutus*.
13 Additional flowering by the knapweed after the period of egg laying by the weevil reduced this
14 percentage somewhat. Using our autumn seedhead data, we estimated that 54% of seedheads
15 at site 1 contained *L. minutus*. This percentage declined in samples obtained away from this
16 area. Seedheads obtained about one km from the release site ('Reference' in Table 4) show
17 continued high seed production in the absence of weevils. Adult *L. minutus* weevils feed on
18 bolting knapweed and can do extensive damage to adult plants (Lang et al. 2000b). We observed
19 this at several sites in Colorado, and this activity was particularly evident at our site in 2001. We
20 believe that this herbivory on the foliage and stems of the knapweed by the adults, in addition to
21 the seed predation by the weevil larvae, contributed to the very low levels of seed production
22 observed in 2001.

23 *Sphenoptera jugoslavica* and *Cyphocleonus achates* larvae were estimated from
24 inspection of roots of rosettes or flowering plants. In midsummer of 2000, all adult flowering plants
25 in six, 1m² quadrats at site 1 were censused for root damage. We found 83% (126 of 155 plants
26 examined) showed root damage from beetle activity, almost all of which appeared to be the result
27 of *S. jugoslavica* activity. In late fall 2000, 31 of 50 rosettes sampled at random at site 2 were
28 found to contain insect damage. Prior to the spring of 2001, less than a dozen *C. achates* larvae

1 were found in knapweed rosettes or bolting plants, and only low numbers of adults of this species
2 had been found at or near release sites. This insect is very large relative to the size of the diffuse
3 knapweed found at our site, and we initially believed that most plants were unable to provide
4 enough resources to complete the development of this insect. However, by late spring, 2001,
5 these larvae were as abundant as *S jugoslavica* (Table 5). While we have no data on the actual
6 impact of the root-feeding insects, the decline in rosette numbers observed at the release site is
7 correlated with the abundance of these insects. Previous studies (e.g., Powell and Myers 1988)
8 suggest that seed production is negatively affected by root feeders, and the seed reduction in
9 seedheads not affected by *L. minutus* at site 1 in 2000 (Table 4) support this contention.

10 *Metzneria paucipunctella*, the spotted knapweed seed head moth, did not establish at our
11 site. Following the release of adults in the summer of 1997, we found no indication that
12 seedheads were used by the moth larvae, and no adults were ever recovered.

13 **Combined biocontrol impacts on diffuse knapweed**

14 Our reference transect vegetation data from a moderately grazed prairie indicated that
15 absolute knapweed cover in September, 2001 was about 121% of June 2000 values. In contrast,
16 knapweed cover on the insect study over the same period declined by 77% from 8.3% to 1.9%.
17 The reduction was larger from transects nearest insect release areas.

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

26 The rather interesting and unexpected increase in knapweed stem densities on the insect
27 release area appeared to be a transient response to insect release (Figure 2). Increased
28 mortality in one portion of a weed's life cycle due to management or biocontrol activities can

1 result in increased survivorship of other stages of the life cycle (Myers et al. 1990). Mowing
2 apparently caused a similar response, with this management activity producing increased
3 densities of flowering plants. Mowing has also been reported to enhance the survivorship of
4 flowering plants through a second growing season, but this response did not appear common at
5 our site.

6 The success of diffuse knapweed is attributed in part to its high reproductive output and
7 relatively high survivorship of seedlings. The plant has been reported to produce an average of
8 about 13 seeds per seedhead (Watson and Renney, 1974), and can produce anywhere from
9 1,000 (Strang et al., 1979) to up to 18,000 seeds per plant (Harris and Cranston, 1979).
10 Compared to these reports, plants at our site were small, even in 1997, and were likely
11 constrained in their growth by some combination of water and nutrient limitations in addition to
12 damage inflicted by *Urophora* spp. Following the conceptual model of McEvoy and Coombs
13 (1999), we envision that, in addition to the potential of enhanced mortality of seeds, rosettes and
14 even bolting plants, biocontrol insects limit the ability of knapweeds to exploit available water and
15 nutrients, thereby allowing other plants to be better competitors for limiting resources. In theory,
16 this increased competition from other plant species further reduces the survivorship and
17 reproduction of the target species. Not all biocontrols function in this manner, however (Callaway
18 et al. 1999), and compensatory responses by the knapweed to moderate herbivory might be
19 expected. Here, however, the decline in cover, stem densities, and seed production observed on
20 the study area relative to the nearby reference site suggests a strong negative cumulative impact
21 of these biocontrol insects.

