



Soil nutrient availability as a mechanistic assessment of carbon addition and biological control of spotted knapweed (*Centaurea maculosa* Lam.)  
by Michel Rene Brockington

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Resources and Environmental Sciences  
Montana State University  
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**Abstract:**

Invasive plant infestations alter indigenous plant communities, and have serious ecological and economic consequences. Management strategies aim to alter the processes driving plant communities to favor desired species. The goal of this research was to investigate a mechanistic approach to large-scale weed problems. Two studies examined: 1) the ability of carbon to reduce soil nutrients and invasive plant growth, and 2) insect biological control agent impacts on a spotted knapweed population and associated soil nutrient availability changes. The objective of the first study was to lower soil nutrients by adding a carbon source. We hypothesized that increasing the amount of sucrose would reduce soil nutrients and spotted knapweed growth. Sucrose addition reduced spotted knapweed biomass, yet led to soil nitrogen increases. This result may be attributed to a flush of nutrients after initial microbial immobilization. Our results suggest that if sucrose is used in research, high amounts (>70 gC/m<sup>2</sup>) must be coupled with frequencies of 30 days or less in order to sustain immobilization of limiting nutrients. The second study investigated the effect of an insect biological control agent, *Cyphocleonus achates* Fahr. (Coleoptera: Curculionidae), on soil nutrients and spotted knapweed (*Centaurea maculosa* Lam.). We hypothesized that spotted knapweed growth would increase with N-addition and decrease with sucrose addition, and that soil N would increase with the addition of *C. achates* where N is most limited. With one year of introduction, spotted knapweed aboveground biomass increased in response to *C. achates*, suggesting spotted knapweed may have compensated for insect infection. In a long-term experiment, soil ammonium decreased and biomass increased in response to *C. achates*. Uninfected individual plants may have responded to available resources prevented from acquisition by infected plants. This may suggest that sufficient insect density must be established to infect a majority of plants for an overall growth decline. Natural enemy impact on a target weed population may potentially be predicted from its influence on soil resources. Prediction accuracy will likely be improved where either weevil densities or infection intensities are considered.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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## ABSTRACT

Invasive plant infestations alter indigenous plant communities, and have serious ecological and economic consequences. Management strategies aim to alter the processes driving plant communities to favor desired species. The goal of this research was to investigate a mechanistic approach to large-scale weed problems. Two studies examined: 1) the ability of carbon to reduce soil nutrients and invasive plant growth, and 2) insect biological control agent impacts on a spotted knapweed population and associated soil nutrient availability changes. The objective of the first study was to lower soil nutrients by adding a carbon source. We hypothesized that increasing the amount of sucrose would reduce soil nutrients and spotted knapweed growth. Sucrose addition reduced spotted knapweed biomass, yet led to soil nitrogen increases. This result may be attributed to a flush of nutrients after initial microbial immobilization. Our results suggest that if sucrose is used in research, high amounts ( $>70 \text{ gC/m}^2$ ) must be coupled with frequencies of 30 days or less in order to sustain immobilization of limiting nutrients. The second study investigated the effect of an insect biological control agent, *Cyphocleonus achates* Fahr. (Coleoptera: Curculionidae), on soil nutrients and spotted knapweed (*Centaurea maculosa* Lam.). We hypothesized that spotted knapweed growth would increase with N-addition and decrease with sucrose addition, and that soil N would increase with the addition of *C. achates* where N is most limited. With one year of introduction, spotted knapweed aboveground biomass increased in response to *C. achates*, suggesting spotted knapweed may have compensated for insect infection. In a long-term experiment, soil ammonium decreased and biomass increased in response to *C. achates*. Uninfected individual plants may have responded to available resources prevented from acquisition by infected plants. This may suggest that sufficient insect density must be established to infect a majority of plants for an overall growth decline. Natural enemy impact on a target weed population may potentially be predicted from its influence on soil resources. Prediction accuracy will likely be improved where either weevil densities or infection intensities are considered.

## CHAPTER 1

### INTRODUCTION

Invasion by nonindigenous, undesirable species is a serious ecological and economic threat to North American rangeland ecosystems. Millions of hectares of grassland throughout western North America are dominated by monotypic stands of invasive weeds (Sheley and Petroff 1999). Large-scale infestations are associated with reduced livestock and wildlife habitat (Olson 1999), increased surface runoff, erosion, and stream sedimentation (Lacey et al. 1989), lower species diversity (Kedzie-Webb et al. 2002) and greatly compromised land use. These effects can have devastating economic costs. In fact, weeds in rangeland cost nearly \$2 billion annually in the U. S. alone, which is more than all other pests combined (DiTomaso 2000). In addition to reducing species diversity at various trophic levels, nonindigenous species infestations are deleterious to the organization, structure and function of plant communities (Olson 1999), and may be responsible for disruption of nutrient cycles (Olson 1999, but see Svejcar and Sheley 2001).

The ultimate goal of large-scale weed management is to develop and maintain healthy, weed-resistant plant communities, while providing for other land use objectives such as forage, wildlife habitat, or recreation (Sheley et al. 1996). Functionally diverse communities with maximum niche occupation capture a greater proportion of the system's resources (Carpinelli 2000). This preempts their use by invasive species, and maintains ecosystem health and productivity (Sheley et al. 1996). To address this goal,

ecologically-based management strategies must focus on directing weed-infested grasslands toward a more desired, functioning plant community. Understanding the influence of land management practices on the organization, structure, and function of plant communities is central to ecologically-based management (Sheley and Rinella 2001). The aim of invasive plant management research must be to understand and modify the mechanisms directing the organization, structure, and function of plant communities (Olson 1999). Mechanistic knowledge of trophic-level interactions should provide an ecologically-based framework for greater management efficacy (Sheley et al. 1996, Davis and Pelsor 2001).

Though complex and dynamic, plant ecology requires an understanding of the mechanisms that drive plant community dynamics that may be manipulated and used to predict management outcomes (Luken 1990, Tilman 1990, Kedzie-Webb et al. 2002). Management and research practices that alter the most important mechanisms driving plant community dynamics will have a greater probability for success (Toner and Keddy 1997). The overall goal of this research is to investigate soil nutrient availability as a mechanism driving an invasive plant population.

In western semiarid grasslands of North America, competition for limited soil resources, especially nitrogen (N) and phosphorus (P), is an important mechanism among interspecific plant interactions (Chapin 1980, Redente et al. 1992, Vitousek and Farrington 1997, Kolb et al. 2002). Competition for nutrients in limited supply may be an important factor driving plant community dynamics (Wedin and Tilman 1990, Redente et al. 1992). Competition models offer phenomenological, rather than

mechanistic, models that are useful for predicting site- and species-specific competitive outcomes. Tilman (1981) provides a more mechanistic theory for predicting the outcome of competition based on the unique abilities of plant species to acquire resources. He proposed that as the limiting resource diminishes below a critical level ( $R^*$ ), only species capable of continuing to acquire that resource persist. Tilman and Wedin (1991b) studied a range of species with different successional niches and found that late seral species were very competitive for N. Early seral species were poor competitors for N, yet persisted by maintaining rapid growth rates and high seed production. When grown in pairwise competition experiments, the late seral species displaced early to mid seral species (Tilman and Wedin 1991a). Species populations, therefore, have differing abilities to lower soil quantities of extractable ammonium and nitrate, and this accounted for the results of the competition trials.

Herron et al. (2000) found in a greenhouse study that two early seral species, bottlebrush squirreltail (*Elymus elimoides* (Nutt.) Smith) and annual rye (*Secale cereale* L.), sequestered soil N in their tissue driving the total plant available soil N to a level lower than the  $R^*$  of spotted knapweed (*Centaurea maculosa* Lam.). Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Scribn. & Smith), a late seral species, may have had a lower  $R^*$  for N than spotted knapweed, and, therefore, competitively dominated in the limited-resource environment (Herron et al. 2000). Field studies are needed to elucidate this and other mechanisms associated with invasive plant ecology (Williamson 1999).

Resource availability associated with invasive plant populations may be an integrator of climatic, abiotic, and plant resource allocation factors contributing to plant community dynamics. Generally, dominance by invasive plants is correlated with excess plant-available soil nitrogen (N) (Alpert and Maron 2000, Lejeune and Seastedt 2001, Kolb et al. 2002), either from human application or small-scale disturbances creating small areas of high N available (Tyser and Key 1988). Traits common to many invasive species such as rapid growth, early emergence, and high seed production allow them to preempt N and other important nutrients from slower-growing, competitive species adapted to low nutrient conditions (Grime 1977, Redente et al. 1992, Lambers et al. 1998, Paschke et al. 2000, Blicker et al. 2002). In addition, invasive and/or early seral species are thought to be poor competitors for nutrients compared to later seral species (Grime 1977, Chapin 1980, Lowe et al. 2002). Spotted knapweed is one of the most aggressive nonindigenous forbs, dominating millions of hectares of western grassland ecosystems (Sheley et al. 1999). Spotted knapweed exhibits many early seral characteristics, yet can invade late seral communities when small disturbances induce patches of high N (Tyser and Key 1988).

Specifically, the overall objective of this research was to investigate the availability of soil N and P as a mechanistic factor affecting spotted knapweed populations, and to assess the potential of using nutrient availability for greater prediction accuracy of management outcomes. Toward this objective, I investigated: 1) the ability of carbon (C) to reduce soil nutrients and invasive plant growth, and 2) the effects of an insect biological control agent on a population of spotted knapweed and associated soil

nutrient availability. In the first experiment, carbon (sucrose) was added to soil in a gradient of sucrose amounts and three application frequencies to better understand the response of spotted knapweed growth to nutrient limitation resulting from immobilization of available nutrients. In the second experiment, manipulations of nutrient supply were combined with an insect biological control agent. Spotted knapweed population growth and soil nutrient responses to insect infection were measured in both the short- and long-term.

The overall objective of study 1 was to manipulate soil resources using carbon (as sucrose) additions to reduce the growth and productivity of spotted knapweed. Carbon addition was used to reduce nutrients low enough to shift the competitive balance in favor of desired species (Paschke et al. 2000). Addition of labile C was expected to limit plant-available soil nutrients and growth via microbial immobilization. The specific objective was to determine the relationship between spotted knapweed growth, and frequency and amount of sucrose addition. We hypothesized that increasing amount and frequency of sucrose application would reduce spotted knapweed aboveground biomass. Additionally, we expected negative effects of both amount and frequency of sucrose on soil nitrate, ammonium, and phosphorus. Currently, there is no available information on whether nutrient manipulation with C addition might be used to manage weed populations (Alpert and Maron 2000). Understanding the effects of nutrient limitation on spotted knapweed populations may allow for better predictive ability of the outcomes of this management tool.

The overall objective of study 2 was to determine the effect of *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae), N-addition, and sucrose addition on plant-available soil nutrient content and spotted knapweed growth. Biological control assumes the competitive ability of the target plant species is compromised by a natural enemy introduction (Maron and Vila 2001). A greater understanding is needed concerning biological control effects on the mechanism of limited nutrient competition. We hypothesized that spotted knapweed biomass, cover, and density would increase with N-addition and decrease with sucrose addition. We also hypothesized that plant-available soil N would increase with the addition of *C. achates*, especially in low-N conditions. As the natural enemy stresses the plant and decreases the competitive ability, spotted knapweed's ability to sequester N would decrease, especially where competition for N is a key factor directing community dynamics. This information may provide an initial indication of the potential to use soil nutrient availability as a mechanistic method for predicting the outcome of plant community dynamics prior to management.

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## CHAPTER 2

SPOTTED KNAPWEED (*CENTAUREA MACULOSA* LAM.) AND SOIL NUTRIENT RESPONSES TO SUCROSE ADDITIONIntroduction

In western semiarid grasslands of North America, competition for limited nutrients is an important factor driving plant community dynamics (Chapin 1980, Wedin and Tilman 1990, Redente et al. 1992, Vitousek and Farrington 1997). Dominance by invasive plants is often correlated with high soil nitrogen (N) availability (Alpert and Maron 2000, Lejeune and Seastedt 2001, Kolb et al. 2002). Traits common to many invasive species such as early emergence, rapid growth, and high seed production allow them to preempt N from slower-growing, competitive species adapted to low nutrient conditions (Grime 1977, Redente et al. 1992, Lambers et al. 1998, Paschke et al. 2000, Blicher et al. 2002). Spotted knapweed (*Centaurea maculosa* Lam.) exhibits many early seral characteristics and rapidly infests disturbed soils high in N (Sheley et al. 1999b). Within *Centaurea*, a nonindigenous genus that has infested western North America, phosphorus (P) can also be an important nutrient directing population dynamics (Lejeune and Seastedt 2001). Late-successional grasslands of the West are often limited by N and/or P (Lambers et al. 1998, Blicher et al. 2002). One potential strategy to reduce the dominance of spotted knapweed would be to reduce soil nutrient content to favor intense competition with desired species. For example, Herron et al. (2000) found available N was sufficiently reduced to shift the competitive balance from spotted knapweed to bluebunch wheatgrass

(*Pseudoroegneria spicata* (Pursh) Scrib. & Smith) in the presence of a cover crop that reduced plant available soil N.

Carbon (C) addition is also used to similarly reduce nutrients low enough to shift the competitive balance in favor of desired species (Paschke et al. 2000). In low nutrient soils, invasive and early seral species are poor competitors with late seral species that evolved with this soil condition (Grime 1977, Chapin 1980, Lowe et al. 2002). Even small changes in nutrient supply can affect species productivity (Wedin and Pastor 1993), so manipulation of soil microorganisms that control mineralization has been used to achieve nutrient reduction (Jonasson et al. 1996, Paschke et al. 2000, Schmidt et al. 2000, Ruess et al. 2001). Soil microorganisms are mainly C limited and its addition stimulates expanding microbe populations to immobilize N, P, and other mineralized nutrients in their tissues. With C addition, microbial population growth may also become limited by N and/or P. Microbes act as both a source and a sink of these mineralized nutrients (Jonasson et al. 1996, Kaye and Hart 1997, Ruess et al. 2001). Microbial populations have been found to preempt plant uptake for N and P, and may be more competitive than plants for these nutrients (Harte and Kinsig 1993, Jonasson et al. 1996, Kaye and Hart 1997, Jonasson et al. 1999). Therefore, addition of labile C is expected to limit plant available soil nutrients and growth of ruderal plant species and invasive plants with similar growth characteristics.

Various forms of C addition have been used to immobilize N to favor indigenous plant communities (Schmidt et al. 1997, Reeve-Morghen and Seastedt 1999, Alpert and Maron 2000, Paschke et al. 2000, Schmidt et al. 2000, Ruess et al. 2001). Simple sugars

have been used for more rapid immobilization relative to sawdust or other more complex C forms. Jonasson et al. (1996) found that sugar addition stimulated microbial biomass production, and usually found a decline in inorganic N. In Colorado, Paschke et al. (2000) used sucrose to reduce plant available soil N via immobilization, and in four years, increased the rate of succession from cheatgrass (*Bromus tectorum* L.) to late seral, perennial species. They applied 1600 kg C/ha/yr of sucrose every three weeks during the growing season causing a decrease in plant available soil N. While sucrose has yielded a decrease in plant available N, few studies have specifically investigated effects of frequency and rate of sucrose application on soil nutrient content and invasive weed growth. This information could provide better prediction accuracy of plant community structure as a result of soil nutrient supply, and provide valuable information about using sucrose as an experimental method.

Wedin and Tilman (1993) found that relatively small changes in nutrient supply produced strong feedbacks on plant productivity and net mineralization. These responses were species-dependant, thus have consequences on competition and plant community structure. Because spotted knapweed is primarily associated with disturbed, high N environments (Blicker et al. 2002), it may be feasible to lower N and/or P to a level which severely impacts plant growth. Conversely, this plant may exhibit both early seral characteristics coupled with the competitive ability to survive and reproduce in low N conditions. An improved understanding of spotted knapweed's ability to compete for limiting resources, and the effect of C addition on nutrient supply could be important

where management aims to shift the competitive balance in favor of desired, late-seral species.

