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Biological Control of Diffuse Knapweed (*Centaurea diffusa*) along the Colorado Front Range[1].

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Abstract: Classical biological control of the noxious weed, diffuse knapweed (*Centaurea diffusa* Lam.), has been an elusive goal of weed managers for many decades. This may now have been achieved. Large reductions in knapweed densities have occurred at several locations in grasslands along the Colorado Front Range and elsewhere in western North America. The lesser knapweed flower weevil, *Larinus minutus,* which reduces plant fitness by consuming seeds and by feeding on foliage and stems of flowering plants, was credited with this result. Four other insects that consume knapweed were also present at these sites, but their impacts appeared modest. While we believe that control of diffuse knapweed has been achieved at our study site, the respective roles of insects, plant competition, and climatic variables as factors contributing to this control remains unresolved.

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Nomenclature: Diffuse knapweed, *Centaurea diffusa* Lam., CENDI; 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; lesser knapweed flower weevil, *Larinus minutus* Gyllenhal.

Additional index words: insect herbivory, plant competition

Introduction

Diffuse knapweed (*Centaurea diffusa* Lam.) is an aster of Eurasian origin that has been a major rangeland weed in North America for many decades. This plant was sufficiently widespread midway through the last century to demand biological control efforts. The first species of biological control insects (*Urophora* spp.) were released in North America in 1970, followed by the release of about 10 additional species of insects over the next two decades (Muller-Scharer and Schroeder 1993). By 2000, 13 insects had been released (Story and Piper 2001). In spite of these efforts, diffuse knapweed continued to spread and by 2000 had occupied 1.4 million hectares of western North America (Duncan 2001). Through the 1990s, biocontrol efforts appeared unsuccessful. As summarized by Carpenter and Murray (1999) "At least nine biological control agents that attack *Centaurea diffusa* are established in parts of the United States. Unfortunately, it appears that none of these agents, alone or in combination, effectively controls diffuse knapweed populations".

By 2001, however, evidence suggested that certain insects were having a strong influence on diffuse knapweed densities in the Colorado (Seastedt et al. 2003), Montana (Smith, in press) and British Columbia (Judith Myers, University of British Columbia, unpublished results). The common factor in this reduction was the addition of a seed head weevil to the existing suite of biological control insects. A concern, however, is that this biological effects might be ephemeral (Myers and Bazely 2002), or that control might be limited to a small subset of vegetation and soil types. Nutrient availability and plant competition represent "bottom-up effects" that have the potential to mediate weed responses to herbivory (e.g., McEvoy et al. 1993, McEvoy and Coombs 1999). Accordingly, tests at multiple sites are warranted to establish the generality of insect impacts.

This report documents diffuse knapweed response for the 2001-2003 interval at three sites along the Colorado Front Range where insects were released in 1997, 1998, and in 2001. In addition, the relationship between knapweed seed production and abundance in weevils in knapweed seed heads was quantified at a number of sites in Colorado.

Materials and Methods.

A monitoring site containing diffuse knapweed that represented 25-30% of plant cover was established in Boulder County in 1997. In that year small numbers of *Sphenoptera jugoslavica* Obenb., (Coleoptera: Buprestidae), *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae), both root feeders of rosettes, and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), the lesser knapweed flower weevil, were released at the site. These species were in addition to existing populations of *Urophora quadrifasciata* Meigen, the knapweed seed head fly, Diptera:Tephritidae). A second species of fly, *Urophora affinis* Frauenfeld, the banded gall fly, had invaded the site by 1999.

The same insects were also added to two native grassland sites in Douglas County, approximately 75 km south of the original site. One site was a pasture bordering on a riparian area, and insect releases began in 1998. A second site was located about 0.5 km from the first in a second pasture, and insects were first released there in 2001. As the second site did not contain sufficient densities of insects to anticipate any significant herbivory impacts during the 2001-2003 interval, it served as a reference plot.

Knapweed abundance and reproduction was obtained by counting rosettes, flowering stems, seed heads per plant, and seeds per seed head at all sites. The abundances of *Larinus minutus* were also obtained by during the inventory of seed heads. Details on data collection procedures at the Boulder site are reported in Seastedt et al. (2003); Douglas site data collection procedures were the same as those used in Boulder except that estimates came from samples obtained in permanent transects rather than plots selected at random.

