

Biological Control Insect Use of Fertilized and Unfertilized Diffuse Knapweed in a Colorado Grassland

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ABSTRACT The responses and impacts of five insect species that feed on *Centaurea diffusa* Lamarck, diffuse knapweed, to soil nitrogen and phosphorus additions were studied in grasslands east of the Colorado Front Range. We predicted that fertilization was unlikely to have a direct effect on herbivory but that if the insects preferentially select vigorous plants, increased tissue nitrogen, plant density, or plant mass resulting from fertilization would increase plant susceptibility to biological control insects. Fertilization caused a modest increase in the biomass of *C. diffusa* adults and the nutritive content of aboveground plant biomass. *Larinus minutus* Gyllenhal, a seed head weevil, was less abundant and exhibited reduced impacts on fertilized *C. diffusa* plants. Abundance of gall fly larvae, *Urophora* spp., was negatively influenced by the presence of *L. minutus* and had no detectable effect on seed abundance. No positive or negative impacts of root feeders on knapweed were observed, but the root-feeding *Cyphocleonus achates* (Fahraeus) selected plants in fertilized plots, whereas the root phytophage *Sphenoptera jugoslavica* Obenberger was more common on knapweed in unfertilized plots. Our results indicate that these specialist biological control insects exhibit little preference based on plant biomass, density, or nutritive quality. However, variables that affect the timing of knapweed seed production, such as fertilization, mowing, and grazing, reduce insect impacts and may explain some of the regional variation in effectiveness of these insects as biological controls on knapweed.

KEY WORDS biological control, fertilization effects, *Centaurea diffusa*, *Larinus minutus*, *Cyphocleonus achates*

UNCERTAINTY ABOUT THE EFFECTIVENESS of biological control insects on invasive weeds is confounded because many of the more problematic plant invaders are capable of growing over a wide range of environments and in a wide range of soil conditions. In theory and practice, insects may be effective control agents under one suite of environmental conditions but fail to affect the abundance of the plant under other conditions (Louda 1982, 1983; White 1984; Mattson and Haack 1987; Pecora and Dunn 1990; Price 1991; McEvoy et al. 1993). Host selection is likely influenced by plant phenology, tissue quality, and plant biomass (Louda 1982). Plants grown in soils of greater nutrient availability should produce biomass of greater nutrient quality, and according to the plant vigor hypothesis (Price 1991), should be more attractive to insect herbivores. Many invading plant species respond more rapidly to nutrient enrichment than do native species, and environmental eutrophication (e.g., increased nitrogen deposition) could be facilitating exotic species invasions (Vitousek et al. 1997, Jefferies and Maron 1997, Dukes and Mooney 1999).

Centaurea diffusa Lamarck is a semelparous forb that has invaded and become a dominant plant species across thousands of hectares of the Colorado Front Range. Based on county and state lists, the genus *Centaurea* is the most abundant noxious weed in the western United States (Skinner et al. 2000), and *C. diffusa* is found from British Columbia to Arizona and New Mexico (Rice 2002, SWEPI 2002). Depending on resource availability, *C. diffusa* can be an annual, biennial, or short-lived perennial (Thompson and Stout 1991), and persistence as a rosette for up to 5 yr has been reported (Myers et al. 1990).

The search for effective biological control agents of *C. diffusa* has been long and, until recently, largely unsuccessful. The Nature Conservancy reports that "At least nine biological control agents that attack (*C. diffusa*) are established in parts of the United States. Unfortunately . . . none of these agents, alone or in combination, effectively controls diffuse knapweed populations" (TNC 1999). By 2001, the number of biological control insect species had risen to 13, but no documented success stories were yet available (Story and Piper 2001).

Five of the biological control insects were released in 1997 or were already established in a *C. diffusa*-invaded grassland in Boulder County, Colorado. Two flies introduced from Europe, *Urophora affinis* Frauenfeld and *Urophora quadrifasciata* (Meigen)

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(Diptera: Tephritidae), have been used in North America for several decades to reduce seed production in *C. diffusa* and *Centaurea maculosa* Lamarck (Müller 1990). *U. affinis* is a woody gall-forming insect that oviposits in immature flower buds (Berube 1980, Harris 1990). Hatching coincides with meristematic activity of the upper receptacle. Woody gall formers generate a metabolic sink that sequesters nutrients from other parts of the plant (Harris 1990, Shorthouse 1990). *U. quadrifasciata* oviposits shortly before the flower is in full bloom but does not form a woody gall, so the resource sink created is smaller than that created by *U. affinis* (Harris 1990). Although *U. quadrifasciata* is thought to avoid seed heads containing *U. affinis*, the two species can coexist (Harris 1990).

Larinus minutus Gyllenhal (Coleoptera: Curculionidae), released in the United States in 1991 (Lang et al. 1996), is a weevil that feeds in the seed heads during the larval stage and on the cuticle and foliage of flowering plants during the adult stage (Lang et al. 2000). *L. minutus* oviposits into the open flower head and the larvae feed on the developing achenes (Harris 1990, Kashefi and Sobhian 1998). One larva can destroy 100% of the seeds in the developing seed head (Kashefi and Sobhian 1998). The adults emerge in midsummer, feed on the mature plants and perhaps on rosettes, and overwinter in the soil. The adults emerge from the soil the next spring and feed extensively on the stems and foliage of bolting knapweed. Bolting knapweed can be completely defoliated by *L. minutus*. The insects may then disperse to areas where flowering has not been suppressed by weevil activity. Seed heads heavily attacked by *U. affinis* do not open, so *L. minutus* might supplement the impact of *U. affinis* by attacking flowers not occupied by *U. affinis* larvae (Harris 1990).