The overall objective of this study was to manipulate soil resources using C (as sucrose) additions to reduce the growth of spotted knapweed. The specific objective was to determine the impact of application frequency and rate of sucrose on spotted knapweed growth. We chose to add sucrose on a monthly application schedule to enable quick microbial immobilization that would persist throughout the experiment (Schmidt et al. 2000). We hypothesized that increasing application frequency and amount of sucrose would reduce spotted knapweed aboveground biomass. Additionally, we expected a decrease in availability of both frequency and amount of sucrose application on soil nitrate, ammonium, and phosphorus.

## Materials and Methods

### Plant system

Spotted knapweed is an extremely aggressive, nonindigenous forb that dominates millions of hectares of western grassland ecosystems (Sheley et al. 1999a). This taprooted, rosette-forming perennial in the Asteraceae family currently invades every county in Washington, Idaho, Montana, and Wyoming. Originating from central to eastern Europe, it was introduced to North America in the late 1800s as an alfalfa contaminant and from discarded ship ballast (Sheley et al. 1999b). Large-scale, monotypic stands of spotted knapweed have detrimental ecological impacts, including reduction of trophic-level diversity, decreased livestock forage, increased soil erosion and

stream sedimentation (Lacey et al. 1989); all of which can have serious ecological and economic impacts (DiTomaso 2000). Large monocultures of spotted knapweed are difficult to control (Sheley and Jacobs 1997), and an integrated approach is necessary for sustainable ecological management (Sheley and Petroff 1999).

### Soil and plant materials

The greenhouse study was conducted from February to July of 2002. Field soil from a spotted knapweed-dominated *Festuca idahoensis/Agropyron spicatum* (Mueggler and Stewart 1980) habitat type was collected from the Red Bluff Agricultural Research Station (45° 34'N, 111° 40'W). The area has an elevation of 4960 m, and receives 305 mm annual precipitation. The soil is a Varney clay loam, and is a fine-loamy, mixed frigid Aridic Argiustoll (Table 1). The A horizon, which is the primary biologically active zone, was collected and used for this study. Soil was sieved to remove >10mm rocks and placed in pots. Prior to initiating the study, aqueous solutions from a variety of the sucrose treatments were analyzed for N, and were all found to have similar, trace quantities of the nutrient (data not shown).

Spotted knapweed seeds were collected from Missoula County, MT, in 2000. Tests revealed 95% seed germination. Twenty seeds were sown in 110 (30 x 20 cm) pots in a growth chamber at 21° C day, 10° C night, with 16 hr full spectrum light bulbs (350  $\mu\text{mol}/\text{m}^2/\text{sec}$ ) placed 15 cm above all pots. To better approximate natural conditions, the pots were moved from the growth chamber to a greenhouse 49 days after plant emergence. Conditions in the greenhouse were 14 hr daylight (natural photoperiod), and temperatures were kept at 22° C day, 18° C night.

Table 1. Soil profile characteristics at Red Bluff Agricultural Experiment Station. Profile was excavated at the site of soil collection, and mostly A horizon soil was used.

Horizon	Depth (cm)	Texture*	Clay (%)	Rock Frag. Kind† (Est. % by volume)	pH	Lime‡
A <sub>1</sub>	0-10	Gr SL	16	HV 15%	7.2	NE
A <sub>2</sub>	10-17	Gr SCL	20	HV 15%	7.0	NE
Bt	17-30	Gr SL	10	HV + QC 25%	7.2	NE
2Bt <sub>1</sub>	30-56	Light CL	28	Trace	7.2	NE
2Bt <sub>2</sub>	56-66	CL	24	Trace	-	NE
2Bk <sub>1</sub>	66-91	Gr L	22	HV 25%	-	SE
2Bk <sub>2</sub>	91-150+	Gr SCL	20	HV 25%	-	VE

\*Texture: Gr=gravelly, S=silt, C=clay, L=loam.

†Rock Fragment Kind: HV=hard volcanic, QC=quartz crystals.

‡Lime classes: NE=not effervescent, SE=slightly effervescent, VE=violently effervescent.

### Experimental Design

Treatments consisted of two factors: amount of sucrose and frequency of sucrose application. A spectrum of seven low to high sucrose amounts based on literature was added to monocultures of spotted knapweed. Sucrose amounts consisted of 0, 10, 70, 130, 190, 250, 310 and 370 pure C/m<sup>2</sup> (determined from calculating the percentage of pure C in sucrose and corrected for the amount pure C in table sugar). Each of the seven sucrose amounts was added once (frequency 1), twice (frequency 2), or three times (frequency 3) at approximately 30 day intervals. Treatments were arranged factorially (7 amounts x 3 frequencies) in a randomized complete block design of 21 sucrose treatments plus a control. The experiment was replicated five times.

## Procedures

Seedlings were allowed to grow for 30 days prior to treatment application. Sucrose frequency treatments were applied on April 11 (all pots but controls, day 31). Treatments were reapplied on May 20 (day 70) for pots receiving two and three applications, and again on June 19 (day 100) in pots receiving three applications. To ensure that soil water was not a limiting factor, pots were watered slightly below pot capacity every two weeks. Pot water holding capacity was determined by weighing each pot after a 24 hr saturation and re-weighing after about 30 days. The difference in these weights was calculated, and pots were watered with a slightly lower amount (900 mL/pot) to prevent leaching. Sucrose treatments were dissolved in and applied with the water application. To minimize leaching during watering and treatment application, the water and/or sucrose amount was slowly poured into pots, and buckets were placed under each pot to collect any solution. The solution was re-added to the pot until retention was complete. Pots were randomly rotated within each of the 5 replicates (blocks) during watering to minimize position effects. To mimic natural field density, approximately 7 plants/pot were maintained by hand-pulling extraneous plants prior to the first treatment (day 30). Plants were grown for 139 days after emergence.

## Sampling

Plant growth and nutrient content. In the highest sucrose amount and frequency treatments, all seven plants in 12 pots died 27 days prior to the scheduled harvest date (day 139); so extant plant material was harvested, dried (60 °C, 48 hr) and weighed on

day 112. Soil nutrients were measured in all 110 pots at this time (Table 2). The remaining 98 pots were harvested for above-ground biomass on July 21 (day 139). Plants were clipped at the soil surface, dried (60 °C, 48 hr), and weighed to determine biomass. Spotted knapweed plant tissue was ground to pass a 1 mm sieve, and analyzed on a Leco® CN2000 analyzer (Leco Corporation, St. Joseph, MO) for percent N by combustion at 1150 °C.

Soil and microbial sampling. Soil was analyzed for extractable nitrate and ammonium at the time of field collection ( $\text{mean}_{\text{nitrate}}=1.2$  mg/kg soil,  $\text{SD}=0.04$ ;  $\text{mean}_{\text{ammonium}}=1.52$  mg/kg soil,  $\text{SD}=0.51$ ). Soil was sampled on 1 July 2002 (day 112) for extractable nitrate and ammonium. At the end of the study (21 July 2002, day 139), soil was re-sampled for extractable nitrate, ammonium, and P. If not indicated, other soil data were obtained on day 139 at final harvest. Soil was sampled using a 1-cm diameter auger bored approximately 10 cm deep. Cores were placed in soil bags and transferred to a drying oven, where samples were kept at 60 °C for 48 hr. The samples were then ground and passed through a 2 mm sieve. Nitrate was determined from a KCl extraction using cadmium reduction, and ammonium was analyzed using flow-injection analysis (Clesceri et al. 1998). Plant available P was analyzed using  $\text{NaHCO}_3$  extraction (Olsen et al. 1954). To investigate trends in microbial population differences, soil was also analyzed by treatment for total microbe numbers. At pot harvest, a single soil sample was obtained for each treatment by combining approximately 0.3 L soil from each of the five replications. These 21 samples were analyzed for plate counts of total colony-forming units. Agar plate counts of colony-forming units are commonly used to measure

microbial growth after C additions (Alden et al. 2001). Total heterotrophic plate counts were performed by soil extraction with sterile 0.1% tetrasodium pyrophosphate dispersant. Ten-fold serial dilutions in P-buffered water were plated on standard plate-count agar and incubated for 7-10 d at 25 °C, and colony-forming units (CFU) were counted (Montana Microbiological Services, Inc., Bozeman, MT). Final colonies were expressed in colony-forming units per gram of soil.

### Data Analysis

To identify significant relationships between predictor variables, linear regression was conducted. A combination of model simplicity, Mallows' Cp values,  $R^2$  and sum of squares values were used to assess the appropriateness of the models. Dependent variables were spotted knapweed biomass, nitrate and ammonium (2 sampling times), P, plant aboveground tissue N, and microbial counts, and independent variables were sucrose amounts and application frequency. Poor-fitting models were obtained for tissue N, N uptake, and soil nutrient variables; thus, analysis of variance was used to test the effects of sucrose amount and application frequency on these variables. Least squares means were separated using Fisher's protected LSD ( $\alpha=0.05$ ). Data were log-transformed for nitrate, ammonium, and P variables to meet assumptions of normality. For transformed data, untransformed means are presented, with log-transformed mean comparisons at a significance of  $p<0.05$ . The relationship between biomass and sucrose amount and frequency is presented as a response surface. Because treatments were pooled for microbe analysis, pooled treatment means of independent variables were regressed with the microbe count data ( $n = 22$ ).

## Results

### Spotted knapweed biomass, uptake, and tissue analysis

Early plant death. Twelve pots in the highest sucrose amounts and application frequencies resulted in complete plant death 27 days prior to final harvest. Per-pot biomass (mean=1.3 g/pot, SD=0.9, Table 2) and per plant biomass (mean=0.23 g/plant, SD=0.13) were low in these pots. Nitrate and ammonium in the 12 pots were variable (mean<sub>nitrate</sub>=3.5, SD=4.1, mean<sub>ammonium</sub>=3.9 mg/kg, SD=2.5). Soil P was within the range of the data for the rest of the pots (mean<sub>P</sub>=14.1, SD=7.5). These 12 pots all received >250 g C/m<sup>2</sup> and 2 or 3 applications of sucrose. Five of the pots received 310 g C/m<sup>2</sup> with four of these receiving 3 applications, and six received 370 g C/m<sup>2</sup> with four receiving 3 applications.

Table 2. Actual data for 12 pots that died prior to termination of the experiment.

Pot #	Pure C added (g/m <sup>2</sup> )	Application frequency	Biomass per pot (g)	Biomass per plant (g)	Soil nitrate (mg/kg)	Soil ammonium (mg/kg)	Soil phosphorus (mg/kg)
78	250	3	3.4	0.5	5.8	0.9	23.6
86	310	2	1.6	0.2	4.8	8.3	8.5
92	310	3	2.7	0.4	2.5	2.2	19.8
93	310	3	1.1	0.2	1.0	4.9	6.7
94	310	3	2.3	0.3	0.2	2.8	10.3
95	310	3	1.6	0.2	1.1	1.8	8.9
102	370	2	0.6	0.1	15.6	3.7	23.3
105	370	2	1.9	0.3	1.7	9.7	10.8
106	370	3	2.0	0.3	6.0	2.2	29.7
107	370	3	0.5	0.1	0.6	3.9	10.5
109	370	3	0.2	0.03	0.6	3.9	6.7
110	370	3	0.5	0.1	2.1	3.3	10.4

**Biomass.** Sucrose amount and application frequency were negatively related to aboveground per plant and total pot spotted knapweed biomass (Figure 1 and 2). For every 70 g C/m<sup>2</sup> added, spotted knapweed per-plant biomass decreased by <0.01 g/plant, and with each application frequency, per-plant biomass decreased by 0.18 g/plant (Figure 1). For every 70 g C/m<sup>2</sup> added, spotted knapweed total pot biomass decreased by 0.01 g/pot, and decreased by 1.20 g/pot with every application frequency (Figure 2).

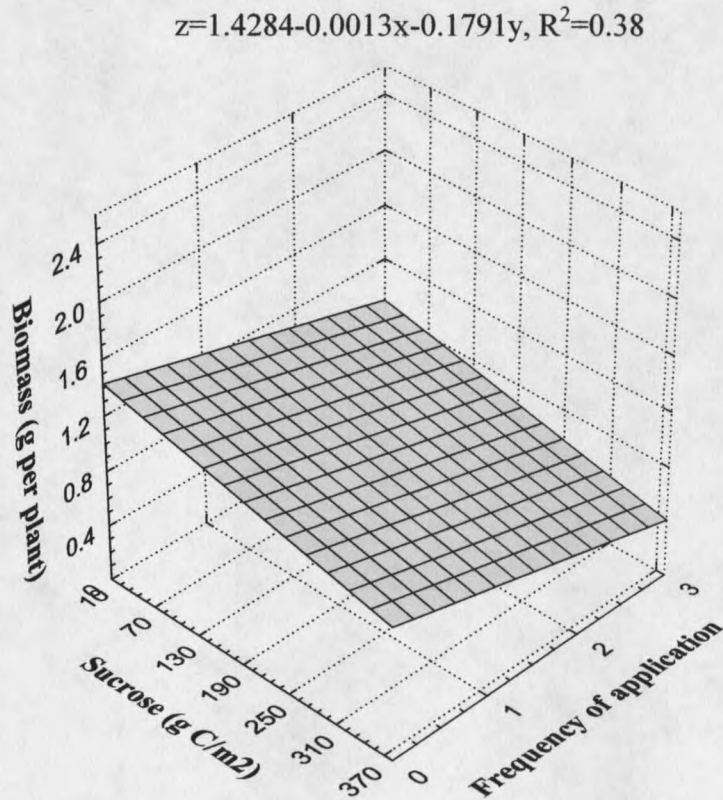


Figure 1. Relationship between spotted knapweed per plant biomass (g/plant, z-axis), frequency of application (0, 1, 2, or 3 applications, x-axis), and sucrose amount (g C/m<sup>2</sup>, y-axis).

$$z=9.7574-0.0088x-1.196y, R^2=0.43$$

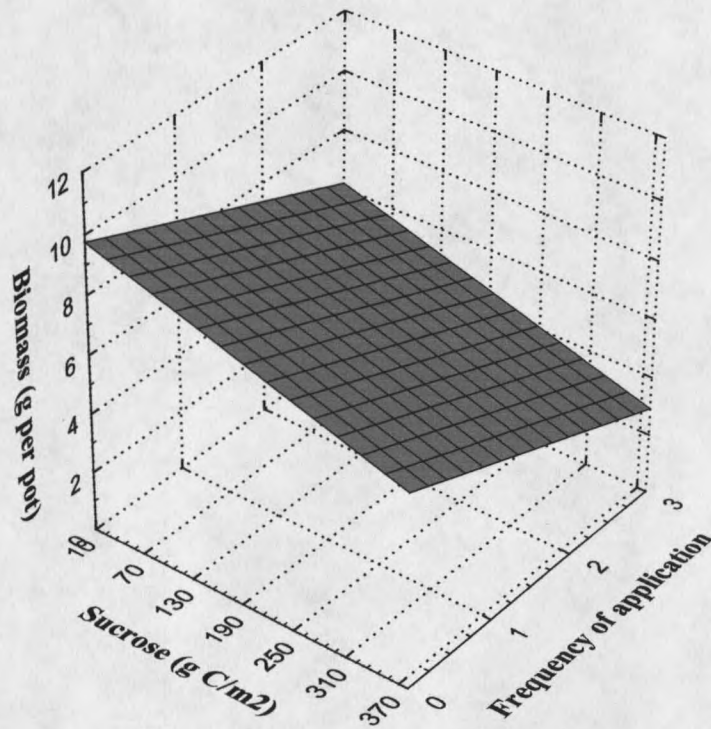


Figure 2. Relationship between spotted knapweed total pot biomass (g/pot, z-axis), application frequency (1, 2, or 3 applications, x-axis), and sucrose amount (g C/m<sup>2</sup>, y-axis).