In addition to quantifying seed production and weevil abundance at the above three sites, additional knapweed infested sites were sampled for seeds and weevils. Sites included three mountain meadow sites and six additional prairie sites at varying distances from the original release site in Boulder County. Data reported here were collected during the 2001-2003 interval. Counts are based on inspection of 180 seed heads from 30 different plants at each site collected in the mid August to mid September interval. At one site this analysis was limited to 108 seed heads from 18 plants.

Results and Discussion

Diffuse knapweed densities declined in all release sites during the 2001-2003 interval.(Table 1). A drought in 2002 undoubtedly influenced results, but, consistent with observations of knapweed following a 2000 drought (Seastedt et al. 2003), knapweed densities recovered in 2003 on areas with few insects, but continued to decline in areas where insects were abundant. The Boulder County site had almost no flowering knapweed in 2003. The Douglas site that had insects released in 1998, site 2, was apparently already on the decline when first surveyed in 2001 as evidenced by weevil numbers in seed heads(Table 1). The other Douglas site, where insects were first released in 2001 (site 3), exhibited large declines in seed production by 2003 but only modest reductions in stem densities relative to 2001. Rosette data provided an index of future knapweed abundance, and in 2003 these sites averaged 1.9, 4.1, and 31.1 rosettes per m^2 at sites 1, 2, and 3, respectively. Rosette densities appear strongly inversely correlated with the time of insect release. For example, rosette densities at the Boulder site in 1997 were about 50 plants $\cdot$ m^{-2} (Seastedt et al. 2003).

The correlation between average seeds produced per seed head and average weevil numbers found per seed head is shown in Figure 2. A single weevil larva will generally consume all of the seeds found in diffuse knapweed seed heads. Thus, the average seed production by knapweed impacted by this insect is determined by seed production in those seed heads not containing weevils. As shown in Figure 2, the relationship is "triangular", i.e., when weevil abundance is high, seed production is uniformly low, but when weevil abundance is low, seed production can be either low or high. *Larinus minutus* appeared to be a good disperser and was observed on knapweed 5-10 km from known release sites.

The reduction in knapweed stem densities at site 1 in this study represents a substantial decline in flowering knapweed from over 10 stems $\cdot$ m^2 in 1997 to under 0.1 stems m^2 in 2003. Harris (1984) suggested that the objective of knapweed control should be to achieve less than 5% cover by the weed on rangelands. This has been accomplished in Colorado, and similar results appear underway in other regions (e.g., Smith, 2004; Judith Myers, Univ. of British Columbia, unpublished results). These results support the hypothesis that the absence of enemies and not novel weapons is an adequate explanation for the ability of diffuse knapweed to invade and dominate grassland areas.

Declines in seed abundance generally co-occur with the abundance of *Larinus minutus* larvae in seed heads (Table 1, Figure 1. Few weevils were found at site 1 in 2002, as were few seeds. This likely resulted from extensive defoliation of the flowering plants by adult *L. minutus* weevils prior to oviposition on the knapweed flowers. For reference, a control plot experiencing no weevil herbivory in 2002 in Boulder County produced an average of almost 8 seeds per seed head (Figure 1). oderate seed production can occur at high weevil densities, as found at site 3 in 2003. This is believed to be the result of weevils migrating to this site after feeding on foliage of areas such as at site 2, where defoliation of flowering plants occurred due to large *L. minutus* densities.

The observed reduction in knapweed densities in Colorado is therefore attributed largely to activities of the lesser knapweed flower weevil, *Larinus minutus*. The other insects present in this study have been unable to control the weed (Myers and Bazely 2002), or, as in the case of *Cyphocleonus achates,* have not been abundant during intervals of knapweed decline (Seastedt, unpublished results). However, these observations do not exclude the possibility that the species collectively have substantially more impact than *L. minutus* operating alone. Seedling mortality appears to be a significant part of the reduction in plant densities, because densities decline when seed production is still moderately high (Seastedt et al. 2003).

Insects alone may not be able to reduce plant fitness to the levels observed in our study. Myers and Risley (2000) conducted a modeling exercise that concluded that knapweed control required a mechanism for reducing rosettes. Our observations in Colorado suggest that *Larinus* attacked rosettes at very high densities, but that rosette abundance can remain very high in areas where *Larinus* are abundant (i.e., site 3 in 2003). Myers and Bazely (2002) indicate that seed predators should only be effective when host plants are poor competitors and have low rosette survival. In Colorado seedling survival appears to be a vulnerable stage for this plant, and soil nutrient availability and plant competition influence survival (LeJeune 2002 and unpublished results). Thus, similar to findings of McEvoy et al. (1993) and research summarized by Muller-Scharer and Schroeder (1993), we hypothesize that resource competition, generated by low soil resource availability or through plant competition, mediate the weed response herbivorous insects. Accordingly, we would predict that in spite of large reductions of knapweed in grassland areas, knapweed will remain common in areas of soil disturbance with high nutrients and little plant competition such as along roadsides. To date, our observations match this prediction.