22 The decline in the viability of knapweed appeared to be particularly enhanced by
23 activities by the seedhead weevil, *Larinus minutus*. Management activities to enhance their
24 numbers and impacts should perhaps receive more emphasis. Knapweed that develops later in
25 the growing season and flowers after the egg-laying interval of *L. minutus* can escape seed
26 predation by this insect. Mowing of knapweed just prior to flowering allowed plants to regrow and
27 flower after the period of egg laying by *L. minutus* had largely terminated (Table 4). Hence,
28 mowing may not be appropriate if control by *L. minutus* is desired. Mowing does, however, offer a

1 refugium for *Urophora* (probably *U. quadrifasciata*, Table 4) and mowing adjacent to insect
2 release sites, while waiting for insects to become established in high numbers, will also keep the
3 plant from tumbling off-site. Grazing of bolting plants by cattle, sheep, goats or even clipping by
4 prairie dogs may cause a similar phenological shift in flowering of diffuse knapweed. Early
5 summer grazing may therefore be undesirable if *L. minutus* is identified as a key biocontrol for
6 diffuse knapweed. Damage by *S. jugoslavica* and *C. achates* to rosette roots also are believed to
7 affect the phenology of flowering in knapweed. In particular, the time that the plant remains as a
8 rosette may be extended. At present we cannot state if *S. jugoslavica* or *C. achates* enhanced or
9 diminished the biocontrol impact of *L. minutus* in terms of seed destruction.

10 Multiple species of insects are believed necessary to reduce the reproduction output of
11 knapweed below replacement levels (Muller-Scharer and Schroeder 1993). The extensive
12 literature on biocontrol impacts on diffuse knapweed and our own observations support this
13 contention. At the same time, the number of biocontrol species used against a weed species
14 should be minimized to reduce interference effects among biocontrols or risks of non-indigenous
15 species additions to nontarget species (McEvoy and Coombs 1999). From our results we
16 speculate that *Larinus minutus* and *Cyphocleonus achates* might be sufficient to reduce diffuse
17 knapweed as effectively as the five species observed here.

18 After a four years, and using small releases of biocontrols, five species of knapweed-
19 feeding insects (*Urophora affinis* and *U. quadrifasciata*, *L. minutus*, *S. jugoslavica*, and *C.*
20 *Achates*) were believed to have substantially reduced the densities and invasive characteristics of
21 diffuse knapweed at a single site along the Colorado Front Range. The extent to which the
22 exclusion of cattle contributed to seedling mortality via enhanced competition from other
23 previously grazed plant species remains unknown. At our site, other nonindigenous species
24 appeared to fill the vacuum left by the reduction in the absolute cover of diffuse knapweed. Also,
25 grazing impacts, as indicated by other local studies (Beck and Rittenhouse 2001), and as shown
26 by our reference transect data on a grazed site, do not appear to enhance knapweed abundance.
27 Our study has shown that - for one ungrazed grassland site east of the Colorado Front Range -
28 biocontrol insects reduced densities of established populations of diffuse knapweed. Whether

1 this response can be sustained and replicated elsewhere remains a focus of ongoing and future
2 research.

3

4

5

6

Acknowledgments

We thank the County Commissioners and Boulder County Open Space for the use of the study site. Particularly, we would like to thank John Evans, Cindy Owsley, Laurie Deiter and Lynn Riedel for their assistance and advice. Katie Suding, Kate LeJeune, and several anonymous reviewers suggested many improvements to the manuscript. Results reported here have been obtained with the help of a number of students from the University of Colorado, and we particularly thank Kate Searle, Mariah Carbone, Megan Greening, Melissa Spannuth, and Kirsty Storey for their assistance with data collection. Boulder County provided funding for the mowing of the site, vegetation surveys, and for the 1997 releases of *Metzneria* and *Larinus*. Subsequent releases were supported by the Colorado Department of Agriculture, Biological Pest Control Section, and we thank Jerry Cochran and Fred Stahl for their efforts in helping us obtain biocontrols and for discussions about the life histories of these insects in Colorado.

Literature Cited

- 1
2 Beck, K. G. and L. R. Rittenhouse. 2001 The influence of cattle grazing on diffuse knapweed.
3 Report to City of Boulder Open Space, Boulder CO.
4
- 5 Callaway, R. M. 1999. Biological-control herbivores may increase competitive ability of the
6 noxious weed *Centaurea maculosa*. Ecology 80: 1196-1201.
7
- 8 DiTomaso, J. M. 2000. Invasive weeds in rangelands: species, impacts and management. Weed
9 Sci. 48:255-265.
10
- 11 Harris, P. 1980a. Establishment of *Urophora affinis* Frfld. and *U. quadrifasciata* (Meig.) (Diptera:
12 Tephritidae) in Canada for the biological control of diffuse and spotted knapweed.
13 *Zeitschrift fur angewandte entomologie* 89:504-514.
14
- 15 Harris, P. 1980b. Effects of *Urophora affinis* Frfld. and *U. quadrifasciata* (Meig.) (Diptera:
16 Tephritidae) on *Centaurea diffusa* Lam. and *C. maculosa* Lam. (Compositae). *Zeitschrift*
17 *fur angewandte entomologie* 90:190-201.
18
- 19 Harris, P. and R. Cranston. 1979. An economic evaluation of control methods for diffuse and
20 spotted knapweed in western Canada. *Can. J. Plant Sci.* 59:375-382.
21
- 22 Harris, P. and J. D. Shorthouse. 1996. Effectiveness of gall inducers in weed biological control.
23 *Can. Ent.* 128:1021-1055.
24
- 25 Lacey, J. R., C. B. Marlow and J. R. Lane. 1989. Influence of spotted knapweed on surface water
26 runoff and sediment yield. *Weed Technol.* 3: 627-631.
27