Tissue analysis. Analysis of variance indicated that spotted knapweed tissue nitrogen content was affected only by sucrose amount (Table 3). Adding 10 or 70 g C/m<sup>2</sup> yielded similar spotted knapweed tissue N as the control (Figure 3). Adding 130, 190, 250, and 370 g C/m<sup>2</sup> all yielded similar tissue N to each other, all of which were higher than that of the control. Unlike the other higher amounts, plants receiving 310 g C/m<sup>2</sup> did not yield significantly higher tissue N than that of the control.

Table 3. P-values of the influence of sucrose amount and frequency on spotted knapweed tissue N percent, soil nitrate, ammonium, P, N uptake, and soil organic matter.

Source of variation	df	Tissue N (%)	N uptake (mg)	Nitrate (day 112)	Nitrate (day 139)	Ammon (day 112)	Ammon (day 139)	Olsen P (mg/kg)	SOM (%)
Rep	4	0.40	0.06	0.29	0.05	<0.01	0.24	0.08	0.06
Amount (A)	6	<0.01	0.38	0.01	<0.01	<0.01	<0.01	<0.01	0.02
Frequency (F)	2	0.23	<0.01	<0.01	<0.01	<0.01	<0.01	0.43	0.53
A x F	12	0.49	0.67	0.26	0.02	<0.01	<0.01	0.33	0.01

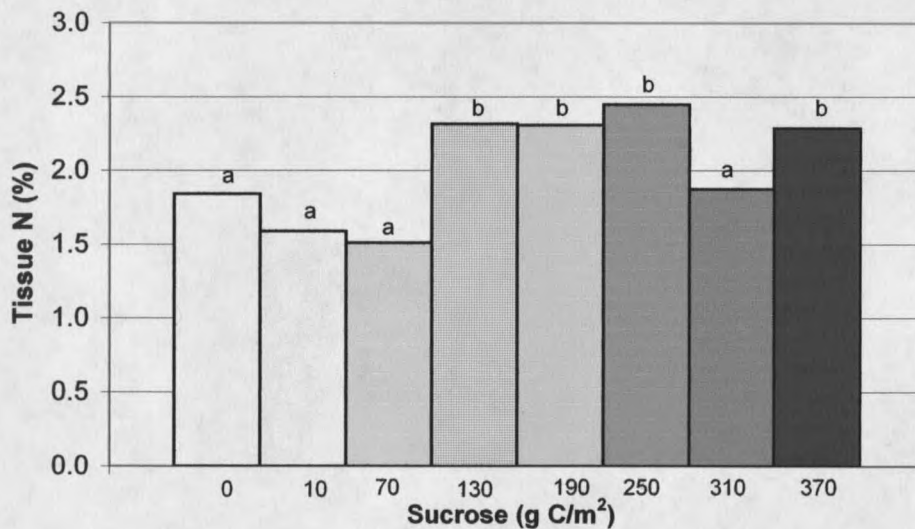


Figure 3. Effect of sucrose amount on spotted knapweed percent N content. Treatments were compared using LSD. Letter differences correspond to significant differences between sucrose amounts.

Plant N uptake. Plant N uptake (N concentration x plant biomass) depended upon application frequency (Table 3). Frequencies 1 and 2 yielded similar N uptake as that of the control (Figure 4). Adding sucrose three times reduced N uptake by spotted knapweed nearly 50% below that of the control.

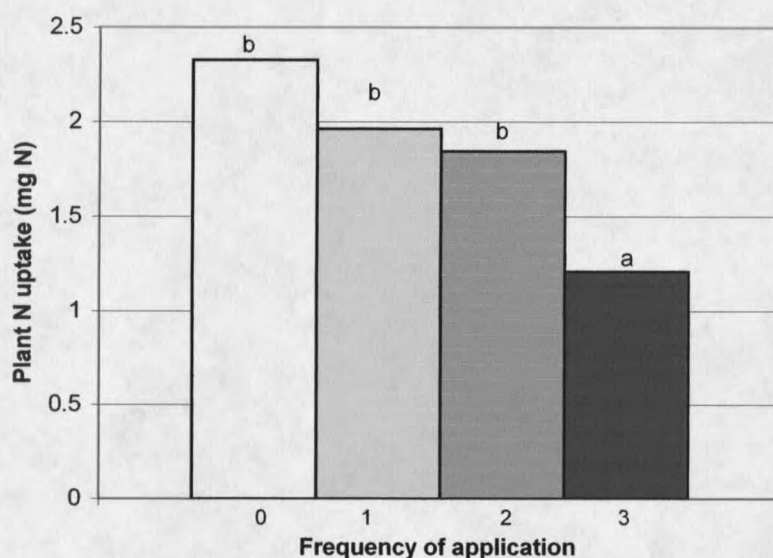


Figure 4. Effect of sucrose application frequency on spotted knapweed N uptake (biomass x concentration). Treatments were compared using LSD. Letter differences correspond to significant differences between sucrose amounts.

### Soil nutrient content

Nitrate. Plant available N was increased by both sucrose amount and frequency of application (Table 3). At the first sampling time (day 112), the main effects of sucrose amount and application frequency affected soil nitrate. Sucrose amounts of 10, 70, 130, 190, 250, and 310 yielded soil nitrate that was similar to the control (Figure 5). Adding 370 g C/m<sup>2</sup> increased nitrate content more than four-fold over the control. Adding 370 g C/m<sup>2</sup> yielded greater soil nitrate than all other amounts. Adding 10 g C/m<sup>2</sup> produced similar soil nitrate as the control, but yielded lower nitrate content than soils receiving 130, 190, 250, and 370 g C/m<sup>2</sup>. The only effect of application frequency was that the

second application of sucrose yielded over three times greater soil nitrate than that of the control and frequency 1, and twice the soil nitrate of frequency 3 (Figure 6).

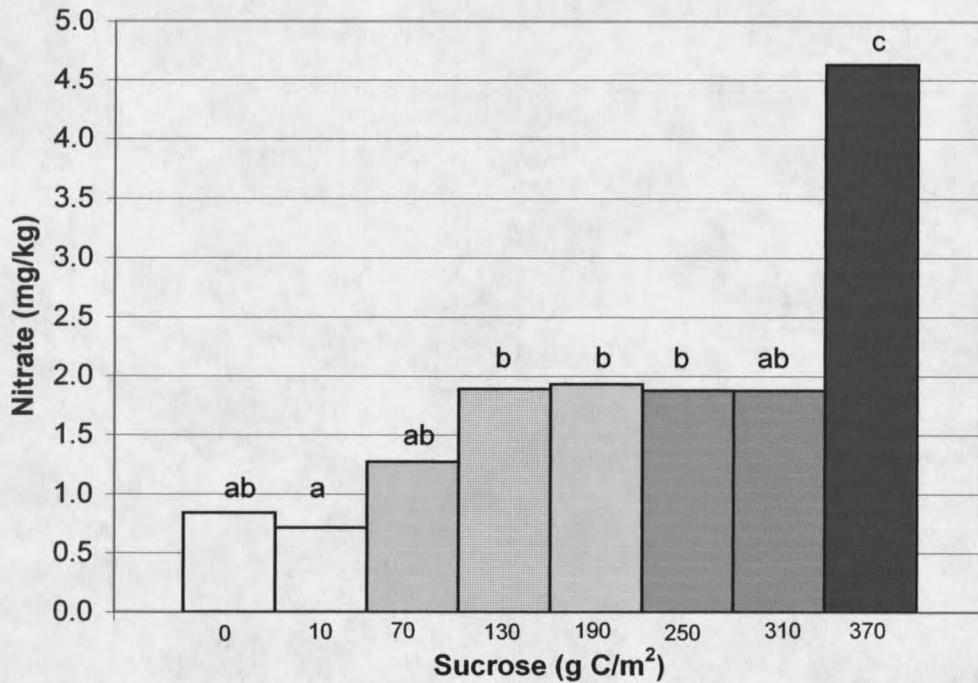


Figure 5. Effect of sucrose amount on soil nitrate, first sample (day 112). Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

When sampled at final harvest (day 139), sucrose amount and frequency impacted extractable soil nitrate (Table 3). Sucrose amounts added once generally yielded similar nitrate values as the control, yet soils receiving 130 g C/m<sup>2</sup> yielded 1.4 mg/kg higher nitrate than that of the control (Figure 7). With two applications, 250 g C/m<sup>2</sup> increased nitrate by 8.3 mg/kg over that of the control. Adding 310 g C/m<sup>2</sup> twice increased soil nitrate 3.6 mg/kg over that of the control. Three hundred seventy g C/m<sup>2</sup>, added twice, yielded a nitrate level over 10 times the control nitrate level. With three applications, 130

g C/m<sup>2</sup> yielded a 2.4 mg/kg nitrate increase. Soils receiving 370 g C/m<sup>2</sup> three times produced over 4 times higher nitrate than the control.

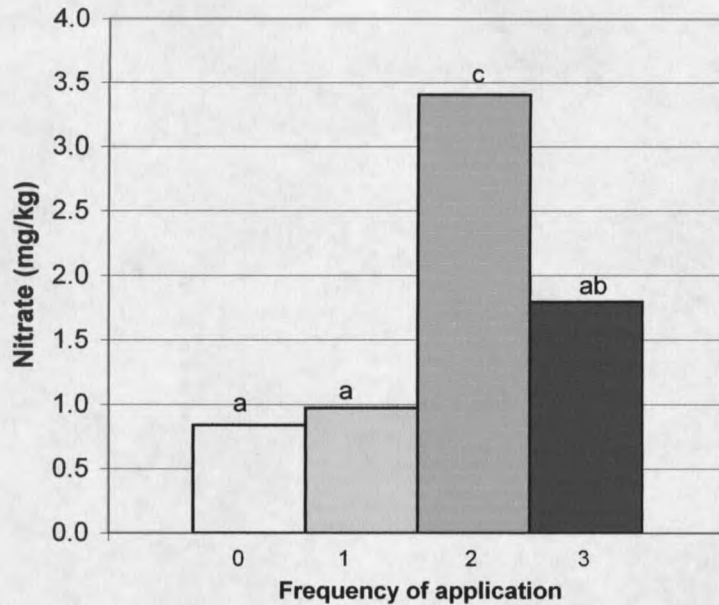


Figure 6. Effect of sucrose frequency on soil nitrate, on day 112. Treatment comparisons were calculated using log<sub>10</sub> transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

Ammonium. Sucrose amount and frequency affected extractable soil ammonium (Table 3). After 112 days, adding 190 or 250 g C/m<sup>2</sup> once produced about 1.3 mg/kg more ammonium than that of the control (Figure 8). Adding one application of 370 g C/m<sup>2</sup> yielded over twice the ammonium level as the control. All amounts >10 g C/m<sup>2</sup>, applied twice, yielded higher ammonium. Seventy and 190 g C/m<sup>2</sup> produced about 1.6 mg/kg greater ammonium than the control. Adding 130, 250, and 310 g C/m<sup>2</sup> twice all produced about 4 mg/kg more soil ammonium than the control. Two applications of 370 g C/m<sup>2</sup> yielded three times the control ammonium level. Only amounts of 130 and 190 g C/m<sup>2</sup>, applied three times, yielded higher ammonium compared to the control, and these

amounts increased soil ammonium by 3.4 and 4.3 mg/kg, respectively. The first sampling of ammonium exhibited less of an increase relative to the second sampling.

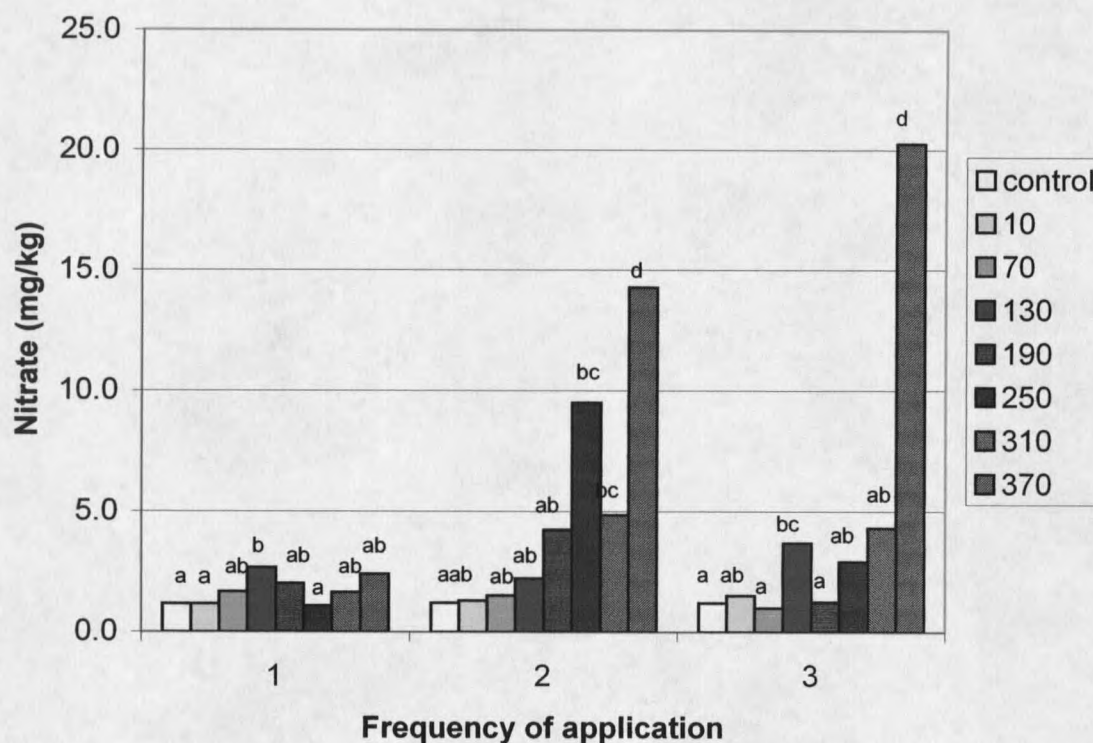


Figure 7. Interaction of sucrose amount and frequency on soil nitrate on day 139. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

Sucrose amount and frequency interacted to effect soil ammonium at 139 days (Table 3). One application of 190 g C/m<sup>2</sup> yielded over twice the ammonium level as the control (Figure 9). Adding 370 g C/m<sup>2</sup> once produced 1.2 mg/kg more ammonium than the control, and adding 250 g C/m<sup>2</sup> twice produced a 1.7 mg/kg increase. Two applications of 310 or 370 g C/m<sup>2</sup> nearly doubled the control ammonium content. Three applications

of all amounts  $>70$  g C/m<sup>2</sup> yielded greater ammonium than the control. Adding 130 or 190 g C/m<sup>2</sup> three times yielded a 1.4 mg/kg increase in ammonium. Amounts of 250 or 370 g C/m<sup>2</sup> more than tripled the control content. Three applications of 310 g C/m<sup>2</sup> produced over 5 times greater ammonium than the control.