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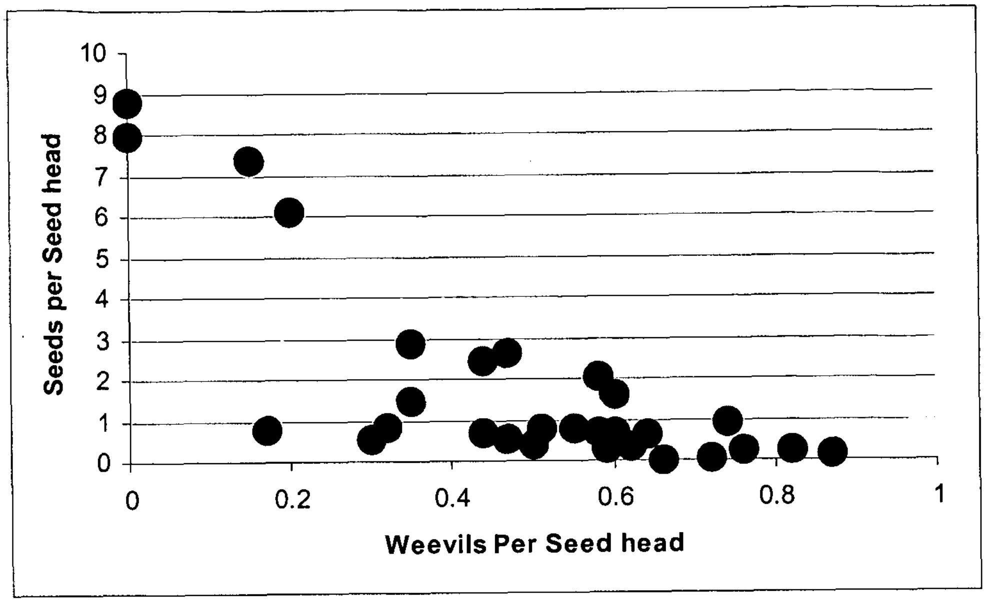
Table 1. Knapweed and *L. minutus* larvae densities at three sites in Colorado[1].

Site[2]	Variable	First year of measurement[3]	2002	2003
1	stem density	11.8 (1.07)	0.2 (0.03)	0.03 (0.00)
	seed heads per plant	71.4 (8.06)	6.5 (0.27)	77.0 (5.50)
	seeds per seed head	4.9 (0.53)	0.8 (0.15)	0.1 (0.05)
	weevils per seed head	0 0	0.2 (0.04)	0.7 (0.04)
2	stem density	30.7 (4.90)	4.9 (1.40)	1.6 (0.56)
	seed heads per plant	21.7 (4.16)	6.1 (1.10)	no data[4]
	seeds per seed head	6.9 (0.51)	2.5 (0.56)	0.7 (0.09)
	weevils per seed head	0.1 (0.00)	0.4 (0.05)	0.6 (0.09)
3	stem density	77.1 (7.51)	8.0 (0.74)	13.3 (1.36)
	seed heads per plant	24.5 (2.95)	11.8 (1.99)	31.0 (2.87)
	seeds per seed head	8.8 (0.50)	2.9 0.29)	1.7 (0.21)
	weevils per seed head	0.0 (0.01)	0.3 (0.04)	0.6 (0.07)

1. Values are means and standard errors of n = 30 or n > 30 samples per collection.
2. Site 1 = site of Seastedt et al. (2003); 2001 data were obtained from that study. Sites 2 and 3 are from Douglas County, CO.
3. First year of measurement = 1997 for site 1, and 2001 for sites 2 and 3.
4. Defoliated plants produced flower heads, but most of these failed to open and, using the procedures described in Seastedt et al. (2003) would not be counted as seed heads.

Figure Legend

Figure 1. Relationship between seed and weevil production in seed heads of diffuse knapweed. Data are means from ten sites along the Colorado Front Range collected during the 2001-2003 interval.



10
9
8
7
6
5
4
3
2
1
0
Seeds per Seed head
0
0.2
0.4
0.6
0.8
1
Weevils Per Seed head