Sphenoptera jugoslavica Obenberger (Coleoptera: Buprestidae), released for weed control in North America >20 yr ago, and *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae), released in the United States in the late 1980s, both feed on the roots of *C. maculosa* and *C. diffusa* rosettes (Müller 1990). *S. jugoslavica* and *C. achates* larvae overwinter on or in the root of *C. diffusa* rosettes. *S. jugoslavica* emerge as adults in late June and egg laying in the rosette leaves begins 1 to 2 wk after emergence (Powell and Myers 1988). The larvae tunnel through the base of the plant and into the root. By the end of the growing season, *S. jugoslavica* larvae are nearly mature. *C. achates* emerge as adults after *S. jugoslavica*, and feed on rosette leaves (Stinson et al. 1994) and on stem tissue of flowering knapweed (T.R.S., unpublished). Eggs are laid in late summer and early autumn in the soil near or on roots of rosettes, and the larvae tunnel into the root. *C. achates* larvae are immature at the end of the growing season and feed mainly during the next spring (Stinson et al. 1994). Multiple larvae may inhabit a single root, and both root-boring species can inhabit the same root. Larval activity of both species induces a large swollen gall and can cause stunting of surviving plants (Powell and Myers 1988, Stinson et al. 1994). Adult *C. achates* are much larger (≈ 20 mm) than *S.*

jugoslavica (≈ 7 mm), exhibit preference for larger plants (Stinson et al. 1994), and might be expected to select larger rosettes and to inflict greater tissue damage.

Here, we report the results of field investigations to examine the effect of soil fertility on plant characteristics and the effect of plant characteristics on the abundance of biological control agents. We quantified the effects of fertilization with nitrogen and phosphorus on *C. diffusa* and on the density and effects of three categories of biological control insects. We asked the following questions: 1) Does increased availability of nitrogen, phosphorus, or both increase plant attributes that might make *C. diffusa* more attractive to insects (plant density, plant biomass, seed head production, or tissue quality)? 2) Is biological control insect density related to fertilization treatment or to *C. diffusa* density, biomass, seed head production, or tissue quality? and 3) Is the effectiveness of the biological control insects in reducing plant fecundity (seed production) related to fertilization, plant size, or tissue quality?

Materials and Methods

Research Site

The research site is located ≈ 10 km east of the Colorado Front Range, near Superior, CO (39° 55' 35" N, 105° 11' 8" W). The site is a degraded parcel of mixed grass prairie, historically grazed, and heavily invaded by *C. diffusa*. The soils of the study area are predominantly clayey over loamy-skeletal montmorillonitic, mesic Aridic Arigustolls formed on old high terraces and benches in gravelly and cobbly loamy alluvium (U.S. Dep. Agric. SCS 1975). The surface layer (0–10 cm) is a grayish brown clay loam that contains varying amounts of cobbles and gravel.

Native grass species include *Agropyron smithii* Rydberg, *Aristida longiseta* Steudel, *Bouteloua gracilis* (Humbolt, Bobland, Kunth) Lagasca ex Steudel, *Buchloe dactyloides* (Nuttall) Engelmann, *Koeleria macrantha* (Ledebour) Schultes, *Poa compressa* L., and *Schedonnardus paniculatus* (Nuttall) Trelease. Non-native grasses include *Bromus japonicus* Thunberg, *Bromus tectorum* L., *Poa pratensis* L., and *Agropyron intermedium* (Host) Beauvois. Native forbs include *Artemisia frigida* Willdenow, *Erigeron flagellaris* Gray, *E. divergens* Torrey & Gray, *Sphaeralcea coccinea* (Pursh) Rydberg, and *Gutierrezia sarothrae* (Pursh) Britton & Rusby (a diminutive shrub at this site). Non-native forbs include *Alyssum minus* (L.) Rothmaler, *C. diffusa*, *Convolvulus arvensis* L., and *Hyperricum perforatum* L. Non-native species comprised slightly <50% of the total vegetation cover (LeJeune 2002). In 2000, *C. diffusa* comprised $\approx 34\%$ of the total vegetation cover (Seastedt et al. 2003).

Field Experiments

Experiment 1. Experiment 1 consisted of a random complete block design with four replications of four treatments in each of two adjacent fields (north and

south). Manipulations included addition of nitrogen, phosphorus, both, or neither (control). The north field set included four additional control plots (i.e., $n = 20$ plots in north field; $n = 16$ plots in south field). In each field, the plots measured 2 by 2 m and were separated by a 1-m walkway.

The south field experiment was established in 199 and the north field in 2000. The south and north field experiments were located ≈ 300 and 200 m southwest of the original insect release site, respectively. Nitrogen (as ammonium nitrate, 34% N), phosphate (as superphosphate, 18% P), or both were added to test plots twice during the growing season each year. Nitrogen was added by hand in May and late June at a rate of 10 g N/m² in 1999 and 2000 and at 5 g N/m² in 2001. Phosphorus was added by hand on the same dates at a rate of 1 g P/m² in both years.

In late August 2001, all mature *C. diffusa* plants were collected from the 16 plots in the north field and the 20 plots in south field, dried, and the aboveground portion was weighed. Aboveground biomass of all other vegetation was clipped in 0.1-m² subplots (one per plot); separated into grass, forb, and *C. diffusa* rosette biomass; dried; and weighed. The number of seed heads per mature *C. diffusa* plant was counted and averaged to estimate the mean number of seed heads per plant and per plot. The number of seeds per seed head was estimated by dissecting six seed heads per plant from a randomly selected subset of the mature *C. diffusa* plants harvested from each plot. Because of our harvest, no knapweed flowered on these plots; however, the number of plants censused in other treatments ranged from a low of 22 to a high of 79. In total, 1,788 seed heads were used in this analysis.

Three "groups" of the five biological control insects were enumerated: gall flies, seed feeding weevils, and root feeders. Gall fly larvae (*U. affinis* and *U. quadri-fasciata*) and *L. minutus* weevils were censused by examining seed heads under a dissecting microscope. Larvae and remains of pupae were easily distinguished from seeds. The *Urophora* species were not distinguished from one another. Most of the *L. minutus* had emerged from the seed head before the census, but the fecal remains and damage caused by *L. minutus* are easy to detect with a dissecting microscope. Earlier occupation of the root by either *S. jugoslavica* or *C. achates* was determined by examining the harvested roots for damage. The larvae were not present, but they cause a thickening of the root, vascular tissue necrosis, and often leave obvious fecal material. Voucher specimens are maintained at the University of Colorado, INSTAAR, by T.R.S.

The dried aboveground biomass of mature *C. diffusa* plants was composited by plot, ground, and analyzed for carbon, nitrogen, and phosphorus. Total carbon and nitrogen was analyzed by Micro-Dumas combustion on an NA1500 C/H/N Analyzer (Carbo Erba Strumentazione, Milan, Italy). Biomass was digested with sulfuric acid-hydrogen peroxide, and the digests were analyzed for phosphorus on a Technicon Auto Analyzer II (Technicon Industrial Systems 1977).