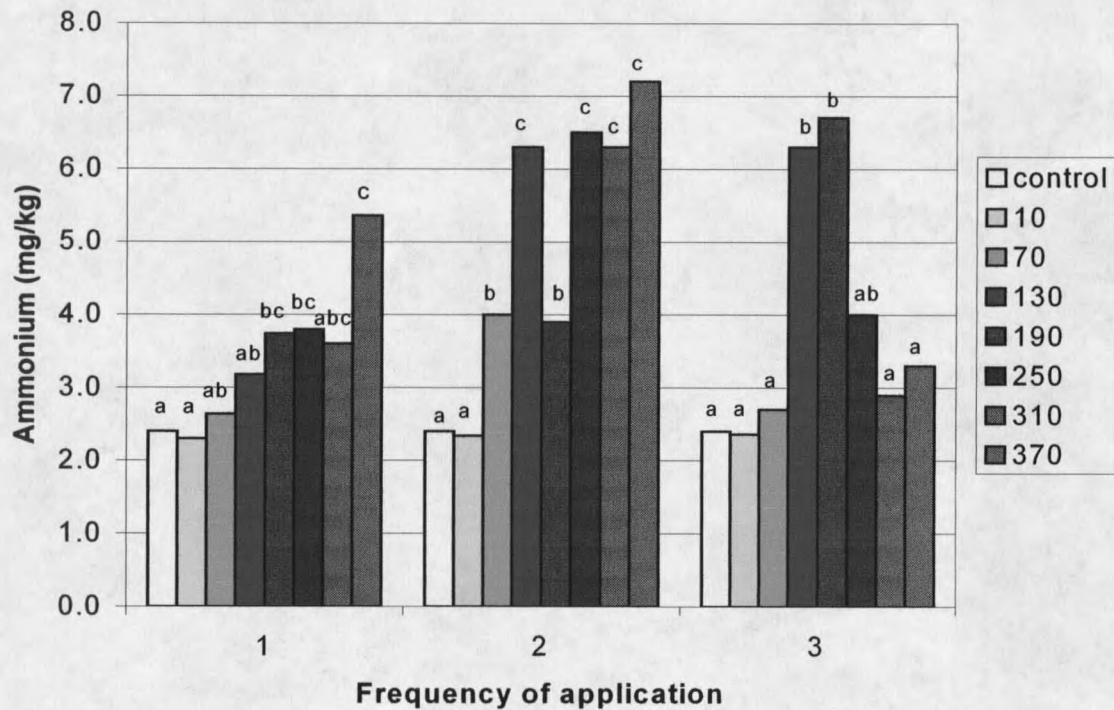


Figure 8. Interaction of sucrose amount and frequency on soil ammonium on day 112. Treatment comparisons were calculated using log<sub>10</sub> transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

Phosphorus. Sucrose amount affected available soil P (Table 3). However, there was no consistent trend in available P with increasing sucrose amounts (Figure 10).

Adding 190 or 250 g C/m<sup>2</sup> produced similar soil P as that of the control. Adding 10, 130, or 370 g C/m<sup>2</sup> yielded about 3 mg/kg more P than the control. Interestingly, 70 or 310 g C/m<sup>2</sup> yielded the highest available soil P, more than doubling that of the control.

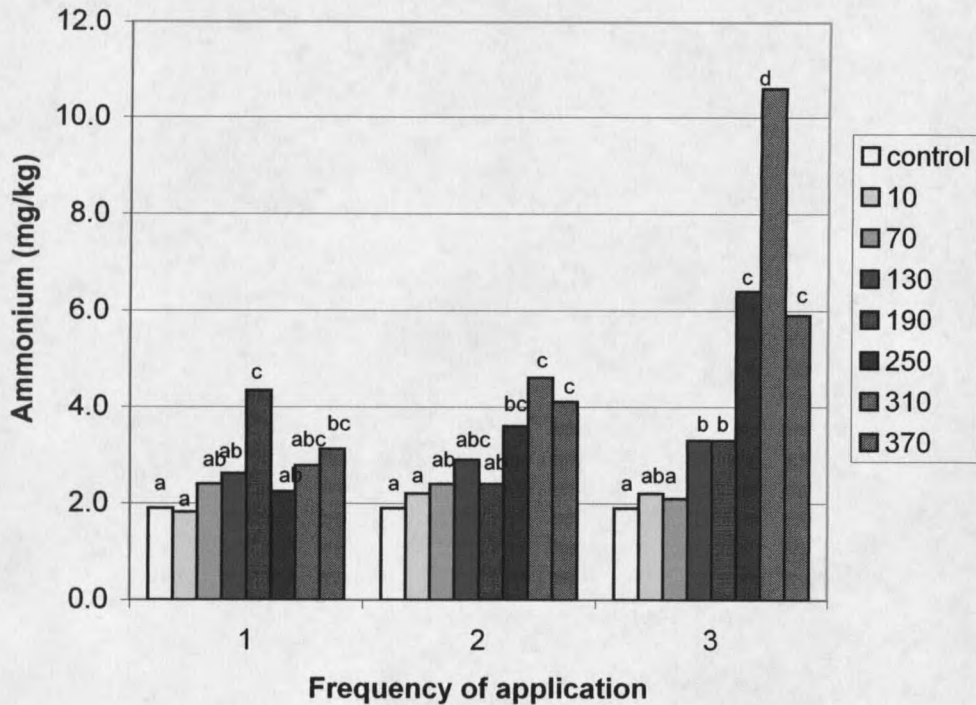


Figure 9. Interaction of sucrose amount and frequency on soil ammonium, second sample (day 139). Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

Organic Matter. Frequency and sucrose amount affected soil organic matter, but the results were variable with respect to frequency, and no consistent patterns emerged (data not shown). Organic matter ranged from 2.6% to about 3.8%.

Microbe counts. Regression indicated that only available soil P was related to microbe counts ( $R^2=0.36$ ,  $P=<0.01$ ). Relative to all other predictors, only soil P demonstrated a slight positive relationship with microbial colony-forming units (Figure 11). With each increase in P, microbe counts increased by  $5.0 \times 10^6$  CFU/g soil.

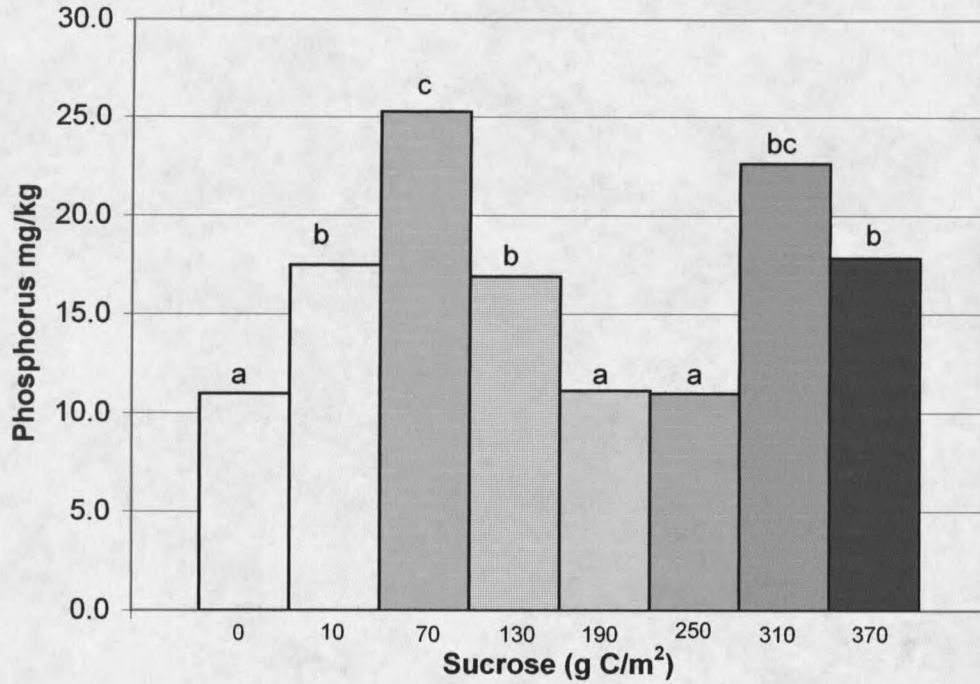


Figure 10. Sucrose amount effects on soil phosphorus. Mean comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

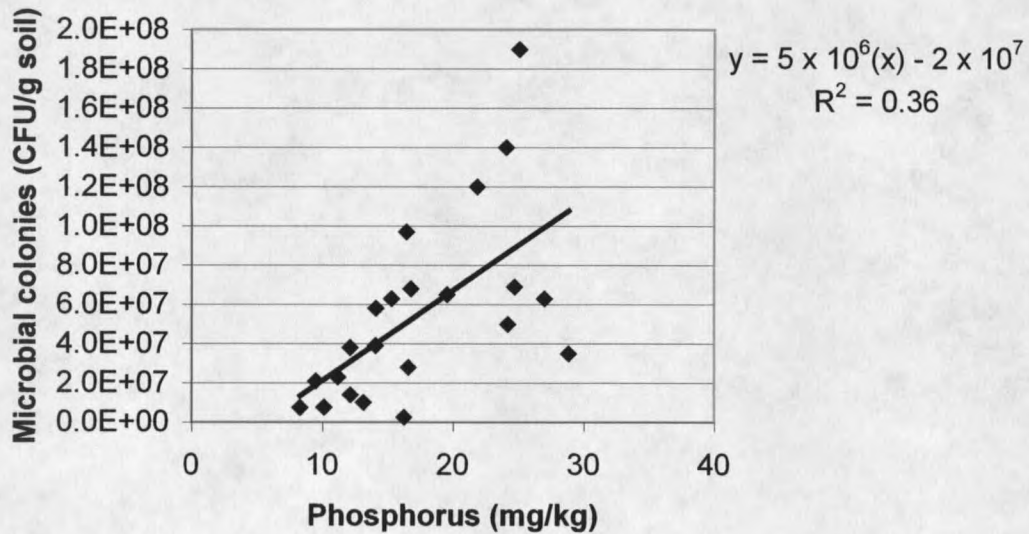


Figure 11. Relationship between available soil P and heterotrophic plate counts.

### Discussion

In this study, increasing amount and frequency of sucrose application negatively affected spotted knapweed biomass, supporting our hypothesis that sucrose inhibits spotted knapweed growth. These results have implications for using sucrose and other sources of C addition to reduce the productivity, and ultimately competitiveness, of spotted knapweed. This evidence compares with C-induced biomass decreases in Colorado grasslands (Reever-Morghen and Seastedt 1999). Because plant death occurred with the highest sucrose amounts and frequencies, it may be that sucrose has the potential to lower nutrients to levels at which spotted knapweed can no longer persist. These results support strategies to reduce the dominance of spotted knapweed by reducing soil nutrient content to favor intense competition with desired species, especially if invasive species are poor competitors with late seral species that evolved with this soil condition (Grime 1977, Chapin 1980, Lowe et al. 2002).

However, I also hypothesized that a decrease in soil N from microbial immobilization would explain the biomass reduction in spotted knapweed, yet both forms of available nitrogen increased rather than decreased as expected. In experiments using  $^{15}\text{N}$  tracers, simple sugar sources have been found to quickly tie up available N, with peak immobilization occurring in only three days (Stevenson 1982). It is likely that the effects of sucrose on microbial immobilization of plant available N are highly ephemeral. Peak immobilization may have occurred prior to sampling, after which populations declined and released nutrients back into the soil. The second sampling exhibited a marked increase in extractable nitrate and ammonium in the higher sucrose amounts relative to

the first sampling. This suggests that with more time, the microbe populations continued to decline and release previously immobilized nitrate and ammonium. Other studies have found an immobilization and subsequent release of nutrients with sucrose application (Jonasson et al. 1999, Reeve-Morghan and Seastedt 1999). However, it is possible that the higher sucrose treatments affected the osmotic component of water, which could have induced a water stress to the plant roots (Metting 1993), despite our attempts to eliminate water stress.

Microorganisms were not extensively measured, as counts were pooled by treatment. In addition, bacterial plate count procedures can preclude the growth of some groups of microorganisms, and can never be interpreted as a total count (Atlas and Bartha 1997). While another method of microbial quantification would be necessary to make any substantial conclusions about microbe response to sucrose addition, our results indicate a positive relationship of available soil P and colony-forming units. Relative to available N and all other variables, available soil P was the only variable that was correlated to microbe count, providing some evidence that P became limited as N immobilization occurred. Schmidt et al. (2000) found that in a high-altitude arctic soil, only adding NPK fertilizer in combination with sucrose yielded an increase in microbial biomass, but they could not determine whether microbes were limited by N, P, or K. Jonasson et al. (1999) also found that episodes of microbial decline yielded a flush of P into the plant available pool.

Another potential explanation for the increase of ammonium is that nitrifying bacteria are very sensitive to oxygen deficiency as well as high C:N environments, and in poorly

aerated soils, ammonium usually increases (Larcher 1975). Thus, potted soil may have lost its structure relative to the field environment, and exhibited a lack of oxygen that could induce a decline in the nitrifier portion of the microbe population. Only more intensive microbial measurements would elucidate this possible effect.

Since inorganic soil N exhibited more of a consistent trend than available soil P in our study, and microbe counts were only slightly correlated to soil P, we cannot support the previous finding that P became limited to spotted knapweed (Lejeune and Seastedt 2001). Perhaps the initial low N conditions associated with our soils made N more limiting to the plants relative to soil P. Percent N content of spotted knapweed demonstrated slight increases with increasing sucrose amount, yet more importantly, N uptake decreased as sucrose application frequency increased. A common plant response to N-limited conditions is to reduce N acquisition, leaving more in the soil (Lambers et al. 1998), and this has been found with spotted knapweed (Blicker et al. 2002). Plants that have evolved in poor nutrient conditions have an increased ability to store and recycle nutrients when they become limited (Blicker et al. 2002). This study may support the hypothesis that *Centaurea* species slow their uptake in response to lower nutrient conditions in order to increase available N for members of their own (often monotypic) populations (Lejeune and Seastedt 2001). If spotted knapweed were able to perpetuate soil N availability in a feedback mechanism, it may self-perpetuate a competitive advantage over indigenous species that may not be favored by higher N. Root uptake:shoot uptake of N and P would be necessary information to identify how resources were distributed within the plant, since root systems tend to be more extensive

in low N conditions, and spotted knapweed root biomass has been observed to increase in low N supply (Blicker et al. 2002). The inclusion of neighboring species in a study similar to this one would be necessary to test this hypothesis.

In conclusion, we accept the hypothesis that sucrose addition reduced the productivity of spotted knapweed, most likely via limiting soil nutrients. An increase in extractable nitrate and ammonium could have resulted from a release of these nutrients from declining microbe populations after an initial immobilization. It is also possible that spotted knapweed further reduced its N uptake rate in response to low nutrient conditions, creating a time trend that resulted in more available soil N. It remains unclear whether spotted knapweed or microbe populations may have induced changes in organic matter in response to sucrose addition. Our higher sucrose amounts and frequencies may have induced the death of all plants in 12 pots, because almost all of the pots were at the highest and most frequent sucrose amounts. N content was variable in these pots, presumably due to the highly ephemeral, treatment-dependant immobilization response of sucrose on soil N. Root:shoot ratios, plant P uptake, and a longer study would be necessary to conclude if spotted knapweed became too limited in either N or P to grow and reproduce.

#### Research and Management Implications

Management of large-scale weed infestations and restoration projects must be both ecologically and economically feasible. Adding sucrose as a management tool may not be economically feasible, as we found frequent applications are necessary to reduce

available N over time to allow for a more desirable plant community trajectory.

However, sawdust and other inexpensive, high C:N materials have been found to provide similar reductions in invasive plant growth. If sucrose is used in research, high amounts ( $>70 \text{ g C/m}^2$ ) must be coupled with frequencies of 30 days or less in order to sustain immobilization of limiting nutrients.

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## CHAPTER 3

INFLUENCE OF *CYPHOCLEONUS ACHATES* (COLEOPTERA: CIRCULIONIDAE),  
NITROGEN, AND SUCROSE ON SPOTTED KNAPWEED (*CENTAUREA*  
*MACULOSA* LAM.)

Introduction

Mechanistic knowledge of trophic-level interactions directing plant community dynamics will be required to predict ecological impacts of invasive plants and the outcome of integrated weed management programs. Infection by host-specific insects can be a sustainable management strategy for large-scale infestations (Mack et al. 2000). A basic paradigm of biological control is that insect infection influences fitness and interspecific competition in favor of non-host species (Carson and Root 1999, Nowierski and Huffaker 1999); thus, biological control assumes the competitive ability of the target plant species is reduced by a natural enemy (Maron and Vila 2001). This paradigm is currently being questioned (Belsky 1986, Crawley 1989, Trumble et al. 1993, Callaway et al. 1999, Maron and Vila 2001). The basis for this reevaluation is that plants have evolved traits allowing them to tolerate insect damage (Stowe et al. 2000), and these traits are expressed species- and site-specifically (Louda and Collinge 1992). For example, in a greenhouse experiment, Callaway and DeLuca (1999) found that Idaho fescue (*Festuca idahoensis* L.) plants grown with spotted knapweed (*Centaurea maculosa* Lam.) that had been attacked by a non-native insect *Trichoplusia ni* had smaller root systems than when they were planted with spotted knapweed that were protected from herbivory. Tilman

(1990) suggests population and community dynamics may be a function of a complex of constraints and tradeoffs. These relationships create variable responses to infection and changing environmental conditions, making prediction of population and community dynamics difficult (Muller and Steinger 1990, Muller-Scharer and Schroeder 1993, Notzold et al. 1998). To a large extent, studies of the interaction among trophic levels involving biological control have been phenomenological, rather than mechanistic, in design. Mechanistic studies have high potential for enhancing prediction accuracy because models are based on the actual cause of the change in competitive relationships (Toner and Keddy 1997).