1 Lang, R. F., R. D. Richard, P. E. Parker and L. Wendel. 2000. Release and establishment of
2 diffuse and spotted knapweed biocontrol agents by USDA, APHIS, PPQ, in the United
3 States. *Pan-Pacific Ent* 76:197-218.
4
5 Lang, R. F., R. W. Hansen, R. D. Richard and H. Ziolkowski. 2000. Spotted *knapweed*
6 (*Centaurea maculosa* Lamarck) seed and *Urophora* spp. gall destruction by *Larinus*
7 *minutus* Gyllenhal (Coleoptera: Curculionidae) combined with *Urophora affinis* Frauenfeld
8 (Diptera: Tephritidae) and *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae).
9 Pages 735-737 in: N.R. Spencer (ed.), Proceedings of the X International Symposium for
10 Biological Control of Weeds, Bozeman Montana.
11
12 Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. Bazzaz. Biotic Invasions:
13 Causes, Epidemiology, Global Consequences, and Control. *Ecol. Appl.* 10:689-710.
14
15 Maddox, D. M. 1979. The knapweeds: their economics and biological control in the western
16 states, USA. *Rangelands* 1:139-141.
17
18 Maddox, D. M. 1982. Biological control of diffuse knapweed (*Centaurea diffusa*) and spotted
19 knapweed (*C. maculosa*). *Weed Sci.* 30:76-82.
20
21 McEvoy, P. B., and E. M. Coombs. 1999. Biological control of plant invaders: regional patterns,
22 field experiments, and structured population models. *Ecol. Appl.* 9:387-401.
23
24 Moreland, D.C. and R. E. Moreland. 1975. Soil survey of the Boulder County area, Colorado.
25 USDA Soil Conservation Service. Colorado Agricultural Experiment Station Report.
26
27 Muller-Scharer, H., and D. Schroeder. 1993. The biological control of *Centaurea* spp. in North
28 America: Do insects solve the problem? *Pesticide Sci.* 37:343-353.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Myers, J. H., C. Risley, and R. Eng. 1990. The ability of plants to compensate for insect attack: Why biological control of weeds with insects is so difficult. Pages 67-73 in: E.S. Delfosse (ed). Proc. VII Int. Symp. Biol. Control Weeds, 1988. Instituto Sperimentale per la Patologia Vegetale, M.A.F., Rome Italy.

Powell, R. D., and J. H. Myers. 1988. The effects of *Sphenoptera jugoslavica* Obenb. (Col., Buprestidae) on its host plant, *Centaurea diffusa* Lam. (Compositae). J. Appl. Ent. 106:24-45.

Rees, N. E., P. C. Quimby, Jr, G. L. Piper, E. M. Coombs, C. E. Turner, N. R. Spencer, and L. V. Knutson. 1996. Biological control of weeds in the west. Western Society of Weed Science, USDA Agric. Res. Serv., Montana Dept Agric. Montana State Univ.

Roche, B.F., and C.T. Roche. 1998. Diffuse Knapweed. Pages 217-230 in: R.L. Sheley and J.K. Petroff. (eds). *Biology and Management of Noxious Rangeland Weeds*. Oregon State University Press, Corvallis OR.

SAS Institute Inc. *SAS/STAT User's Guide*, Release 6.03 Edition. Cary, NC: SAS Institute Inc., 1988. 1028 pp.

Sheley, R. L., J. S. Jacobs and M. F Carpinelli. 1998. Distribution, biology and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technol. 12:353-362.

Strang, R. M., K. M. Lindsay, and R. S. Price. 1979. Knapweeds: British Columbia's undesirable aliens. Rangelands 1:141-143.

- 1 Watson, A. K. and A. J. Renney. 1974. The biology of Canadian weeds. Canadian Journal of
2 Plant Science 54:687-701.
3
- 4 Wester, D. B. 1992. Replication, randomization, and statistics in range research. J. Range
5 Manage. 45:285-290.
6
- 7 Woodall, C., A. Handler, and L. Broberg. 1999. Social dilemmas in grassland ecosystem
8 restoration. Ecological Restoration 18: 39-44.