Experiment 2. A second fertilization experiment was conducted to test whether the two root-feeding insects, *C. achates* and *S. jugoslavica*, exhibited preferences for fertilized or unfertilized *C. diffusa*. The experiment was established in 1999 in the north field ≈ 300 m northeast of the release site and consisted of 36 1-m² plots. Eighteen of the plots were randomly selected for fertilization with 10 g of N and 1 g of P in May 1999 and in May 2000. Vegetation production was increased from an average of ≈ 80 g m⁻² to 130 g m⁻² in these plots by the end of summer 2000, substantially altering both the chemical and physical environment for knapweed (Scott 2001). This produced a markedly different chemical and physical environment for subsequent knapweed growth. In early June 2002, all of the *C. diffusa*, including the tap root, was removed from each of the plots. Plants were separated into rosettes or bolting plants (plants in the process of flowering). The roots were examined for signs of current or previous insect damage. If insects were present in the root, the insect species was identified and recorded.

Statistical Analyses

We analyzed the results separately by field (north and south) because the two fields had distinct grazing management and land use histories. Soil analyses indicated that the north field contained less organic carbon and nitrogen and more clay (LeJeune 2002). The analysis of variance (ANOVA) model to assess the effects of fertilization on *C. diffusa* included treatment two factors (addition of nitrogen and addition of phosphorus) and two levels (with and without addition). We recognized that fertilizer treatment could affect responses on both a per plant and per plot basis. These responses would be similar if individual plant characteristics and plant densities responded to treatments in similar ways or if the response was dominated by individual plant responses. All individual plant characteristics were averaged on a plot basis before analysis to avoid pseudoreplication. Response variables included plant biomass (grams per *C. diffusa* plant, averaged per plot), density (*C. diffusa* plants per square meter), relative biomass of *C. diffusa* (percentage of plant community biomass), seed heads per *C. diffusa* plant averaged, seeds per seed head, and tissue concentrations of nitrogen and phosphorus. Seed production data from individual seed heads were averaged on a per plant basis and then on a per plot basis before statistical analysis. Aboveground plant biomass, seed head number, and seed number were square-root transformed for analyses to satisfy model assumptions.

The ANOVA model to assess the effects of fertilization on biological control insect density included two factors (addition of nitrogen and addition of phosphorus) and two levels (with and without addition). Response variables included weevil density, fly density, and root feeder density. We examined relationships between plant tissue quality, density, biomass, seed production, and biological control agent density by using simple regression. We compared the results

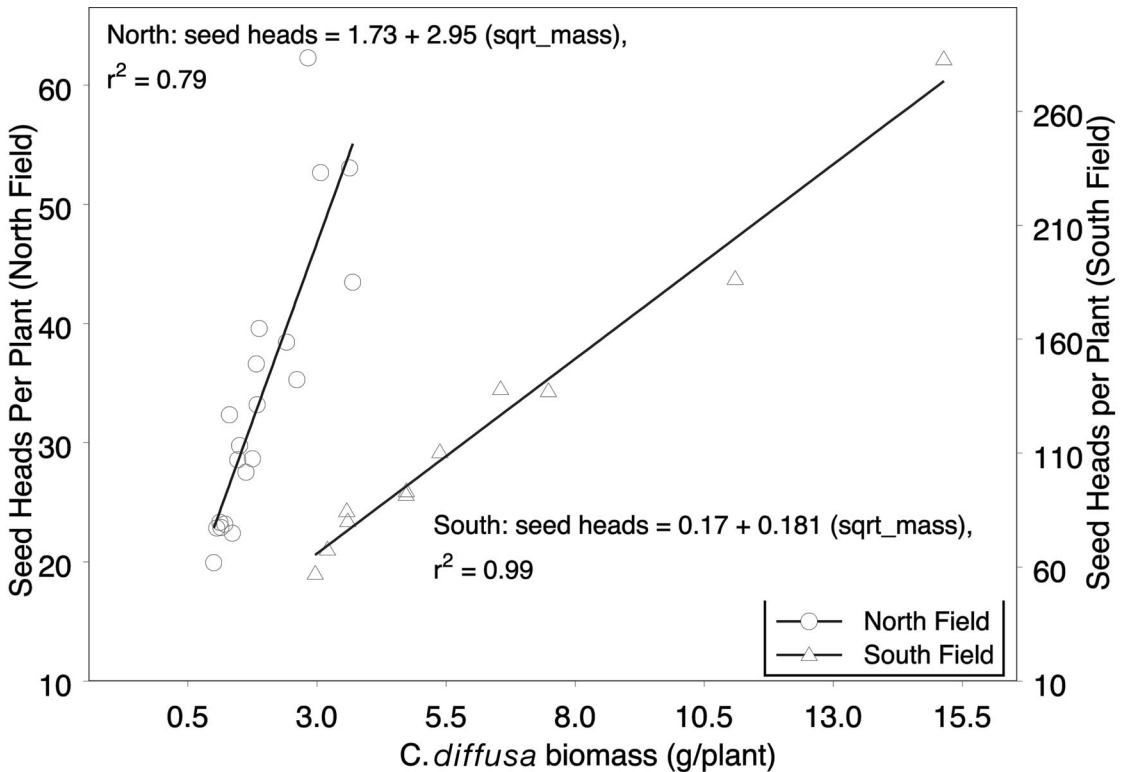


Fig. 1. Number of viable seed heads per mature *C. diffusa* is related to the aboveground biomass of mature *C. diffusa*. Data are from plants harvested from control plots and were log transformed for analyses.

of the regressions to results of the ANOVAs, testing relationships between fertilization treatment and biological control insect density to assess whether fertilization seemed to have a direct effect on biological control abundance or an indirect effect via one of the affected plant characteristics. Relationships between biological control insect density and *C. diffusa* seed production were analyzed using simple regression.

Post hoc tests to evaluate ANOVA interaction terms and pairwise comparisons were conducted on least-squares means by using a Bonferroni adjustment for multiple comparisons, with initial protection levels set at 5%. Analyses were performed in SAS version 8.02 (SAS Institute 1999–2001). In reporting *F* values, error terms sometimes reflect numbers below those of a balanced design for each site because individual cells lacked knapweed.

Effects of fertilizer on *C. diffusa* rosette and bolting plant abundance, density, and root feeder use were summarized on a per plant and density basis. We used a χ^2 test to examine expected frequencies for *C. achates* and *S. jugoslavica* abundance in roots of *C. diffusa* plants.