Competition for nitrogen (N) and/or phosphorus (P) can be an important mechanism among plant interactions (Chapin 1980, Redente et al. 1992, Vitousek and Farrington 1997, Kolb et al. 2002). Tilman (1981) provides a mechanistic theory for predicting the outcome of competition. He proposed that as the limiting resource diminishes below a critical level ( $R^*$ ), only species capable of continuing to acquire that resource persist. Tilman and Wedin (1991b) studied a range of species with different successional niches and found that late seral species were very competitive for N. Early seral species were poor competitors for N, yet persisted by maintaining rapid growth rates and high seed production. When grown in pairwise competition experiments, the late seral species displaced early to mid seral species (Tilman and Wedin 1991a). The ability of a species to lower soil quantities of extractable ammonium and nitrate at a soil depth of 16 cm accounted for the results of the competition trials. Similarly, Herron et al. (2000) found in a greenhouse study that two early seral species, bottlebrush squirreltail (*Elymus elimoides* (Nutt.) Smith) and annual rye (*Secale cereale* L.), sequestered soil N in their

tissue driving the total plant available soil N to a level lower than the  $R^*$  of spotted knapweed. Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Scribn. & Smith), a late seral species, may have had a lower  $R^*$  for N than spotted knapweed, and, therefore, competitively dominated in the limited-resource environment (Herron et al. 2000).

Based on the  $R^*$  theory, the success of spotted knapweed is dependant upon the nutrient supply rate. Resource supply rate is dependant on resource input, climatic influence, and microbial decomposition of organic matter. Nitrogen fertilization has been used to increase spotted knapweed (Story et al. 1989) and meadow hawkweed (*Hieracium praetense* Tausch.) (Wilson 1999) biomass. Adding carbon to decrease plant N availability via microbial immobilization has been implemented in research (Schmidt et al. 1997, Reeve-Morghen and Seastedt 1999, Alpert and Maron 2000). Carbon ( $>70$  g C/m<sup>2</sup>), added as sucrose, decreased spotted knapweed biomass below that of the untreated control (Brockington 2003). In that study, higher rates of carbon addition amount (310 and 370 g C/m<sup>2</sup>) and increased frequency immobilized nutrients sufficiently to induce an overall spotted knapweed biomass decrease, and complete death of some plants.

Most biological control agent selection, host specificity testing, and release monitoring occurs from the insect perspective (Harris 1973, Crawley 1989, Wilson 1999), and the target weed perspective (Burdon and Marshall 1981). Biological control has advanced to consider population level efficacy (Hopper 2001), as well as safety and effectiveness of a potential agent on an individual plant (Wapshere 1974). While rigorous host specificity testing reliably judges risk of non-target plant infection prior to release (Pemberton 2000), there is limited risk assessment for judging agent impact on a

plant population in new environments (Louda and Potvin 1995, Simberloff and Stiling 1996, Waage 2001). A major factor in the success of an agent release could be how it affects the plant population's ability to acquire resources in limited supply. Assessing how a plant populations' ability to acquire resources changes in response to insect damage may streamline future agent searches, and reduce risk of failure and non-target impacts in biological control programs.

In addition to individual plant and plant population fitness, insects may influence interspecific competitive ability among associated populations (Nowierski and Huffaker 1999). However, plant community dynamics in response to insect predation of a dominant population have been largely overlooked in biological control research (Crawley 1989, Muller-Scharer and Schroeder 1993, Wiman 2001). Understanding the influence of natural enemies on the ability of populations to acquire resources, or their  $R^*$ , may provide a method for predicting plant community response to biological control by comparing various species within the community and incorporating known changes in  $R^*$  because of agent introduction. This approach may elucidate whether a natural enemy is able to shift the competitive balance enough to favor desired species prior to their release in North America.

The overall objective of this study was to determine the effect of *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae), N addition, and sucrose addition on plant-available soil nutrient content and spotted knapweed growth. This information may provide an initial indication of the potential to use soil nutrient availability and the relative ability of species to acquire nutrients as a mechanistic method for predicting the outcome of plant community dynamics prior to management. We hypothesized that

spotted knapweed biomass, cover and density would increase with N addition and decrease with sucrose addition. We also hypothesized that plant available soil N would increase with the addition of *C. achates*, especially in a low N environment. The rationale for this hypothesis is that as the natural enemy stresses the plant, decreasing spotted knapweed's ability to sequester N.

### Materials and Methods

#### Plant-Insect System

Spotted knapweed is a taprooted, rosette-forming perennial in the Asteraceae family. Originating from central to eastern Europe, it was introduced to North America in the late 1800s as an alfalfa contaminant and from discarded ship ballast (Sheley et al. 1999). This aggressive plant currently invades every county in Washington, Idaho, Montana, and Wyoming, and has dominated millions of hectares of western grassland ecosystems (Sheley et al. 1999). Large-scale monotypic stands of spotted knapweed have detrimental ecological impacts including reduced associated trophic-level diversity, decreased livestock forage, increased soil erosion, and stream sedimentation (Lacey et al. 1989); all of which can have serious economic impacts (DiTomaso 2000). Spotted knapweed dominates large areas of land, and thus is difficult and expensive to manage (Sheley and Jacobs 1997), and an integrated approach is necessary for sustainable ecological management (Olson 1999).

*Cyphocleonus achates* is one of the 13 insect biocontrol agents released in North America for knapweed control (Clark et al. 2001). This root weevil's host plant preference is spotted knapweed, but will also feed on diffuse knapweed (*Centaurea*

*diffusa* L.) (Wikeem et al. 1999). Introduced to North America in 1987, *C. achates* is indigenous to central, eastern, and southern Europe and western Asia. Successful establishment has occurred in several western states and provinces in the U.S. (Montana, Washington, Colorado, Oregon) and Canada (British Columbia) (Wikeem et al. 1999). Females of this univoltine weevil lay an average of 65 eggs per season, and adults emerge from mid-July to mid-September. Adult *C. achates* feed on knapweed leaves, and the larval stage is most destructive. Pupating larvae, sometimes several in one taproot, mine and destroy the central vascular tissue of the roots.

This research includes a short- and long-term component. Study 1 refers to the short-term (single season) investigation. Study 2 tests portions of our hypotheses after 7 years of insect release, and is presented following a complete description of study 1.

### Study 1: Materials and Methods

#### Study Sites

This study was conducted at two research sites located in Missoula County, Montana, during 2001 and 2002. Both sites were selected for soil and plant homogeneity, lack of existing biological control agents, and presence of a spotted knapweed monoculture.

ANOVA of ammonium and nitrate quantities sampled (10 cm depth) prior to treatment application (September 2001) indicated no significant differences across the study sites (data not shown). O'Keefe Creek is 15 km NW of Missoula, MT (46° 59'N, 114° 7'W), at an elevation of 1400 m. The soil is a Bigarm gravelly loam (Loamy-skeletal, mixed, frigid, Typic Haploxerall). Since the plots were adjacent to a highway, the surface layer consists of gravel from the fill process. A soil profile analysis was conducted in 2002

(Table 1). Mean annual precipitation is 406 mm, and a direct-read rain gauge indicated 112 mm rain fell during 2 July – 13 August 2002. Miller Creek is located 10 km SW of Missoula, MT (46° 48'N, 114° 4'W), at an elevation of 1160 m. The soil is a Biglake gravelly sandy loam (Clayey-skeletal, mixed Typic Eutroboralf), and a soil profile analysis was conducted in 2002 (Table 1). This site receives 381 mm of annual precipitation, and 233 mm of precipitation was recorded from 2 July to 13 August, 2002.

Both sites are on a *Festuca idahoensis/Agropyron spicatum* habitat type (Mueggler and Stewart 1980) infested with a near-monoculture of spotted knapweed. These sites contained mature spotted knapweed populations that had been established for over 10 years. Based on models of population dynamics and observations developed by Jacobs and Sheley (1998), each population appeared to be at equilibrium with oscillatory dynamics about a relatively constant mean. Sweeps of the area, random root excavation, inspection, and lack of prior releases in the area indicated that *C. achates* was absent.

#### Experimental design

Treatments consisted of two factors. The N amendment factor consisted of three levels: an untreated control, sucrose addition, and N fertilizer addition. The second factor consisted of the presence or absence of *C. achates*. Treatments were arranged factorially with 3 N levels x 2 *C. achates* levels in a randomized complete block design. The experiment was replicated 5 times (5 blocks) at both sites.

Table 1. Soil profile characteristics at O'Keefe and Miller Creek.

Site	Horizon	Depth (cm)	EC (ds/m) 1:1	Sand, silt, clay (%)	Kind, course fragments† (%)	Lime‡
O'Keefe Creek	A <sub>1</sub>	0-18	0.15	46, 29, 25	AG, 48	NE
	A <sub>2</sub>	18-25	0.22	46, 28, 26	AG, 46	NE
	(auger to C layer)	25-30	0.36	---	AG, 44	NE
Miller Creek	A <sub>1</sub>	0-8	0.57	44, 32, 24	RG, 11	NE
	A <sub>2</sub>	8-25	0.31	44, 31, 25	RG, 16	NE
	Bt <sub>1</sub>	25-38	0.56	47, 34, 19	RG, 26	NE
	Bt <sub>2</sub>	38-50	0.90	48, 33, 19	RG, 39	SE
	Bt <sub>3</sub>	50-56	0.61	46, 36, 18	RG, 36	SE
	Bk <sub>1</sub>	56-64	0.34	44, 32, 24	RG, 33	ST
	Bk <sub>2</sub>	64-76	0.35	46, 34, 20	RG, 18	VE
	Bk <sub>3</sub>	76-89	0.28	46, 37, 17	RG, 15	VE

† Course fragment abbreviations: AG=angular gravel, RG=round gravel

‡ Effervescence classes: NE=not effervescent, SE=slightly effervescent, ST=strongly effervescent, VE=violently effervescent

### Procedures

Plots were established July 2001. Circular 1.54 m<sup>2</sup> plots were constructed using galvanized tin "corrals" to contain *C. achates* (Story and Wood 1996). Corrals were 50 cm high, buried 10 cm into the soil, and secured with wooden stakes. The upper 5 cm of each corral was folded over 140° to prevent *C. achates* escape (Story and Wood 1996), and corrals were not covered with bird netting. Sucrose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) was broadcast at a rate of 100 g/m<sup>2</sup> pure C (375g sugar per plot, sugar = 40% pure C). Ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) fertilizer was broadcast at a rate of 112 kg/ha N (48 g/plot, 35% pure N). Treatments were applied 19 October 2001 and 22 May 2002. For study 1, *C. achates* were obtained from an established release site near Hamilton, MT, and released into corrals on 8 August 2001 at a rate of 30 weevils per corral. Based on research by Wikeem et al. (1999) this was a high release density, and thus provided adequate

numbers for mating pairs, and maximized effects on plants. Monocultures of spotted knapweed were maintained at each site by hand pulling and/or carefully painting other species with a 10% glyphosate (N-(phosphonomethyl)glycine) solution three times during the spring of 2002.

### Sampling

Plants and insect larvae. Spotted knapweed cover was estimated fall 2001 and spring 2002 for each corral using a randomly placed 31.7 cm x 31.7 cm square frame. At peak standing crop (O'Keefe Cr: 10-13 July 2002, Miller Cr: 16-17 July 2002), density was counted, aboveground biomass harvested, and the number of *C. achates* larvae per adult plant was counted within the same square frame. Cover, density, and biomass were determined separately for juvenile and adult spotted knapweed plants. Rosette plants were considered juveniles. Clipped plant material was dried for 48 hrs at 60 °C, and weighed. *Cyphocleonus achates* were counted by excavating all mature spotted knapweed taproots within the frame, dissecting and identifying all larvae and/or larval cases (Wikeem et al. 1999, Clark et al. 2000).

Soil nutrients. Soils were sampled from the A horizon for nutrient analysis because it is the primary biologically active zone of nutrient cycling. Soils were sampled for available N, P, K, S, and other nutrients using soil core extractions, and for nitrate and ammonium using ion-exchange membrane probes. In each corral, randomly located soil cores were collected 16 September 2001, and three times throughout the growing season of 2002 (21-22 May, 10-11 July, and 9 September) using a 2-cm diameter auger, bored approximately 10 cm deep. Cores were placed in soil sample bags placed in a drying

oven at 60 °C for 48 hr. The samples were then ground and passed through a 2 mm sieve. Nitrate was determined from a KCl extraction using cadmium reduction, and ammonium was analyzed using flow-injection analysis (Clésceri et al. 1998). Plant available P was analyzed using the Bray HCl and NH<sub>4</sub>F extraction method (Pace et al. 1982). Plant available K, S, Ca, Mg, Zn, Na, CEC, pH, and organic matter were analyzed following the methods outlined in Gavlak et al (1994).

Anion exchange membranes in probe form (Plant Root Simulator, or PRS®-probes; Western Ag Innovations, Saskatoon, SK, Canada) were utilized in addition to the traditional soil core extraction method to provide a nutrient status assessment over 6 weeks' time. Preliminary tests indicated low plant available N levels, and this allowed a six-week nutrient measurement because there was low risk of ion saturation during that time period. Two cation and two anion probes (2 x 4 cm ion membrane stakes) were buried in each corral from 2 July – 13 August 2002. The probes were inserted into the soil surface carefully to ensure complete soil contact with the membrane. A direct-read rain gauge captured precipitation during the probe burial time. At the end of six weeks, probes were carefully removed and scrubbed with de-ionized water to remove all soil particles. Mean soil temperatures at probe removal were 38 °C at O'Keefe and 23 °C at Miller Creek. Nutrients were eluted with a 0.5M HCl extraction, and the extract was analyzed using an automated colorimeter (Qian and Schoenau 1995).

### Data Analysis

Data were analyzed using analysis of variance to test the effects of *C. achates*, N, and sucrose on spotted knapweed and soil nutrients. Sites were analyzed separately because

they had unequal variances. All nutrient data were analyzed as a split-plot in time. Biomass, cover, and density were analyzed as a randomized complete block with rep, weevil, and nutrient additions and *C. achates* x nutrient additions interaction included in the model. All other interactions comprise the error term. Main effects, sucrose or N addition, and *C. achates* and their interactions were included in the model as whole-plots, while sampling time (May, July, September) and interactions involving time were considered subplots. Whole-plots were tested using the rep x weevil x addition interaction as the error term, and subplots were tested using the overall model mean square error term. Nitrate, ammonium, and biomass data were log-transformed to meet the assumptions of normality, and significant differences were designated at the  $P < 0.05$  level. Untransformed means were compared using Fisher's protected LSD ( $\alpha = 0.05$ ), and transformed means were separated using Tukey's multiple comparisons (Peterson 1985). For transformed data, untransformed means are presented with log-transformed mean comparisons. ANOVA indicated that treatments had no influence on organic matter, K, S, Ca, Zn, and CEC; therefore, these data are not presented.

PRS<sup>TM</sup>-Probe Analysis. ANOVA was used to test the effects of sucrose, N, and weevil treatment effects on soil nitrate and ammonium. Nitrate and ammonium data were log-transformed to meet the assumptions of normality. Fisher's protected LSD ( $\alpha = 0.05$ ) was used to compare means. It should be noted that the ion-exchange membrane technique measures nutrient flux over time, as opposed to a static soil core sample. They behave similarly to a plant root and usually more highly correlated with plant nutrient concentrations than with soil nutrient concentrations (Western Ag Innovations, Inc.,

PRS™ Operations Manual). Correlation matrices were calculated to test this attribute of the membranes. SAS and Statistica software were used for all calculations.