1 Table 1. Releases of biocontrol insects, 1997-2000.

2

3

4 Year	Number of adults released			
5	<i>Larinus</i>	<i>Sphenoptera</i>	<i>Cyphocleonus</i>	<i>Metzneria</i>
6				
7				
8 1997	200	400	50	2000
9				
10 1998	50	250	-	-
11				
12 1999	300	850	100	-
13				
14 2000	-	-	50	-
15				

16

1 Table 2. Sampling schedule and sampling activities

YEAR	VARIABLE	SITE		
		Site 1	Site 2	Site 3
		Release	Reference	Mowed, 1997-2000
1997	Stem Densities	August	August	no data
	Seedhead counts	no data	September	September
	Seeds per Seedhead ¹	no data	September	September
	Rosettes	no data	October	no data
1998	Stem Densities	September	September	September
	Seedhead counts	September	September	September
	Seeds per Seedhead ¹	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 ¹	September	September	September
	Rosettes	no data	October	no data
2000	Stem Densities	September	September	September
	Seedhead counts	September	September	September
	Seeds per Seedhead ¹	September	late July	September
	Rosettes	October	October	no data
2001	Stem Densities	August	August	August
	Seedhead counts	August	August	August
	Seeds per Seedhead ¹	August	September	September
	Rosettes	June	June	no data
2002	Rosettes	April	April	no data

1. *Urophora* spp and *Larinus* abundance also estimated from these data.

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

Vegetation	Transect 1		Transect 2		Transect 3		Transect 4		Average		Reference ¹	
	Relative Absolute Cover	Cover	Relative Absolute Cover	Cover	Relative Absolute Cover	Cover	Relative Absolute Cover	Cover	Relative Absolute Cover	Cover	Relative Absolute Cover	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	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 ²	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 ²	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 ²	100	35.5	100	42.0	100	56.5	100	54.5	100.1	47.1	100	58.5
----- Sept. 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 ²	100	36.5	100	40.0	100	49.5	100	53.5	100	44.9	100	56.0

1. Transect located about 1 km from main research area.

2. Total vegetation cover includes succulents and shrubs not shown.

1 Table 4. Impact of *Larinus minutus* weevils on seed production and *Urophora* spp. in
 2 seedheads of *Centaurea diffusa* in 2000 and 2001.

4 Site	5 All Data (Weevils Present)			6 Seedheads Lacking Weevils	
	7 % Seedheads 8 with weevils	9 Seeds per 10 Seedhead	11 <i>Urophora</i> per 12 Seedhead	13 Seeds per 14 Seedhead	15 <i>Urophora</i> per 16 Seedhead
17 -----2000-----					
18 Site 1	53.9	0.9 ± .22	0.28 ± .04	1.8 ± .29	0.59 ± .08
19 Site 2	35.5	4.1 ± .41	0.28 ± .09	6.5 ± .60	0.41 ± .08
20 Mowed	14.6	2.9 ± .22	0.59 ± .06	3.1 ± .24	0.65 ± .10
21 Reference	9.6	6.4 ± .38	0.74 ± .08	7.10 ± .39	0.82 ± .10
22 -----2001-----					
23 Site 1	56.1	0.68 ± .13	0.16 ± .03	1.53 ± .27	0.35 ± .06
24 Site 2	50.5	0.65 ± .14	0.34 ± .05	1.30 ± .27	0.69 ± .09
25 Previously Mowed	60.0	0.52 ± .13	0.11 ± .04	0.42 ± .15	0.24 ± .06
26 Reference	18.3	5.95 ± .40	0.40 ± .06	7.29 ± .42	0.50 ± .07

26 Values are means ± std.errors. Sample size =180 seedheads for all data, and 83, 116, 153, and
 27 163 seedheads lacking weevils in site 1, site 2, mowed and reference sites, respectively, in 2000
 28 and 79, 89, 72, and 147 for seedheads lacking weevils in these same sites 2001.

1 Table 5. *Cyphocleonus achates* and *Sphenoptera jugoslavica* larvae in roots of bolting
2 knapweed, 31 May 2001.

3

4

5 Site	Sample size	Plants with Larvae	<i>Cyphocleonus</i>	<i>Sphenoptera</i>
7 Site 1	30	28 (93%)	15 (50%)	13 (43%)
8 Site 2	60	27 (45%)	14 (23%)	13 (22%)
9 Total	90	55 (61%)	29 (32%)	26 (29%)

10

11

12

1 Figure Legends.

2

3 Figure 1. Main study site which consisted an area that was used as the insect release area (site
4 1), and a reference area (unmowed, without insect releases; site 2), and a swath of prairie that
5 was mowed once per summer during the 1997-2000 interval. Vegetation transects were placed
6 within the release and reference areas, and at two locations outside of knapweed and insect
7 measurements. An additional vegetation transect was located about 1 km from the release area.

8

9 Figure 2. Stem densities (A), seedhead production per plant (B), and seeds per seedhead (C) of
10 *Centaurea diffusa*, 1997-2001. Values shown are means \pm one std. error of 30 samples per site
11 per date.