Results

Experiment 1

Effect of Fertilization on *C. diffusa*. The biomass of individual mature *C. diffusa* in the north field in-

creased relative to plants in control plots, which averaged 18.4 ± 4.5 g, with added nitrogen (mean plant biomass of 49.7 ± 8.6 g; $F_{1,16} = 38.1$; $P < 0.0001$), phosphorus (mean plant biomass of 26.7 ± 5.7 g; $F_{1,16} = 12.09$; $P = 0.003$), and both nutrients (mean plant biomass of 62.6 ± 22.1 g; $F_{1,16} = 5.77$; $P = 0.029$). *C. diffusa* density was unrelated to fertilization treatment in the north field. There was no significant response in *C. diffusa* biomass to fertilization in the south field. The site differences observed here likely reflect that the south field was initially higher in nutrient content and had a texture more conducive to providing water to plants (LeJeune 2002). Fertilization increased the aboveground plant community biomass in both fields, but fertilization did not increase the relative biomass of mature *C. diffusa* within the communities.

In the north field, *C. diffusa* %N (in aboveground biomass) increased relative to control %N (mean of $0.85 \pm 0.06\%$) with added nitrogen (mean %N = $1.36 \pm 0.12\%$; $F_{1,16} = 36.3$; $P < 0.001$). Mean %P increased relative to control %P (mean of $0.10 \pm 0.01\%$) with added phosphorus (mean %P = $0.17 \pm 0.01\%$; $F_{1,16} = 12.2$; $P = 0.003$). In the south field, there was no significant response of mature *C. diffusa* tissue quality to fertilization, i.e., plants increased in biomass but not in nutrient concentration.

As average plant size increased, the number of seed heads per plant increased (Fig. 1) and the seed density (seeds per square meter) increased (north field, $F_{1,18}$

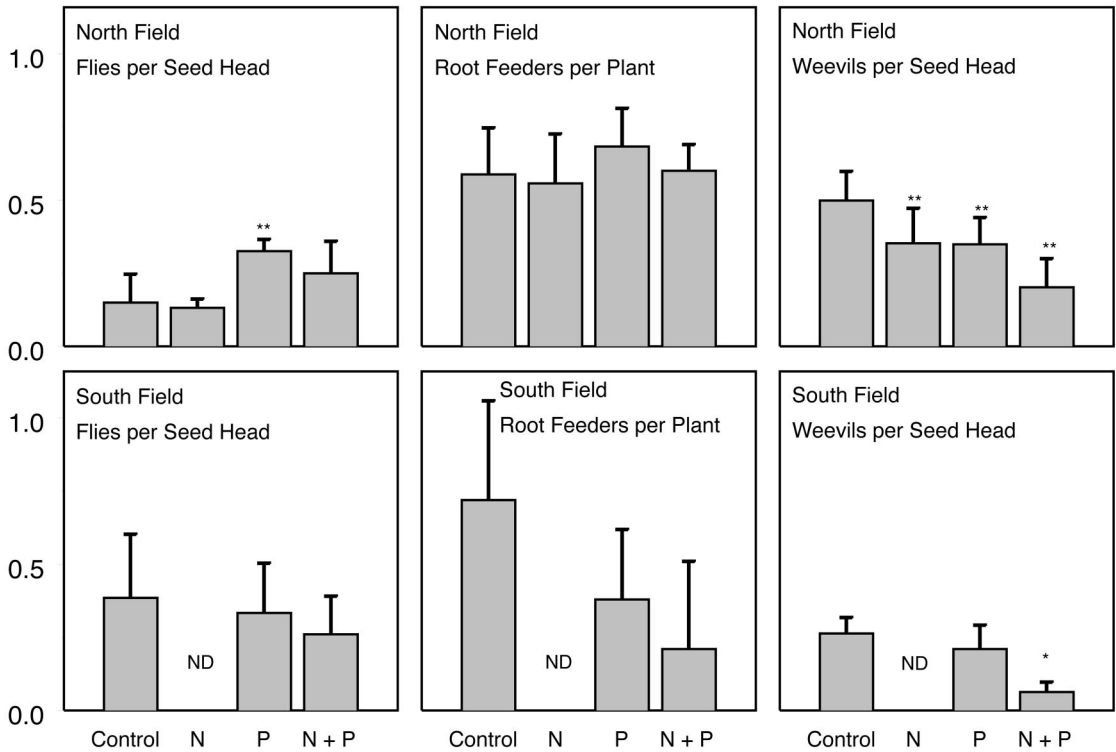


Fig. 2. Mean and standard error of insect use by field and fertilization treatment. Vertical axes vary from left to right; label in each box indicates field and measurement. Asterisks indicate significant difference from the control (least-squared means post hoc pairwise tests by field, by using rank-transformed data and a Bonferroni correction for multiple comparisons). * $P < 0.05$; ** $P < 0.01$. ND, in the south field, there were no mature *C. diffusa* in plots with added nitrogen only.

= 5.86; $P = 0.026$; $r^2 = 0.25$; south field, $F_{1, 14} = 11.6$; $P = 0.004$; $r^2 = 0.45$). In the north field, as aboveground tissue nitrogen concentration increased, number of seed heads increased ($F_{1, 18} = 5.01$; $P = 0.038$; $r^2 = 0.22$) and seed head density increased ($F_{1, 18} = 5.56$; $P = 0.03$; $r^2 = 0.24$). There were no significant relationships between tissue nitrogen and seed head production in the south field, and there were no significant relationships between tissue phosphorus concentration and seed head production in either field.

Biological Control Insect Density and Fertilization. Fertilization reduced seed head weevil density. In the north field, there was a negative effect of added nitrogen ($F_{1, 16} = 9.27$; $P = 0.008$) and phosphorus ($F_{1, 16} = 9.82$; $P = 0.0064$) on weevil density (Fig. 2). The combined effect of nitrogen and phosphorus did not differ significantly from the effect of either nutrient alone, but post hoc tests indicated that where both nutrients were added, weevil density was significantly lower than in control plots. In the south field, weevil density in plots with both nutrients added was reduced relative to the control.

Seed head weevil density in the north field was negatively correlated with mature *C. diffusa* biomass (grams per plant) ($F_{1, 18} = 18.54$; $P = 0.0004$; $r^2 = 0.51$) and with tissue nitrogen concentration ($F_{1, 18} = 5.39$;

$P = 0.032$; $r^2 = 0.23$). In the south field, weevil density was unrelated to biomass and nitrogen content, and in both fields, weevil density was unrelated to tissue phosphorus concentration, plant density, and seed head number.