### Study 1: Results

#### *C. achates* larval numbers

Mean larval number across treatment where weevils were released was 0.7 weevils/m<sup>2</sup> at O'Keefe Creek and 7.3 weevils/m<sup>2</sup> at Miller Creek. At O'Keefe Creek, only one of 15 plots had *C. achates*, whereas the weevils were present in six of 15 plots at Miller Creek. ANOVA was not conducted because low weevil establishment resulted in non-normal probability distributions.

#### O'Keefe Creek: Spotted knapweed cover, density, and biomass

Spotted knapweed total and adult percent cover was affected by *C. achates* or nutrient addition (Table 2). *C. achates* increased spotted knapweed total cover from 55 to 78%, and adult cover from 44 to 61% in the control and weevil plots, respectively (Figure-1). Adding N increased spotted knapweed total and adult cover from 58 to 93% and 38 to 88% in the control and N plots, respectively (Figure 2). N addition decreased spotted knapweed juveniles 19 and 5% in the control and N plots, respectively (Table 2; Figure 2). Adding sucrose did not alter spotted knapweed total, adult, or juvenile cover compared to the control, but decreased total and adult cover below that of N addition by about one-half (Figure 2). In contrast, sucrose increased juvenile spotted knapweed cover over plots receiving N.

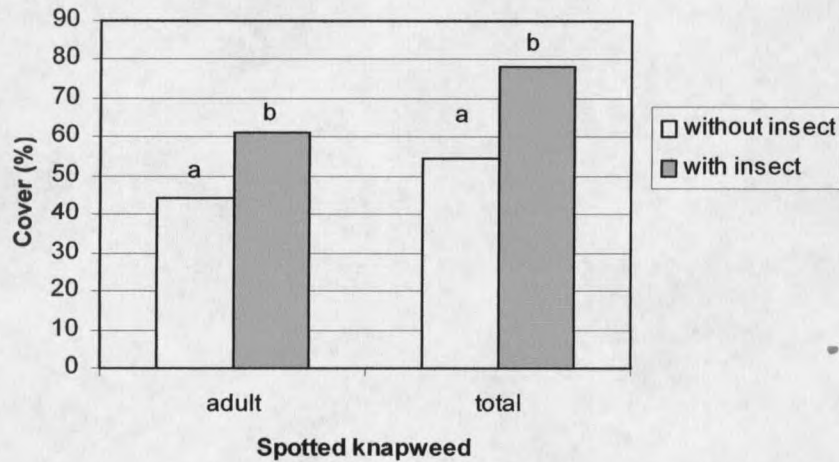


Figure 1. Influence of *C. achates* on adult and total spotted knapweed cover at O'Keefe Creek. Treatment comparisons were calculated using square-root transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments within growth stage.

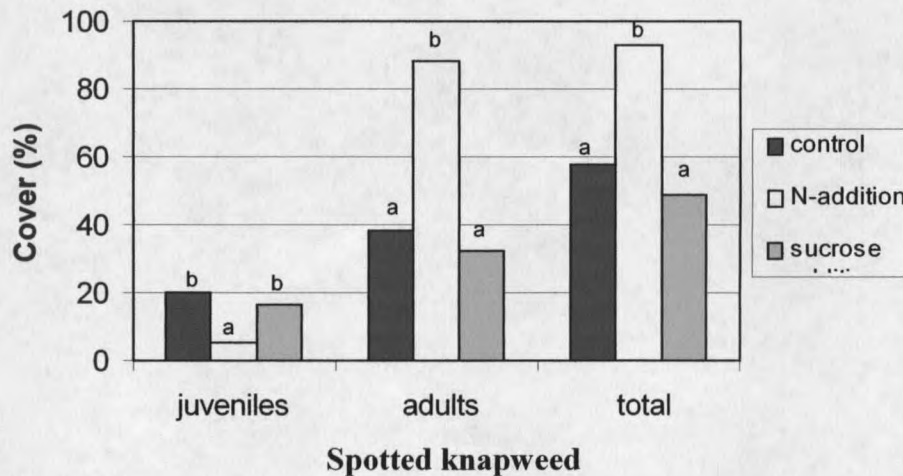


Figure 2. Influence of nutrient addition on juvenile, adults, and total spotted knapweed cover at O'Keefe Creek. Treatment comparisons were calculated using square-root transformations, and mean comparisons were calculated using LSD (0.05). Letter differences correspond to significant differences between treatments within each growth stage.

*C. achates* did not affect spotted knapweed juvenile or adult density measurement at O'Keefe Creek (Table 2). Nitrogen addition and sucrose affected total and juvenile spotted knapweed density, but not adult density. Total and juvenile spotted knapweed density was decreased with N addition by about one fourth of the control density (Figure 3). Sucrose addition increased both juvenile and total spotted knapweed from 500 to 900 plants/m<sup>2</sup>. In addition, sucrose also increased juvenile and total spotted knapweed by 797 plants/m<sup>2</sup> over that of the N-treatment.

*Cyphocleonus achates* affected adult and total spotted knapweed biomass at O'Keefe Creek (Table 2). Total spotted knapweed biomass was 113 g/m<sup>2</sup> higher in plot with *C. achates* compared to controls (Figure 4). Adult biomass increased by about 102 g/m<sup>2</sup> in the presence of *C. achates*. Juvenile spotted knapweed biomass was not affected by nutrient amendments, but total and adult knapweed biomass was affected at this site (Table 2). Biomass response followed a similar pattern as cover. N addition more than doubled total and adult biomass over that of the control (Figure 5). Sucrose yielded spotted knapweed adult and total biomass similar to that of the control, but decreased biomass by about 580 g/m<sup>2</sup> below that of the N addition.

Table 2. Results for spotted knapweed cover, density, and biomass with *C. achates* and nutrient additions at O'Keefe Creek.

Source of variation	df	Cover P>F			Density P>F			Biomass P>F		
		Juvenile	Adult	Total	Juvenile	Adult	Total	Juvenile	Adult	Total
Rep	4	0.33	0.74	0.90	0.69	1.00	0.70	0.58	0.92	0.95
Weevil (W)	1	0.44	0.03	<0.01	0.60	0.10	0.75	0.29	0.05	0.05
Nutrient (N)	2	<0.01	<0.01	<0.01	<0.01	0.09	<0.01	0.09	<0.01	<0.01
W x N	2	0.96	0.49	0.25	0.75	0.51	0.81	0.78	0.47	0.49

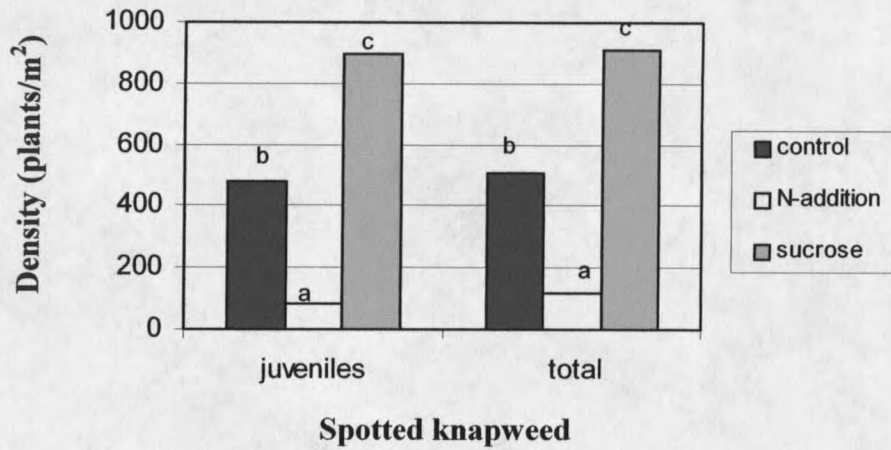


Figure 3. Influence of nutrient additions on spotted knapweed juvenile and total density at O'Keefe Creek. Treatment comparisons were calculated using square root transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments within each growth stage.

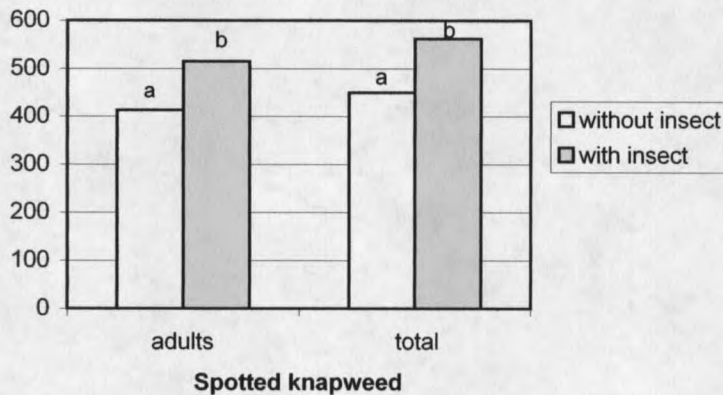


Figure 4. Influence of *C. achates* on spotted knapweed adult and total biomass at O'Keefe Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means compared using LSD. Letter differences correspond to significant differences between treatments at each growth stage.

O'Keefe Creek: Soil nutrients

Soil core extractions. The influence of soil amendments on soil nitrate depended upon time of sampling (Table 3). In May, N addition increased soil nitrate by 15.0 mg/kg over that of the control, and by 16.5 mg/kg over that of sucrose addition (Figure 6). Sucrose addition yielded nitrate values similar to the control. Nitrate means were 4.5 mg/kg in the control, 19.5 mg/kg in the N addition treatment, and 3.0 mg/kg with the sucrose treatment in May. In July, N addition increased nitrate by 22.3 mg/kg over that of the control and by 23.5 mg/kg over soils receiving sucrose (Figure 6). Sucrose addition reduced nitrate 1.2 mg/kg below that of the control. Means for nitrate were 2.3 mg/kg in the control, 24.6 mg/kg with the N treatment, and 1.1 mg/kg with sucrose in July. Similarly, in September, N addition increased nitrate by 20.2 mg/kg over that of the control and by 27.5 mg/kg over soils receiving sucrose. Sucrose addition reduced nitrate content 7.3 mg/kg below that of the control (Figure 6). Nitrate means were 11.7, 31.9, and 4.4 mg/kg in the control, N addition, and sucrose addition treatments, respectively, at this time.

The influence of *C. achates* on soil ammonium depended upon nutrient amendment and time of sampling (Table 3). *C. achates* did not affect soil ammonium in May (Figure 7), N addition increased soil ammonium 11.5 mg/kg over that of the control. Sucrose did not affect soil ammonium at this sampling time. In July, *C. achates* increased soil

Table 3. Soil nutrient results with nutrient and *C. achates* addition at both study sites.

Site	Source of variation	df	Nitrate	Ammonium	Bray
			P>F	P>F	Phosphorus
O'Keefe Creek	Rep	4	0.92	0.32	0.25
	Weevil (W)	2	0.14	0.12	0.60
	Nutrient (N)	1	<0.01	<0.01	0.31
	W x N	2	0.17	0.16	0.19
	Time (T)	2	<0.01	<0.01	0.25
	T x N	4	<0.01	0.61	0.48
	T x W	2	0.29	0.32	0.58
	T x W x N	4	0.62	0.05	0.29
Miller Creek	Rep	4	0.70	0.03	0.05
	Weevil (W)	2	0.97	0.65	0.89
	Nutrient (N)	1	<0.01	<0.01	0.43
	W x N	2	0.89	0.88	0.94
	Time (T)	2	<0.01	0.12	0.75
	T x N	4	0.08	0.87	0.25
	T x W	2	0.77	0.07	0.34
	T x W x N	4	0.38	0.93	0.52

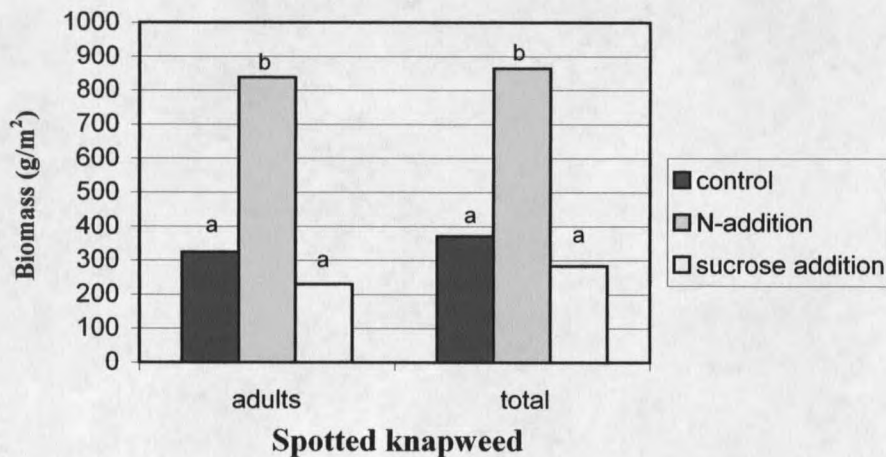


Figure 5. Influence of nutrient additions on spotted knapweed adult and total biomass at O'Keefe Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments at each growth stage.

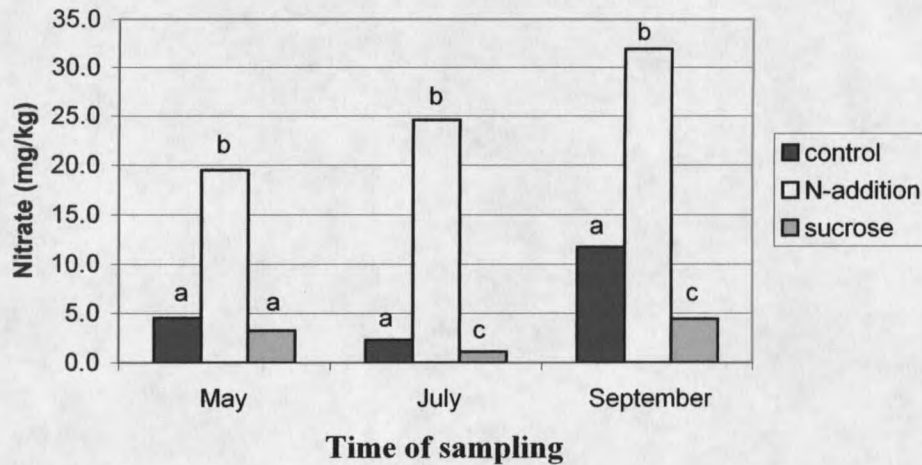


Figure 6. Influence of nutrient addition on soil nitrate at O'Keefe Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and mean separations were calculated using the LS means procedure. Letter differences correspond to significant differences within addition treatments.

ammonium 3-5 times over all other treatments where N was added. In the absence of the weevil, N addition also increased ammonium over that of the control and soils receiving sucrose at this sampling time. Soils receiving sucrose were similar to the control. In September, the effect of *C. achates* on soil ammonium was no longer significant. Soil ammonium with N addition was about 2 times higher than the control or the sucrose-treated soils. Sucrose-treated soil ammonium was no different than that of the control (Figure 7).

Phosphorus was not affected by nutrient amendment, weevil addition, or time of sampling at O'Keefe Creek (Table 3).

PRS™ Probe Results. *C. achates* addition had no effect on ion-exchange membrane results for nitrate or ammonium (Table 4). Nitrate in soils receiving N addition was nearly 10 times greater than that of the control, and 40 times greater than soils receiving sucrose (Table 4, Figure 8). With sucrose added, soil nitrate was  $4.9 \mu\text{g}/\text{cm}^2/6\text{wk}$  lower than that of the control, and  $59.3 \mu\text{g}/\text{cm}^2/6\text{wk}$  lower than soils receiving N addition. Soil ammonium content with N addition was  $8.7 \mu\text{g}/\text{cm}^2/6\text{wk}$  higher than that of the control (Table 4). Soils treated with sucrose had similar ammonium values to the control (Figure 8). Correlation matrices showed that ion membrane rates had moderate correlation with soil core extraction values, depending on soil core extraction time (Table 5). Generally, summing May and July core extraction values yielded higher  $r$  values. Nitrate values showed slightly greater correlation with ion membranes than ammonium.