12

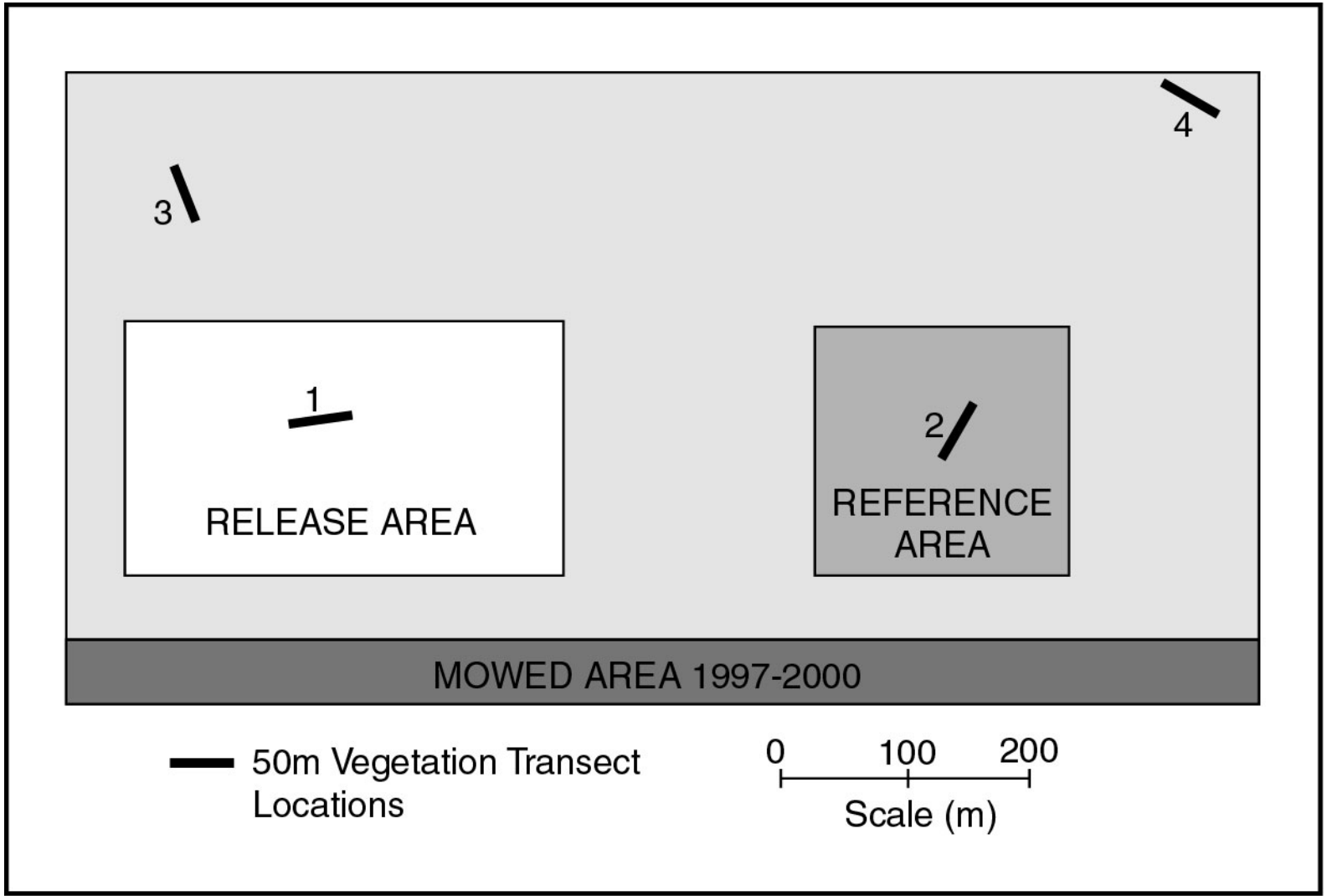
13 Figure 3. Estimates of seed density (seeds per m²), 1997-2001. Values shown are calculated
14 from data shown in Figure 2.

15

16 Figure 4. Rosette densities (number per m²), 1997-2001. Values shown are the means \pm one std.
17 error (n=30 per date, except 1999 when n=8). All data collected in autumn, except for 2001,
18 which was collected in late spring and includes bolting rosettes.

19

20 Figure 5. *Urophora* spp. abundance in seedheads of diffuse knapweed, 1997-2001. Data shown
21 are means \pm one std. error of 30 samples per site per date.