In the north field, gall fly larval density was greater in plots with added phosphorus ($F_{1, 16} = 14.92$; $P = 0.0017$) and was positively correlated with tissue phosphorus concentration ($F_{1, 18} = 5.63$; $P = 0.029$; $r^2 = 0.24$). Fertilizing had no significant effect on gall fly larval density in the south field. Gall fly larval density was unrelated to plant phosphorus concentration in the south field, and unrelated to biomass, tissue nitrogen concentration, plant density, and seed head number in both fields.

The average densities of *Cyphocleonus* and *Spinoptera* in knapweed roots were unaffected by fertilization treatment in both fields (Fig. 2). Root feeder densities were unrelated to plant biomass, tissue quality, and plant density in both fields.

Effect of Biological Control Insects on *C. diffusa* Fecundity. Seed head weevils significantly reduced seed production. As weevil density increased, the mean number of seeds per seed head decreased in the north ($F_{1, 18} = 5.31$; $P = 0.033$) and the south ($F_{1, 7} = 5.74$; $P = 0.048$) fields. The mean number of seeds per seed head in mature *C. diffusa* in the north field by

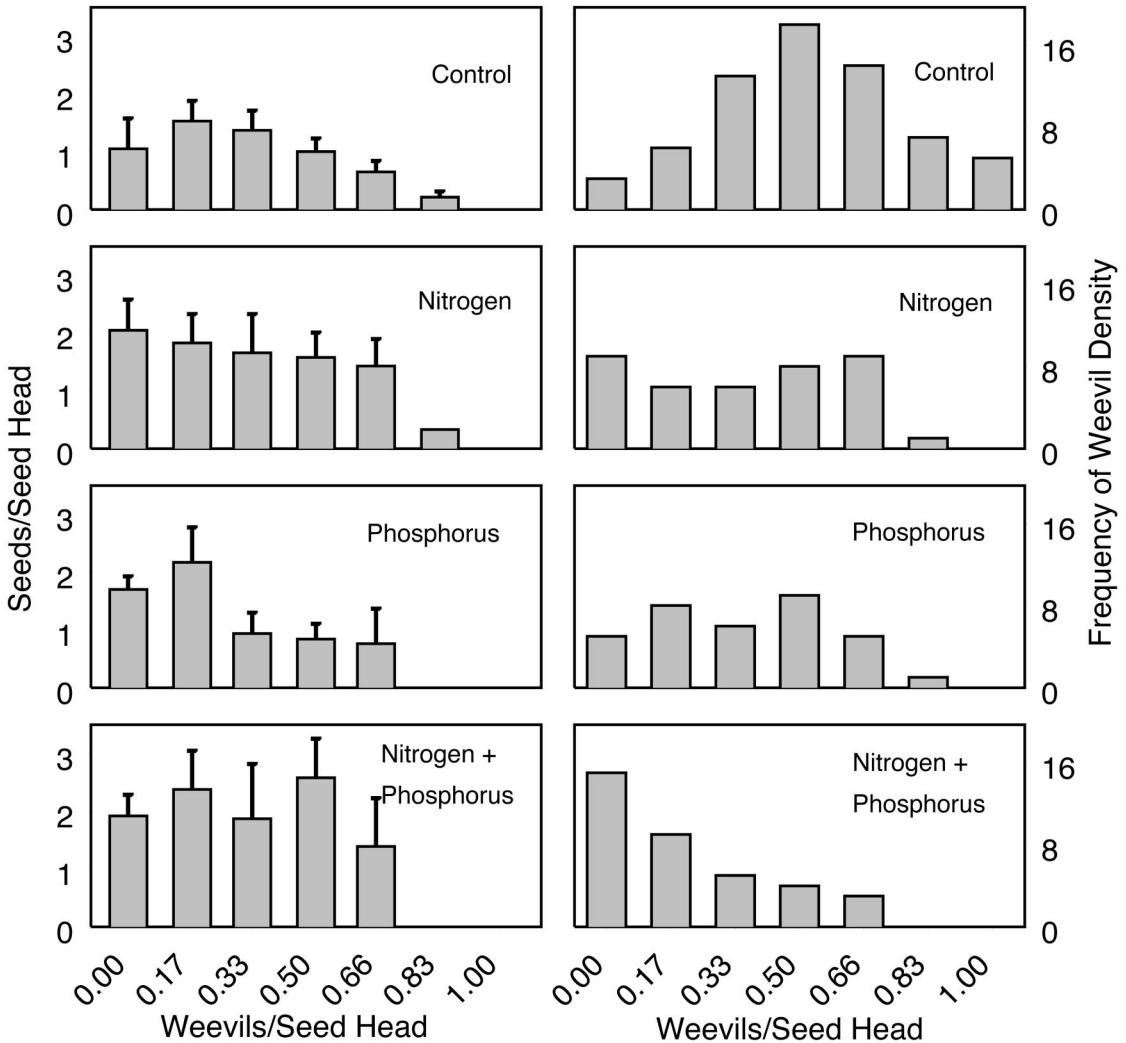


Fig. 3. (Left) Mean (standard error) number of seeds per seed head, by fertilization treatment and weevil density. (Right) Fraction of total plants having a given range of weevil densities in seed heads (i.e., a frequency distribution) separated by treatment. Control *C. diffusa* supported the greatest density of weevils and exhibited the strongest negative seed response to increasing weevil density. Data presented are from the north field where weevil densities were highest.

treatment and weevil density is illustrated in Fig. 3. Weevil density is expressed as the fraction of seed heads containing weevils, averaged per plot. The frequency distribution of weevil density in mature *C. diffusa* plants is also shown in Fig. 3.

In the north field, the average number of seeds per plant decreased from a range of 60–25 seeds per plant in plants with low weevil density (<0.5 weevils per seed head), and to six to zero seeds per plant in plants with high weevil density (≥ 0.5 weevil per seed head). In the south field, the average number of seeds per plant decreased from ≈ 35 –75 per plant at low weevil density to zero to 10 seeds per plant at high weevil density.

Seed production was unrelated to gall fly and root feeder density. Root feeders in the presence of seed head weevils did not enhance the negative relation-

ship between weevils and seed production, and the combined presence of root feeders and flies had no significant effect on seed production.