#### Miller Creek: Spotted knapweed cover, density, and biomass

*C. achates* did not influence cover at Miller Creek (Table 6). Adult and juvenile spotted knapweed cover was affected by N addition. N addition produced adult knapweed cover similar to that of the control, but was 3 times greater than in soils receiving sucrose (Figure 9). N addition reduced spotted knapweed juvenile cover from 26% compared to 9% with the control. Sucrose produced juvenile spotted knapweed cover similar to that of the control, but reduced adult cover 3 times below that of soil receiving N addition.

Table 4. Ion exchange membrane results of soil nitrate and ammonium with *C. achates* and nutrient addition at both study sites.

O'Keefe Creek	Source	df	Nitrate P>F	Ammonium P>F
	Rep	4	0.47	0.25
	Weevil (W)	1	0.60	0.98
	Nutrient (N)	2	<0.01	0.05
	W x N	2	0.60	0.99
Miller Creek	Source	df	Nitrate P>F	Ammonium P>F
	Rep	4	0.32	0.05
	Weevil (W)	1	0.84	0.41
	Nutrient (N)	2	<0.01	0.03
	W x N	2	0.80	0.15

*C. achates* did not influence spotted knapweed density at Miller Creek (Table 6). N addition decreased both juvenile and total density counts, but spotted knapweed adults were not affected (Table 6). Nitrogen addition reduced juvenile and total density by about 700 plants/m<sup>2</sup> (Figure 10). Sucrose addition decreased juvenile spotted knapweed density by about 200 plants/m<sup>2</sup> less than that of the control, and increased juvenile plants by over six times that in the N treated soil. Sucrose addition total density results were similar to that of the control.

Table 5. Correlation (r) between ion exchange membrane and extraction results of soil nitrate and ammonium.

O'Keefe Creek	Method	Core July	Core September	Core May+July	Core May+July+September
Nitrate	PRS	0.68***	0.54**	0.69***	0.63***
Ammonium	PRS	0.81***	0.46*	0.64***	0.62**
Miller Creek	Method	Core July	Core September	Core May+July	Core May+July+September
Nitrate	PRS	0.68***	0.85***	0.78***	0.86***
Ammonium	PRS	0.07	0.12	0.06	0.09

\* P=&lt;0.05

\*\* P=&lt;0.01

\*\*\* P=&lt;0.005

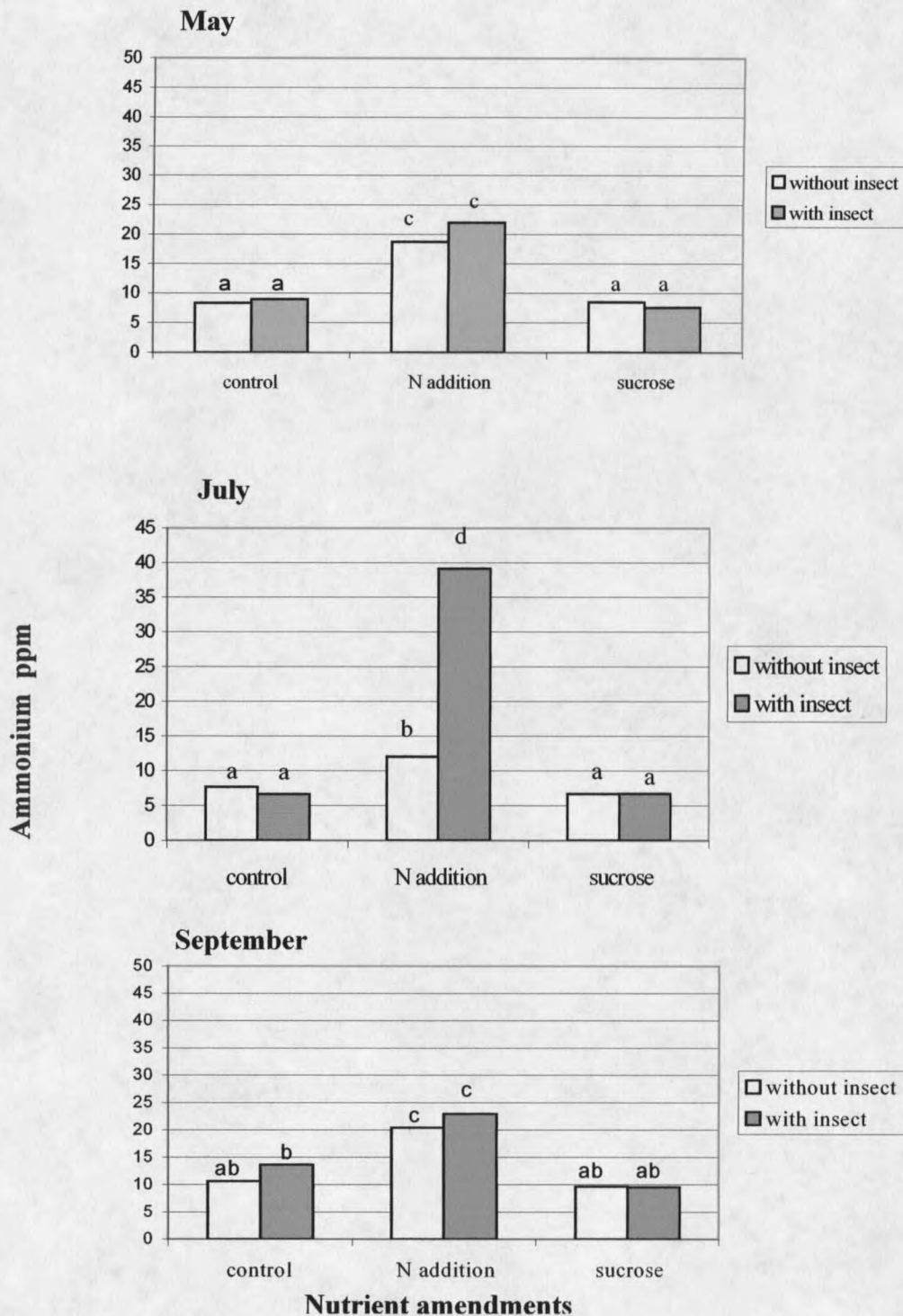


Figure 7. Interaction between time of sampling, nutrient and weevil addition on soil ammonium. Treatment mean comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

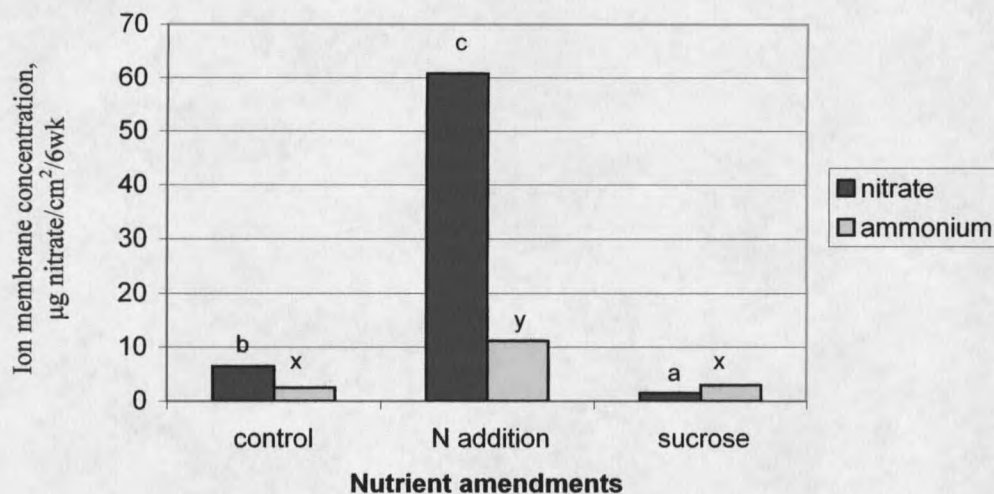


Figure 8. Influence of nutrient addition on ion exchange membrane nitrate and ammonium results at O'Keefe Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments within each N form.

Table 6. Spotted knapweed cover, density, and biomass results with *C. achates* and nutrient additions at Miller Creek.

Source of variation	df	Cover P>F			Density P>F			Biomass P>F		
		Juvenile	Adult	Total	Juvenile	Adult	Total	Juvenile	Adult	Total
Rep	4	0.02	0.84	0.17	0.69	0.73	0.44	0.11	0.51	0.28
Weevil (W)	1	0.56	0.52	0.26	0.60	0.98	0.49	0.13	0.36	0.14
Nutrient (N)	2	<0.01	0.05	0.41	<0.01	0.34	<0.01	0.06	<0.01	0.05
W x N	2	0.70	0.50	0.88	0.75	0.67	0.94	0.59	0.21	0.78

*C. achates* did not affect spotted knapweed biomass at Miller Creek (Table 6). Total and adult spotted knapweed biomass was affected by nutrient amendments (Table 6). Juvenile biomass appeared to decrease with sucrose treatments, but the effect was not

significant ( $P=0.06$ ). N addition yielded total and adult biomass that was similar to the control, yet increased total and adult biomass over those plots receiving sucrose by 179 g/m<sup>2</sup> and 199 g/m<sup>2</sup>, respectively (Figure 11). Sucrose addition yielded similar total and adult biomass to the control, yet was lower than total and adult biomass of soils receiving N.

#### Miller Creek: Soil nutrients

Soil core extractions. Nutrient addition and time significantly affected nitrate measurements, though there was no interaction of the two (Table 3). Nitrate levels with N addition were over 4 times higher than that of the control, and over 10 times higher than soils receiving sucrose (Figure 12). Sucrose additions decreased nitrate values by 4.6 mg/kg below that of the control, and 34.5 mg/kg below that of the N addition soils. Interestingly, May nitrate values were about half the amount of both July and September samplings, which had similar nitrate values (Figure 13).

Soil ammonium was not affected by *C. achates* or time of sampling, but was affected by nutrient amendments (Table 3). N addition increased ammonium values by almost 2 times over that of the control and sucrose treatments (Figure 14), while sucrose addition yielded ammonium values similar to the control.

Phosphorus was not affected by nutrient amendment, weevil addition, or time of sampling at Miller Creek (Table 3).

**PRS<sup>TM</sup>-probe results.** *C. achates* addition did not affect nitrate or ammonium levels at Miller Creek, but the ion probes detected differences due to nutrient additions (Table 4). N addition increased nitrate supply rates by 212.4  $\mu\text{g}/\text{cm}^2/6\text{wk}$  over that of the control, and by 243.7  $\mu\text{g}/\text{cm}^2/6\text{wk}$  over that of the sucrose-treated soil (Figure 15). Sucrose addition diminished nitrate rates below that of the control by approximately 50%.

Ammonium supply rates were increased with N addition by 6.3  $\mu\text{g}/\text{cm}^2/6\text{wk}$  over the control (Table 4). Sucrose addition yielded similar ammonium supply rates as the control, but decreased rates by 4.7  $\mu\text{g}/\text{cm}^2/6\text{wk}$  over that of soils receiving N (Figure 16). Correlation matrices showed lower correlation between PRS<sup>TM</sup> probe and core values than O'Keefe Creek, especially with respect to ammonium (Table 5).

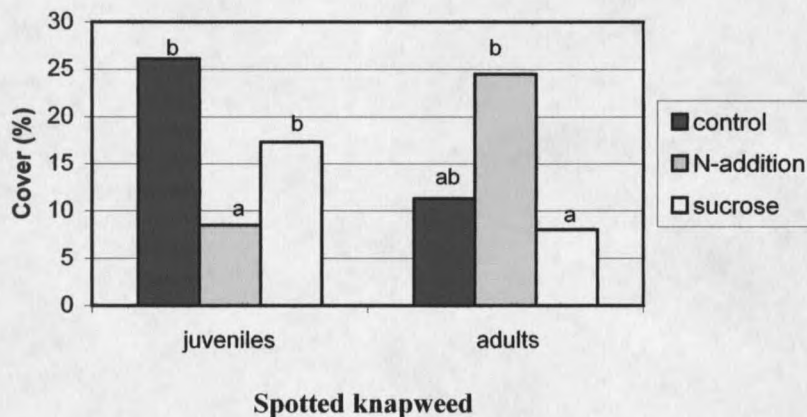


Figure 9. Nutrient addition influence on spotted knapweed percent cover at Miller Creek. Treatment comparisons were calculated using square-root transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments at each growth stage.

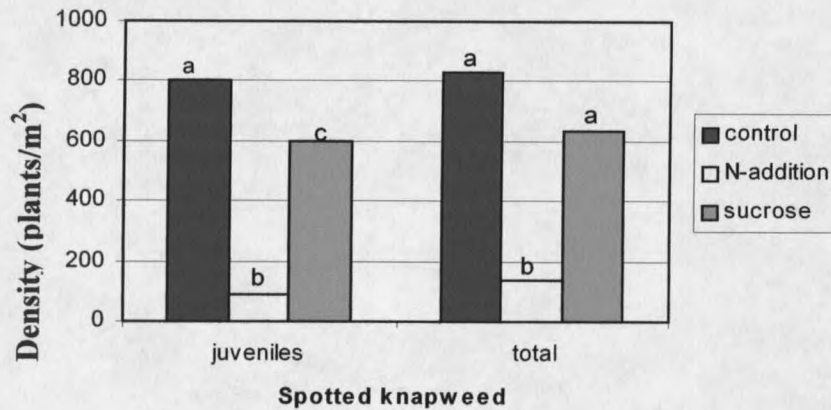


Figure 10. Nutrient addition influence on spotted knapweed density at Miller Creek. Treatment comparisons were calculated using square root transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments at each growth stage.

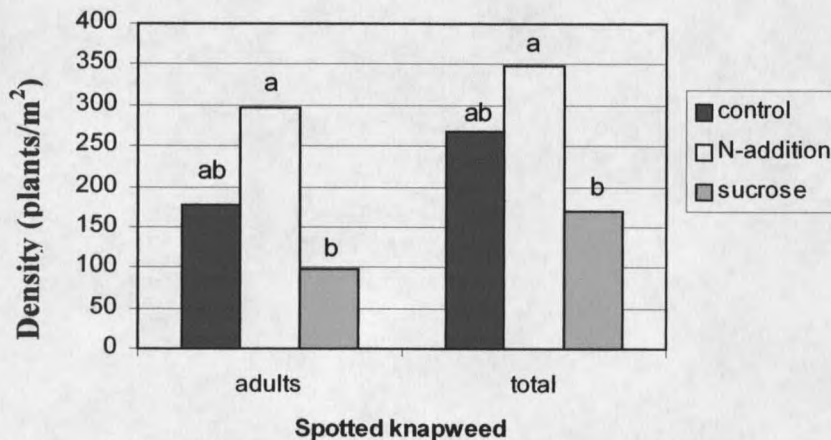


Figure 11. Influence of nutrient addition on spotted knapweed biomass at Miller Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments at each growth stage.