1
2 Figure 1

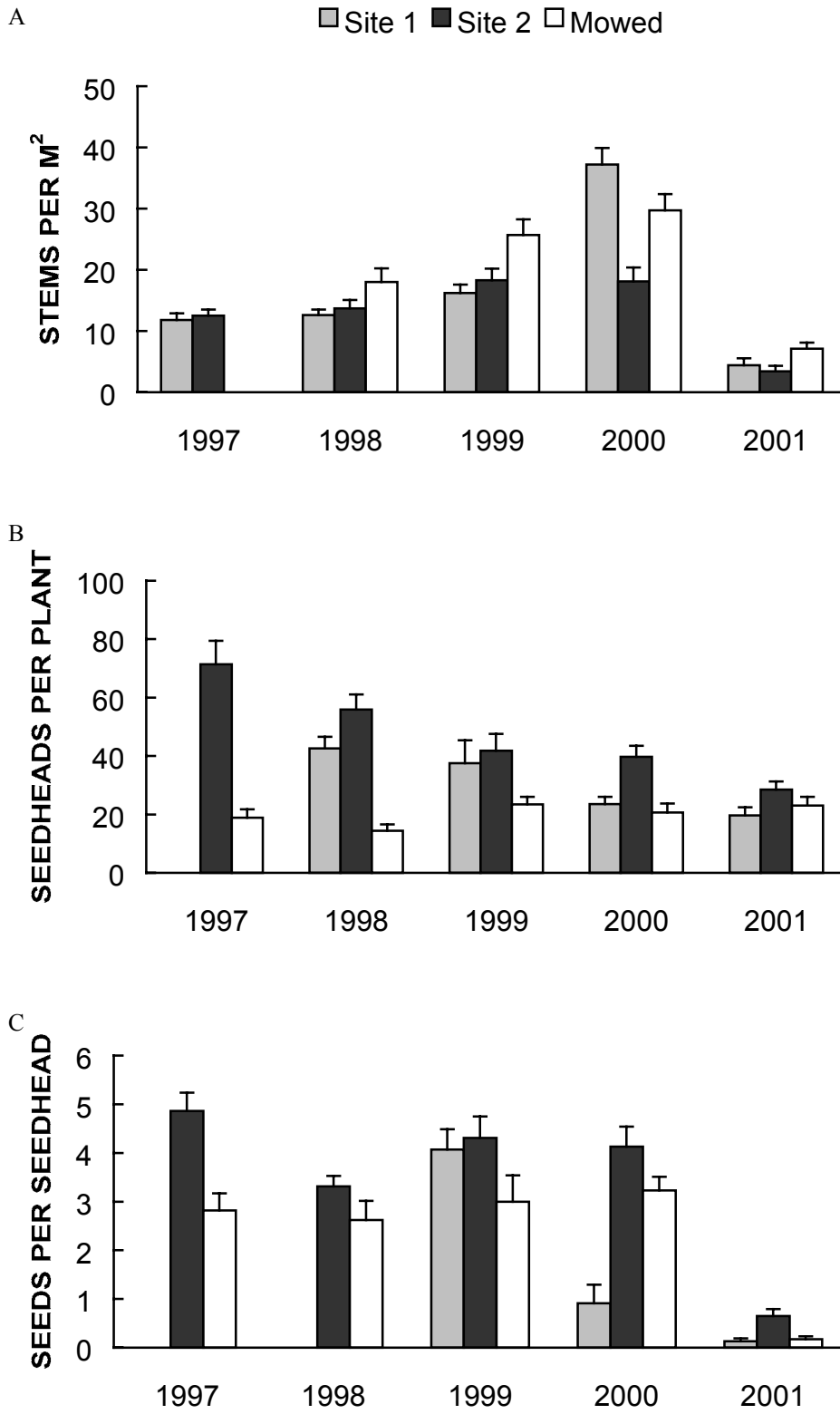