Where both weevils and gall fly larvae were present, the weevils preyed on both the seeds and the fly larvae within the galls. Less than 2% of the 1,788 seed heads examined contained both fly and weevil larvae, and in those cases our collections may have prevented the weevil from consuming the fly larvae. Across fields, there was a negative relationship between *L. minutus* weevils and *Urophora* gall fly larvae ($F_{1,27} = 5.35$; $P = 0.03$; $r^2 = 0.17$).

Experiment 2

The average density of *C. diffusa* rosettes and bolting plants was similar in fertilized and control plots

Table 1. Summary statistics describing *C. diffusa* rosette and bolting plant abundance and density of root-feeding *C. achates* and *S. jugoslavica* in control and fertilized (nitrogen plus phosphorus) plots, experiment 2

Measure	% of all plants [mean (SE)]		No./m ² [mean (SE)]	
	Control	Fertilized	Control	Fertilized
<i>C. diffusa</i> rosettes	83.6 (2.8)	76.9 (3.7)	8.22 (3.4)	5.72 (2.8)
Bolting <i>C. diffusa</i>	16.4 (2.7)	23.1 (3.7)	1.61 (0.71)	1.72 (0.91)
<i>C. achates</i>	4.0 (1.5)	13.4 (3.0)	0.38 (0.16)	1.00 (0.54)
<i>S. jugoslavica</i>	22.0 (3.1)	2.3 (1.3)	2.17 (1.0)	0.17 (0.12)

For percentages, $n = 311$ *C. diffusa*. For density, $n = 18$ plots per treatment (control, fertilized).

(Table 1). Current or past occupation by a root feeder was recorded in 39.5% of control plants and 20.9% of fertilized plants. Of the occupied control plants, 83.5% were occupied by *S. jugoslavica* and 16.5% by *C. achates*. Of the occupied fertilized plants, 19.7% were occupied by *S. jugoslavica* and 80.3% by *C. achates*. Thus, there was clear selection of the fertilized plants by *C. achates* ($\chi^2 = 5.76$, $P < 0.02$), whereas *S. jugoslavica* preferred control plants ($\chi^2 = 26.0$, $P < 0.0001$). *S. jugoslavica* density averaged $2.17 \pm 1.0/m^2$ in control plots and $0.17 \pm 0.12/m^2$ in fertilized plots. *C. achates* averaged $0.389 \pm 0.16/m^2$ in control plots and $1.0 \pm 0.536/m^2$ in fertilized plots.

In both control and fertilized plots, *C. achates* was found more frequently in bolting plants than in rosettes. In control plots, *C. achates* was found in 10.3% of the bolting plants and in only 2.7% of the rosettes. In fertilized plots, *C. achates* was found in 41.9% bolting plants and in only 4.8% of the rosettes. *S. jugoslavica* was found in a greater percentage of bolting plants than rosettes as well (34% bolting and 19.5% rosettes), but only in control plots. *S. jugoslavica* was found in very few fertilized plants. The number of plants with evidence of past root feeder damage but without a larva present was greater in control plots ($n = 24$, 13.6%) than in fertilized plots ($n = 7$, 5.2%).

Discussion

Recent findings show *C. diffusa* to be an aggressive invader and one that may use allelopathic chemicals to achieve community dominance (Hierro and Callaway 2003, Vivanco et al. 2004). The allelopathic chemical identified for *C. diffusa* contains nitrogen, implying that greater nitrogen availability could confer greater competitive ability for this species. Suding et al. (2004) showed that the competitive abilities of the rosettes of *C. diffusa* to be greatest under the highest nitrogen treatments used in that study. Thus, we suggest that it is reasonable to speculate that biological insect responses to the nutrient status of *C. diffusa* could amplify or attenuate this nutrient effect that seems to enhance the competitive abilities of this species.

Recent studies also have provided evidence of classical top-down biological control of *C. diffusa* (Seastedt et al. 2003, Smith 2004). Because the weevil *Larinus minutus* has been identified as the key insect in this biological control effect (Myers 2004), the response of this insect to resource availability is particularly relevant. The negative relationship we ob-

served between fertilization and *L. minutus* abundance in seed heads therefore deserves emphasis. Fertilization prolonged the growing period and delayed bolting and maturation of reproductive plant parts. The lowest weevil density was in plots fertilized with both nitrogen and phosphorus (Fig. 3). These plants were the largest and had the most seed heads, but they matured later, and the timing of seed head development possibly lagged behind the peak timing of *L. minutus* oviposition in 2001. Seastedt et al. (2003) observed that *L. minutus* use was reduced in plants that were mowed in late June, presumably because mowing delayed initiation of flowering in the mowed plants. In a subsequent study, the effects of goat grazing on knapweed in June resulted in higher seed production per seed head and reduced *L. minutus* abundance in grazed plants (T.R.S., unpublished).

Predation of seeds by *L. minutus* weevil larvae significantly reduced seed production by *C. diffusa*. This effect was most evident in unfertilized plants. In control plots, as the number of weevils increased, seeds per seed head decreased (Fig. 3). *L. minutus* densities in seed heads were not correlated with plant biomass in control plots, suggesting that within control plots, weevils did not select plants based on size or that weevil presence did not reduce plant biomass or vigor beyond the consumption of seeds.

Fertilization seemed to buffer the negative effect of *L. minutus* on seed production, possibly by supplying the resources necessary for compensation of those lost to herbivory. At the same weevil density, fertilized plants maintained a higher seed production than unfertilized plants (Fig. 3). In plots with added phosphorus and nitrogen plus phosphorus, there was a suggestion of a compensatory response in number of seeds per seed head. The response was not significant, but it is possible that at low use rates, feeding by *L. minutus* could increase *C. diffusa* seed production. Compensatory responses in fertilized and unfertilized *C. maculosa* plants in response to herbivory by *C. achates* were reported by Steinger and Müller-Scharer (1992): aboveground biomass reduction by *C. achates* was much greater in nitrogen-deficient plants than in plants with high nitrogen supply (Steinger and Müller-Scharer 1992).