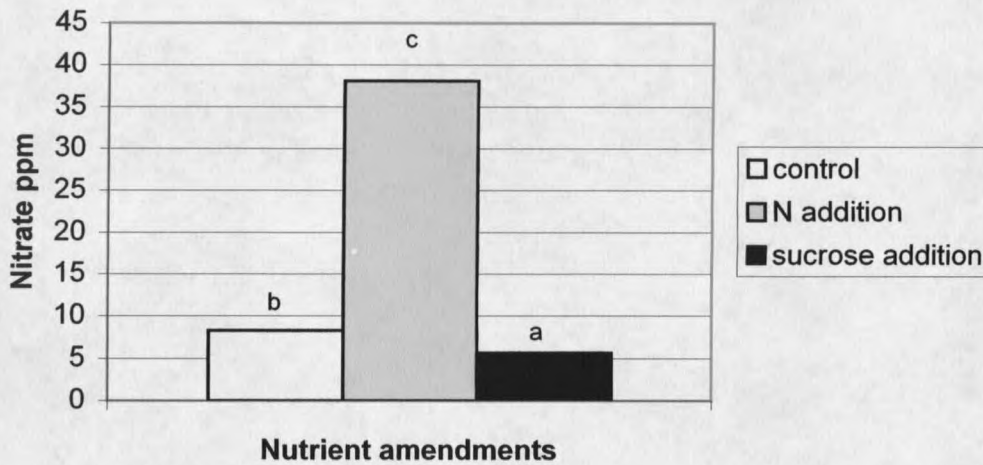


Figure 12. Influence of nutrient addition on soil nitrate at Miller Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

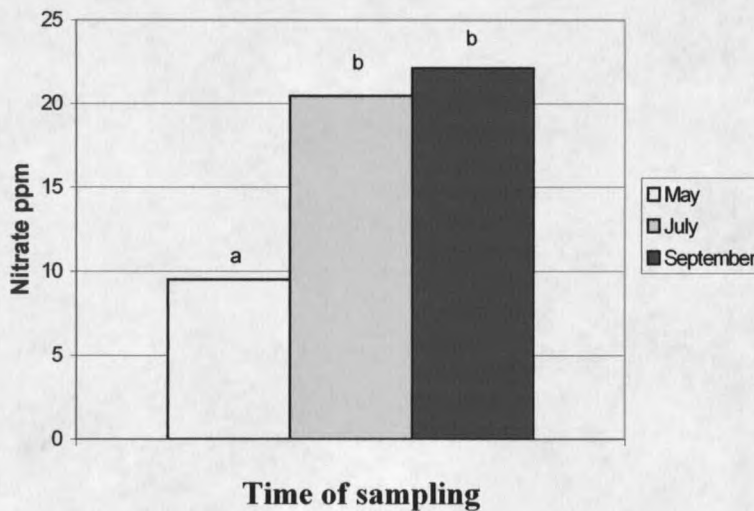


Figure 13. Influence of sampling month on soil nitrate at Miller Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

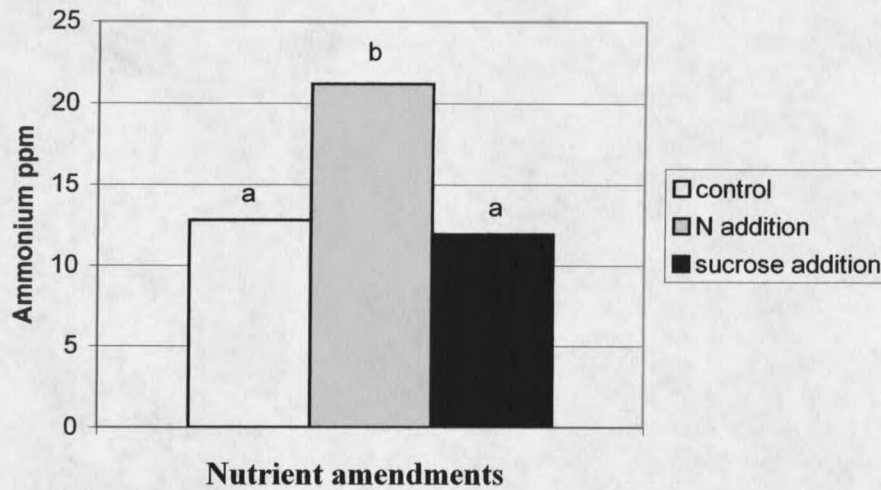


Figure 14. Influence of nutrient addition on soil ammonium at Miller Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

## Study 2: Materials and Methods

### Study site

This long-term study was initiated in 1995 at a site about 11 km east-northeast of Hamilton, Montana, ( $46^{\circ} 17' N$ ,  $114^{\circ} 1' W$ ) with an elevation of 1341 m. The site was level and uniform at establishment. Soils are Stecum stony loamy coarse sand (mixed typic Cryorthents) and are moderately deep. Annual precipitation ranges from 406 to 457 mm and mean annual temperature is  $6.6^{\circ} C$ . This site is a *Festuca scabrella/Agropyron spicatum* habitat type (Mueggler and Stewart 1980), and was dominated by spotted knapweed with few other grass or forb species represented in 1995.

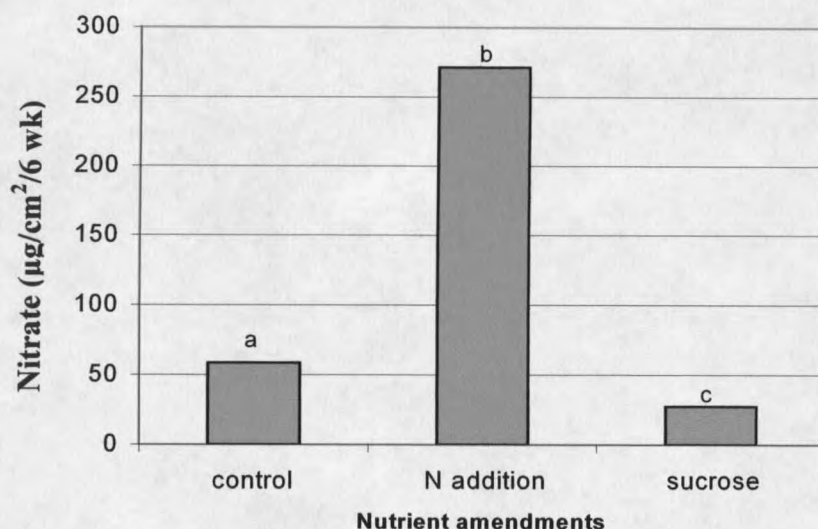


Figure 15. Influence of nutrient addition on nitrate levels measured with ion exchange membranes at Miller Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant differences between treatments.

#### Procedures and experimental design

This study tested the effects of *C. achates* on spotted knapweed and soil nutrients within a long-term experiment. This study was established in 1995 to test the use of low picloram (4-amino-3,5,6-trichloropicolinic acid) rates to enhance establishment of *C. achates* on spotted knapweed-infested rangeland (Jacobs et al. 2000). In 1995, eighteen treatments (six picloram rates, three weevil release densities) were applied to 2 m<sup>2</sup> plots,

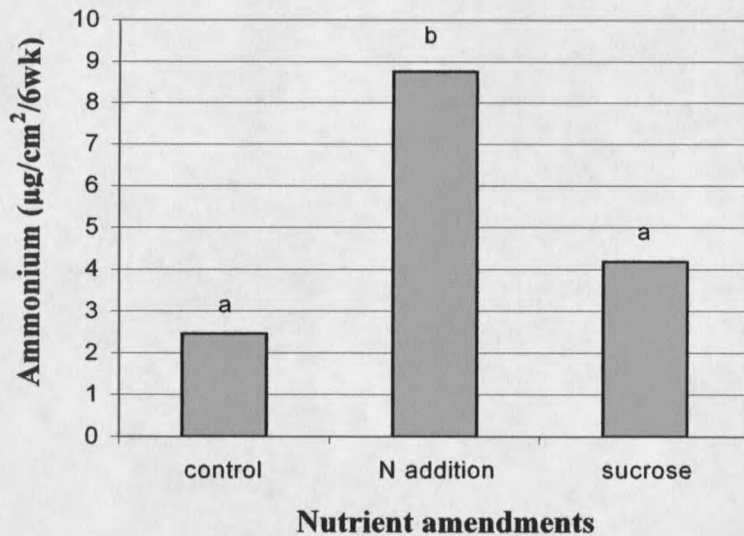


Figure 16. Influence of nutrient addition on ammonium levels measured with ion exchange membranes at Miller Creek. Treatment comparisons were calculated using  $\log_{10}$  transformations, and means were compared using LSD. Letter differences correspond to significant treatment differences.

with circular enclosures (corrals) constructed in each plot to contain *C. achates* (Story and Wood 1996), as described in Study 1. Each corral was applied to the extant spotted knapweed population, and separated from adjacent plots by 4 m. Weevil release densities were 0, 6, and 12 weevils per corral, and picloram rates were 0, 0.03, 0.06, 0.09, 0.12, and 0.15 kg/ha.

Treatments were arranged in a randomized complete block design and replicated four times. This study was sampled each year from 1996-2001 for visual weevil counts, spotted knapweed density and cover, and grass cover. In 1998, weevil estimate results in the control corrals were significantly lower than either weevil density treatment ( $\text{mean}_{\text{control}} < 0.5 \text{ weevil/m}^2$ ). Additionally, weevil numbers were lowest at low and high percentages of spotted knapweed cover, and the range of highest spotted knapweed cover (30-70%) was with the 0.03, 0.06, and 0.09 kg/ha picloram rates. Only control (n=12) and high weevil release treatments (n=18) were included in the analysis.

### Sampling

In 2001, this study was sampled similarly to Study 1, except that adult and juvenile plants were not separated for biomass measures. Total biomass of combined adult and juvenile plants is presented. In addition, only soil cores were used for nutrient sampling.

### Data analysis

The influence of *C. achates* on spotted knapweed and soil nutrients was determined using ANOVA with replication and weevil main effects in the model.

Replication x weevil interaction was used as the error term.

Table 7. Long-term results of soil N and P; total spotted knapweed biomass, density and cover; and larval counts with and without *C. achates* at Hamilton.

Source of variation	df	Total biomass	Nitrate	Ammon	Bray P	Density P>F		Cover P>F		Larval counts
		P>F	P>F	P>F	P>F	Juvenile	Adult	Juv.	Adult	P>F
Rep	3	0.17	0.03	0.05	0.89	0.05	0.16	0.05	0.06	0.79
Weevil	1	<0.01	0.55	0.05	0.41	0.42	0.28	0.89	<0.01	<0.01

## Study 2: Results

In this long-term study, *C. achates* affected total (adult + juvenile) biomass and ammonium (Table 7). *C. achates* increased total spotted knapweed biomass from 92 to 177 g/m<sup>2</sup> over that of the control. Conversely, available ammonium was decreased from 6.2 mg/kg in controls to 5.6 mg/kg in weevil (infected) plots. Spotted knapweed adult cover was increased with *C. achates* from 5.8 in the control to 12.8 plants/corral. *C. achates* mean density in the presence plots was 2.3 weevils/corral, and 0.08 in the control.

## Discussion

The ability of plants to recover or enhance growth following insect infection is considered a common occurrence in both individual plant systems and plant communities (Belsky 1986), though these interactions have been found to be species dependant, spatially variable, and a result of complex associated mechanisms (McNaughton 1983, Carson and Root 1999). Contrary to our hypotheses, we found a single season of infection increased spotted knapweed aboveground biomass and cover at O'Keefe Creek, but not at Miller Creek. It is possible that spotted knapweed had a compensatory response to infection. Plant species show differences in C allocation, growth rates, and photosynthetic rates in response to herbivory (Notzold et al. 1998). Plant compensatory response has been found to be a complex interaction of endogenous and exogenous resource effects, plant habit and tolerance, as well as the intensity, frequency, and season of infection (Trumble et al. 1993). If compensatory growth were the case, our hypotheses

would have predicted a corresponding decrease in soil N with enhanced growth. In the higher N environment, soil ammonium actually increased with addition of *C. achates*. This suggests that infection may have altered the allocation of N from roots to shoots, at least in the higher N environment at O'Keefe Creek.

Insect survival and establishment were higher at Miller Creek than at O'Keefe Creek during this short-term study. We speculate that the lack of increased spotted knapweed biomass may be because of greater infection at Miller Creek, reinforcing suggestions that the magnitude of insect density has implications for its impact on plant populations (Gassmann 1996, Nowierski and Huffaker 1999). Kennett et al. (1992) found that spotted knapweed aboveground biomass was not affected by single or multiple clippings, but root biomass was reduced three-fold by frequent defoliation. The response of spotted knapweed infestations may largely be determined by infection intensity and *C. achates* density.

Spotted knapweed biomass was also greater where *C. achates* had been established for seven years than in areas lacking the weevil. In addition, soil ammonium decreased in the presence of the weevil. This could provide further evidence for a compensatory response to *C. achates* by spotted knapweed. However, Steinger and Muller-Scharer (1992) found that individual plant biomass was reduced by 30% by *C. achates*, and 63% in high N environments. In their study, *C. achates* reduced individual spotted knapweed biomass in all cases. Sheley and Jacobs (1997) found that moderate control of spotted knapweed (45%) did not affect its competitive ability, but 90% control shifted the competitive balance in favor of bluebunch wheatgrass. Their data suggested that at moderate levels of control, remaining spotted knapweed plants captured the majority of

the newly available resources (Sheley and Jacobs 1997). Even low amounts of root herbivory have been found to release C and N to the soil, facilitating absorption by non-infested neighboring roots (Bardgett et al. 1999). In our study, overall increases in biomass may be the response of uninfected individuals within the population responding to available resources otherwise acquired by infected plants (Harper 1977). If this hypothesis is correct, it suggests that a majority of plants must be affected by biological control agents to cause an overall population decline.

Spotted knapweed developed higher biomass and cover with N addition compared to controls. Similarly, (Story and Wood 1996) found increased spotted knapweed biomass with N fertilizer additions. This supports evidence that N is often limiting to spotted knapweed in environments low in that resource (Blicker et al. 2002). In high N environments, the rapid growth rate of spotted knapweed may allow it to preempt resource use from neighboring species (Redente et al. 1992, Lambers et al. 1998, Dukes and Mooney 1999, Blicker et al. 2002). Carpinelli (2000) found spotted knapweed absolute growth rates to be higher than that of alfalfa (*Medicago sativa* L.). Aggressive non-indigenous plants commonly invade highly disturbed systems (Sheley and Petroff 1999), and high N availability may be an important mechanism promoting this invasion (Alpert and Maron 2000, Herron et al. 2000).

We expected sucrose to decrease spotted knapweed cover, density, and biomass. In a greenhouse study, Brockington (2003) found that sucrose applied at 100 g pure C/m<sup>2</sup> reduced spotted knapweed biomass. In that study, there was a slight increase in plant available soil N. It was proposed that the transitory effect of sucrose on microbial growth was too ephemeral to provide sustained and detectable reductions in extractable nitrate

and ammonium. In this field study, sucrose decreased plant available soil nitrate and ammonium only as a function of time of sampling at O'Keefe Creek, and only slightly decreased nitrate at Miller Creek. The ion exchange membrane method provided an indication of a slight decrease in cumulative soil nitrate with sucrose addition at O'Keefe Creek. Spotted knapweed parameters were not consistently affected by sucrose treatments, and there was a positive affect on spotted knapweed density at O'Keefe Creek. This suggests that the amount and frequency of sucrose in this experiment was lower than that needed to have a sustained increase of microbial populations to immobilize soil N or affect spotted knapweed.

Little evidence supported our hypothesis that plant available N would increase in the presence of *C. achates*, with the exception that soil ammonium increased in the high N environment with weevil addition after a single season of defoliation. However, spotted knapweed biomass also increased in this treatment. In the long-term study, soil ammonium decreased and biomass increased. We expected a decrease in biomass and a corresponding increase in nitrate and ammonium in the soil. Although the direction of influence was reversed, it appears that the effects of a natural enemy on a target weed population may be predicted using its influence on soil resources. In this study, the decrease in plant available soil N in the presence of *C. achates* would have suggested a positive response by spotted knapweed to the weevil. Based on the long-term biomass data, the prediction would have been accurate. The prediction accuracy will likely be improved where either weevil densities or infection intensities are considered.

Assessing the impact of natural enemies on plant available soil nutrients may be a useful method to predict how an insect will influence plant community dynamics prior to

their release. With further investigation, it may be possible to calculate  $R^*$  for various invasive plants and changes in  $R^*$  due to biological control agents, then compare these value with known  $R^*$  of various indigenous or desired species. This approach may be more useful than assessing biomass changes across habitat types and years, since biomass varies widely spatially and temporally, and is difficult to assess because plant material is removed by herbivores (Shierenbeck et al. 1994). Individual species ability to sequester N and/or their  $R^*$  may remain relatively constant and comparable for populations where growth rates and nutrient supply rates have equilibrated (Tilman 1981).

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