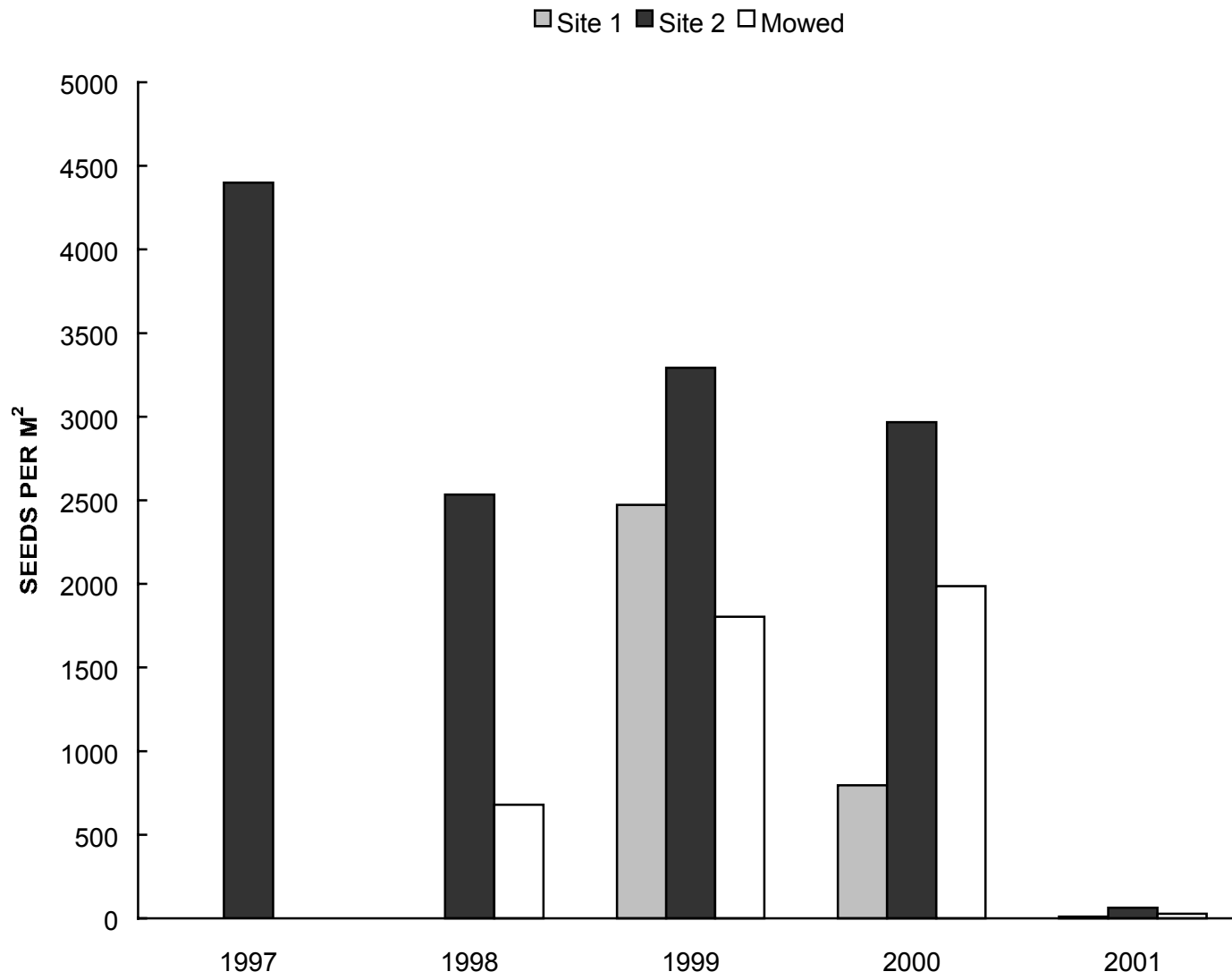
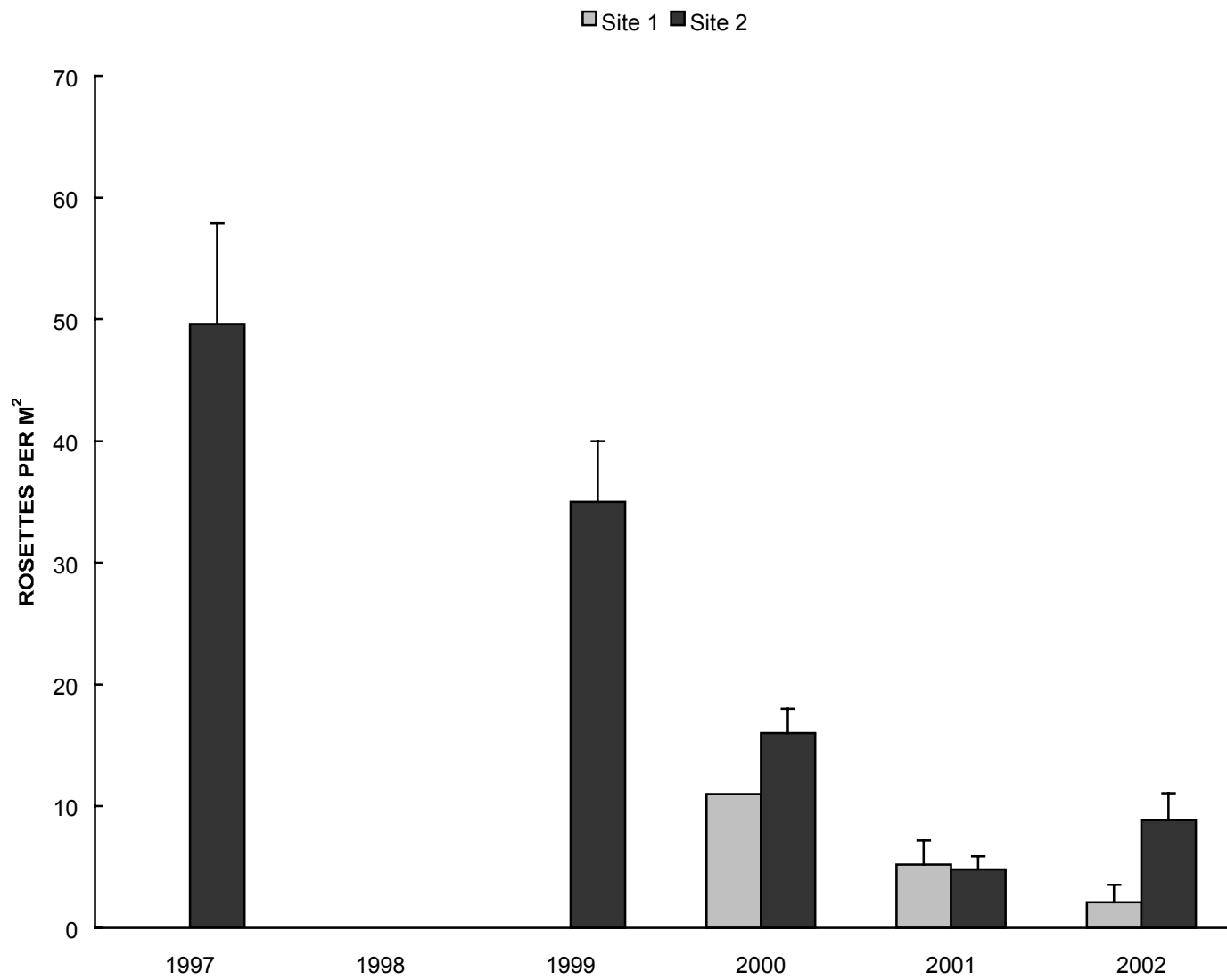