We detected no significant effect of *Urophora* gall flies on seed production. At this site, *Urophora* flies may supplement the effect of *L. minutus* by providing an additional nutrient drain in seed heads and plants attacked by both insects, and as an additional but

lesser drain on plant resources in seed heads that are missed by *L. minutus*. We did not detect significant additive effects of the combined presence of *Urophora* species and *L. minutus*, but we did not design the study to address species interactions. Our data indicate that *Urophora* spp. persist in the presence of *L. minutus*, albeit at lower densities per plant. The negative impacts of the flies reported previously (Harris 1980) probably continues at a reduced level.

S. jugoslavica showed a clear preference for rosettes in control plots and strongly avoided rosettes in fertilized plots. *C. achates* exhibited the opposite response and was more abundant in fertilized plots than in control plots. We cannot discern from these data whether plant chemistry, plant size, or some other environmental changes due to the fertilizer effect caused this selection. Harris and Clapperton (1997) noted that plants colonized by vesicular-arbuscular mycorrhizal fungi were more heavily attacked by *S. jugoslavica* than uncolonized plants, but the mechanisms for host selection—both in that study and in ours—remain unclear.

We did not find any negative or positive effect of *S. jugoslavica* or *C. achates* on *C. diffusa* biomass or seed production, but the presence of many rosettes exhibiting previous insect damage at our site strongly suggests that these insects suppress bolting (Powell and Myers 1988; T.R.S., unpublished). Herbivory of root tissues by insects may alter the competitive abilities of the plant by a number of direct or indirect mechanisms (Callaway et al. 1999, Newingham 2001). No net effects of these were detected with the measurements made in our study.

The plant vigor hypothesis predicts that herbivorous insects will prefer and perform better on rapidly growing plants and plants growing in resource-rich environments (Price 1991). This hypothesis is thought to be most applicable to insects whose development is closely associated with host plant growth processes, such as the seed head gall-forming *Urophora* species and the soft achene-feeding *L. minutus*. According to the plant vigor hypothesis, weedy invaders of more fertile soils should be more susceptible to attack by specialist biological control insects than weedy invaders of less fertile soils. We did not find consistent evidence to support this hypothesis. Instead, our data suggest that plants growing in the more infertile soils, with a higher C:N tissue ratios and lower overall plant biomass were more susceptible to *L. minutus* and *S. jugoslavica*. Only *C. achates* seemed to prefer the fertilized rosettes. However, our data do not fully support the plant stress hypothesis, that stressed plants provide greater nutritional quality, either. The unfertilized mature *C. diffusa* in the north field, which were the smallest and lowest in nitrogen content, were the most heavily attacked by *L. minutus*.

Long-term monitoring of *C. diffusa* at the insect release site shows that biological control insects have significantly reduced *C. diffusa* densities (Seastedt et al. 2003; T.R.S., unpublished). In this study, we found the greatest impacts on knapweed to be caused by *L. minutus*, and the effects of *L. minutus* were generally

the most sensitive to nutrient amendments. We hypothesized that fertilization would alter plant characteristics that would attract specialist predators, but instead, we found that the specialist predators of *C. diffusa* exhibited little preference for vigorous, nutritive plants. This negative relationship between enhanced nutrient abundance seems to be best explained by a delay in plant maturation on higher nutrient sites. Variables that are rarely considered with respect to biological control effectiveness, such as variations in resource availability, mowing, or spring grazing that could delay *C. diffusa* flowering, may prove to be significant in explaining the ability of insects to achieve rapid or effective biological control on invasive species such as *C. diffusa*.

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References Cited

- Berube, D. E. 1980. Interspecific competition between *Urophora affinis* and *U. quadrifasciata* (Diptera: Tephritidae). *Entomophaga* 234: 331-337.
- Callaway, R. M., T. H. DeLuca, and W. M. Belliveau. 1999. Biological-control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. *Ecology* 80: 1196-1201.
- Dukes, J. S., and H. A. Mooney. 1999. Does global change increase the success of biological invaders? *Trends Ecol. Evol.* 14: 135-139.
- Harris, P. 1980. Effects of *Urophora affinis* Frfld. and *U. quadrifasciata* (Meig.) (Diptera: Tephritidae) on *Centaurea diffusa* Lam. and *C. maculosa* Lam. (Compositae). *Angew. Entomol.* 90: 190-201.
- Harris, P. 1990. Feeding strategy, coexistence and impacts of insects in spotted knapweed capitula, pp. 39-47. In E. S. Delfosse [ed.], *Proceedings of the VII International Symposium on Biological Control of Weeds*, March 6-11, 1988, Rome, Italy. Inst. Sper. Patol. Veg. MAF.
- Harris, P., and M. J. Clapperton. 1997. An exploratory study on the influence of vesicular-arbuscular mycorrhizal fungi on the success of weed biological control with insects. *Biocontrol Sci. Technol.* 7: 193-201.
- Hierro, J. L., and R. M. Callaway. 2003. Allelopathy and exotic plant invasions. *Plant and Soil* 256: 29-39.
- Jefferies, R. L., and J. L. Maron. 1997. The embarrassment of riches: atmospheric deposition of nitrogen and community and ecosystem processes. *Trends Ecol. Evol.* 12: 74-78.
- Kashefi, J. M., and R. Sobhian. 1998. Notes on the biology of *Larinus minutus* Gyllenhal (Col., Curculionidae), an