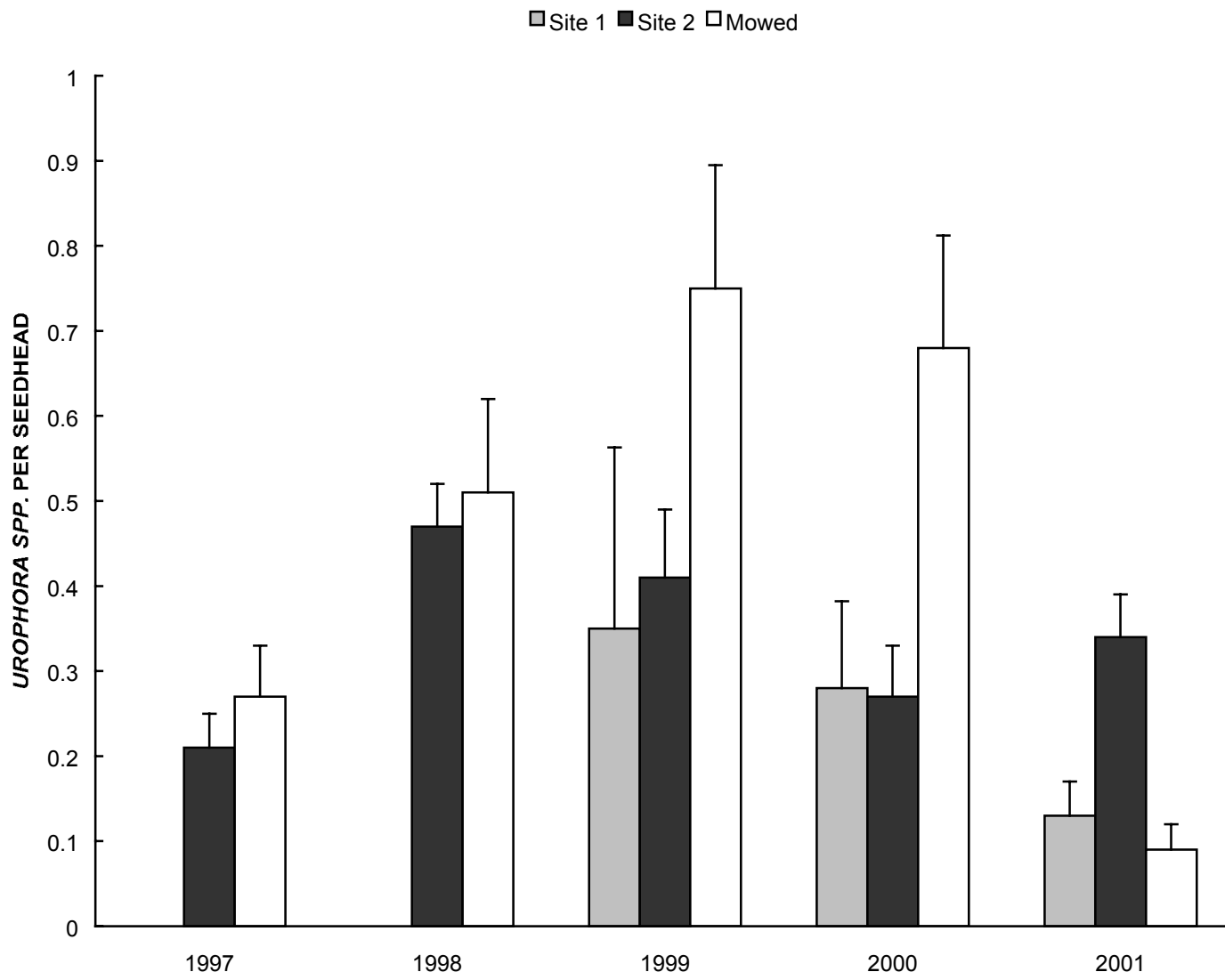
Figure 2



1 Figure 3



1 Figure 4



1 Figure 5