- agent for biological control of diffuse and spotted knapweeds. *J. Appl. Entomol.* 122: 547–549.
- Lang, R. F., J. M. Story, and G. L. Piper. 1996. Establishment of *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) for biological control of diffuse and spotted knapweed in the western United States. *Pan-Pac. Entomol.* 72: 209–212.
- Lang, R. F., R. W. Hansen, R. D. Richard, and H. Ziolkowski. 2000. Spotted knapweed (*Centaurea maculosa* Lamarck) seed and *Urophora* spp. gall destruction by *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) combined with *Urophora affinis* Frauenfeld (Diptera: Tephritidae) and *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae), pp. 735–737. In N. R. Spencer [ed.], Proceedings of the X International Symposium on Biological Control of Weeds, 4–14 July 1999, Montana State University, Bozeman, MT.
- LeJeune, K. D. 2002. An investigation of relationships between soil resource availability and the invasion and dominance of Colorado Front Range Prairies by the non-native *Centaurea diffusa* Lam. Ph.D. dissertation, University of Colorado, Boulder, CO.
- Louda, S. M. 1982. Distribution ecology: variation in plant recruitment over a gradient in relation to insect seed predation. *Ecol. Monogr.* 52: 25–41.
- Louda, S. M. 1983. Seed predation and seedling mortality in the recruitment of a shrub, *Happlopappus venetus* (Asteraceae), along a climatic gradient. *Ecology* 64: 511–521.
- Mattson, W. J., and R. A. Haack. 1987. The role of drought in outbreaks of plant-eating insects. *BioScience* 37: 110–118.
- McEvoy, P. B., N. T. Rudd, C. S. Cox, and M. Huso. 1993. Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaea* populations. *Ecol. Monogr.* 63: 55–75.
- Müller, H. 1990. An experimental and phytocentric approach for selecting effective biological control agents: insects on spotted and diffuse knapweed, *Centaurea maculosa* and *C. diffusa* (Compositae), pp. 181–190. In E. S. Delfosse [ed.], Proceedings of the VII International Symposium on Biological Control of Weeds, March 6–11, 1988, Rome, Italy. Inst. Sper. Patol. Veg. MAF.
- Myers, J. H. 2004. A silver bullet in the biological control of diffuse knapweed. ESA 2004 Annual meeting (abstr.). <http://abstracts.co.allenpress.com/pweb/esa2004> (accessed 10/20/04).
- 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, pp. 67–74. In E. S. Delfosse [ed.], Proceedings of the VII International Symposium on Biological Control of Weeds, March 6–11, 1988, Rome, Italy. Inst. Sper. Patol. Veg. MAF.
- Newingham, B. A. 2001. Effects of defoliation on the invasive species, *Centaurea maculosa*: neighbors, allelopathy, compensatory growth and mycorrhizae. Ecological Society of America, Madison, WI.
- Pecora, P., and P. H. Dunn. 1990. Insect associations on leafy spurge in Europe: implications for strategies for releases of biological control agents in North America, pp. 75–82. In E. S. Delfosse [ed.], Proceedings of the VII International Symposium on Biological Control of Weeds, March 6–11, 1988, Rome, Italy. Inst. Sper. Patol. Veg. MAF.
- Powell, R. D., and J. H. Myers. 1988. The effect of *Sphenoptera jugoslavica* Obenb. (Col., Burprestidae) on its host plant *Centaurea diffusa* Lam. (Compositae). *J. Appl. Entomol.* 106: 25–45.
- Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* 62: 244–251.
- Rice, P. M. 2002. INVADERS database aystem (<http://invader.dbs.umt.edu>). Division of Biological Sciences, University of Montana, Missoula, MT 59812–4824. Accessed June 13, 2002.
- SAS Institute. 1999–2001. Release 8.02. The SAS Institute. Cary, NC.
- Scott, A. B. 2001. Effects of cattle grazing on diffuse knapweed (*Centaurea diffusa*) and other exotic plant species in Boulder County, Colorado grasslands. M.S. thesis, University of Colorado, Boulder.
- Seastedt, T. R., N. Gregory, and D. Buckner. 2003. Reduction of diffuse knapweed using insects in a Colorado grassland. *Weed Sci.* 51: 237–245.
- Shorthouse, J. D. 1990. Modification of flowerheads of diffuse knapweed by the gall inducers *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae), pp. 221–228. In E. S. Delfosse [ed.], Proceedings of the VII International Symposium on Biological Control of Weeds, March 6–11, 1988, Rome, Italy. Inst. Sper. Patol. Veg. MAF.
- Skinner, K., L. Smith, and P. Rice. 2000. Using noxious weed lists to prioritize targets for developing weed management strategies. *Weed Sci.* 48: 640–644.
- Smith, L. 2004. Impact of biological control agents on diffuse knapweed in central Montana, pp. 589–593. In J. M. Cullen, D. T. Briese, D. J. Kriticos, W. M. Lonsdale, L. Morin, and J. K. Scott [eds.], Proceedings of the XI Symposium on Biological Control of Weeds, April 27–May 2, 2003, Canberra, Australia.
- Steinger, T., and H. Müller-Schärer. 1992. Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nitrogen availability. *Oecologia (Berl.)* 91: 141–149.
- Stinson, C.S.A., D. Schroeder, and K. Marquardt. 1994. Investigations on *Cyphocleonus achates* (Fabr.) Col., Curculionidae, a potential biological control agent of spotted knapweed (*Centaurea maculosa* Lam.) and diffuse knapweed (*C. diffusa* Lam.) (Compositae) in North America. *J. Appl. Entomol.* 117: 35–50.
- Story, J. M., and G. L. Piper. 2001. Status of biological control efforts against spotted and diffuse knapweed, pp. 11–17. In L. Smith [ed.], The 1st International Knapweed Symposium of the Twenty-First Century, 15–16 March 2001, Coeur d'Alene, ID.
- Suding, K. N., K. D. LeJeune, and T. R. Seastedt. 2004. Competitive impacts and responses of an invasive weed: dependencies on nitrogen and phosphorus availability. *Oecologia (Berl.)* 141: 526–535.
- [SWEPIC] Southwest Exotic Plant Information Clearinghouse 2002. Southwest Exotic Plant Information Clearinghouse. U.S. Geological Survey, Biological Resources Division, Forest and Rangeland Ecosystem Science Center, Colorado Plateau Field Station. <http://usgssrv.usgs.nau.edu/swepic/>. Accessed 12/27/02.
- Technicon Industrial Systems. 1977. Individual/simultaneous determination of nitrogen and/or phosphorus in a BD (block digester) acid digests. Industrial Method No. 334/74W/B+. Technicon Industrial Systems, Tarrytown, NY.
- Thompson, D. J., and D. G. Stout. 1991. Duration of the juvenile period in diffuse knapweed (*Centaurea diffusa*). *Can. J. Bot.* 69: 368–371.
- TNC. 1999. The Nature Conservancy, Wildland Invasive Species Team. www.tncweeds.ucdavis.edu/esadocs/documts/centdif.pdf. (Accessed 10/04).

- U.S. Dep. Agric. SCS. 1975. Soil survey of Boulder County area, Colorado. Prepared by the United States Department of Agriculture Soil Conservation Service in cooperation with the Colorado Agricultural Experiment Station.
- White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia (Berl.)*. 63: 90-105.
- Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of global nitrogen cycle: sources and consequences. *Ecol. Appl.* 7: 737-750.
- Vivanco, J. M., H. P. Bias, F. R. Stermitz, G. C. Thelen, and R. M. Callaway. 2004. Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecol. Lett.* 7: 285-292.

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