Influence of an exotic species, *Acroptilon repens* (L.) DC. on seedling emergence and growth of native grasses

Douglas W. Grant[1,2], Debra P.C. Peters[1,3,*], George K. Beck[4] and Harold D. Fraleigh[1]
[1]*Graduate Degree Program in Ecology & Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, USA;* [2]*Current address: Crops Research Laboratory, USDA-ARS, Fort Collins, CO 80523, USA;* [3]*Current address: Jornada Experimental Range, USDA-ARS, Box 30003, MSC 3JER, NMSU, Las Cruces, NM 88003-0003, USA;* [4]*Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523, USA;* *Author for correspondence (e-mail: debpeter@nmsu.edu)*

Abstract

Our objective was to evaluate the effects of an invasive perennial forb, *Acroptilon repens* (Russian knapweed), on seed emergence and seedling survival of four native grass species that are important in semiarid grasslands of North America. Greenhouse experiments and field studies conducted at three sites in Colorado, USA were used to examine the response by four perennial grasses: *Bouteloua gracilis, Koelaria cristata, Sporobolus cryptandrus*, and *Agropyon smithii* to *A. repens*. In the greenhouse, seeds of each species were sown in pots that contained either live *A. repens* roots, *A. repens* litter on the soil surface, or both roots and litter. Field transects were placed inside stands of *A. repens* with adjacent control transects in the surrounding grass-dominated community. Emergence and initial survival were decreased by the presence of *A. repens* roots for *K. cristata* (35%), *B. gracilis* (31%), and *S. cryptandrus* (44%) in the greenhouse, and 57, 32, and 36%, respectively, in the field. Root weight was decreased by the presence of *A. repens* roots for both *B. gracilis* and *K. cristata* by more than 55% in the greenhouse. *A. smithii* survival and growth were unaffected by *A. repens* in either the greenhouse or the field. These species-specific responses to the presence of *A. repens* may explain, at least in part, differences in susceptibility and recovery of sites with different native grass species composition.

Introduction

Invasion of native plant communities by exotic weeds has become a major ecological concern. Invasive exotic weeds may replace native species and alter ecosystem structure and function (Usher 1988; Vitousek 1990; Mack et al. 2000). An ecological understanding of the interactions between invasive weeds and native plants helps identify the key processes that either promote invasion or limit recovery of native plants (Mooney and Drake 1986; Sheley et al. 1996, 1999). Although this process-based approach has been used infrequently experimentally, it has great potential for broad applicability beyond the specific native and exotic species being studied. This under-

standing could potentially enhance control and reduce management costs. Our goal was to improve our understanding of the ecological processes occurring during invasions by studying the effects of an invasive perennial forb on the recruitment and initial growth of native grasses.

Some invasive weeds modify natural and seminatural habitats by replacing diverse communities with single species stands (Cronk and Fuller 1995). Knapweeds, such as Russian (*Acroptilon repens* (L.) DC.), spotted (*Centaurea maculosa* Lam.), and diffuse (*Centaurea diffusa* Lam.), are known to be aggressive invaders that often dominate native communities following their invasion (Tyser and Key 1988; James et al. 1991). Recent experiments indicate that

both *C. diffusa* and *C. maculosa* negatively affect growth of neighboring North American grasses (Callaway and Aschehoug 2000; Ridenour and Callaway 2001). *A. repens* is another species of importance, particularly in semiarid grasslands. It is an aggressive perennial that reproduces from seed and adventitious buds on a creeping root system (Fletcher and Renney 1963; Moore and Frankton 1974). Management of *A. repens* is difficult with herbicides alone, and may be enhanced using biological control and integrated pest management. Reseeding with grasses is recognized as a method of weed control that complements other methods, in particular herbicide applications (Bottoms et al. 1996; Bottoms and Whitson 1998; Benz et al. 1999). However, reseeding is not always successful and our knowledge of the controls on this variability in success is very limited. Variable effects of *A. repens* on seed emergence and seedling establishment of different species may explain patterns in grass revegetation and persistence of this invasive weed.

Allelopathy may allow invasive species to form a dense patch and disrupt community dynamics (Gentle and Duggin 1997). *A. repens* shoots and roots both produce secondary compounds with allelopathic potential (Stevens and Merrill 1985; Stevens 1986). Competition may work synergistically with allelopathy to allow this species to dominate over native grasses through time. The individual influences of competition and allelopathy may be difficult or impossible to separate under field conditions (Inderjit and Del Moral 1997; Inderjit and Dakshini 1999). However, direct competition may be relatively more important under natural field conditions when available resources are limited (Ridenour and Callaway 2001).

The influence of allelochemicals may depend on soil texture (Inderjit and Dakshini 1994). Colloidal particles in soil adsorb some volatile allelochemicals, preventing leaching and volatilization and allowing concentrations to accumulate to biologically active levels, particularly in soils with high clay content (Muller and Del Moral 1966; Del Moral and Muller 1970). Loss of volatile allelochemicals, such as polyacetylenes, that are highly sensitive to heat and light decomposition (Stevens 1986) may be higher in sandy soils with more large pore space compared to clay soils. Field observations of *A. repens* stands on sites with different soil texture support this theory (K. G. Beck personal observation), but the mechanisms involved have not been examined. Thus, we hypoth-

esized that *A. repens* infested soils with high clay content would have greater negative effects on native plants than sandy soils. We also hypothesized that *A. repens* roots would have a greater negative effect on grass seedling emergence and growth than *A. repens* litter and that grass species would respond differently.

Methods

Greenhouse experiments

A full factorial randomized complete block design was used for the greenhouse experiments with four treatments, three soil textures, four bioassay species, and four replications. Each experiment was repeated in time to ensure that results were replicable. Treatments consisted of adding grass seeds to soils containing either live roots of *A. repens*, which had shoot material removed to reduce aboveground competition, *A. repens* litter on the soil surface, or both roots and litter. Control pots did not have roots or litter added. Three textures representative of soils found throughout semiarid grasslands of North America were used for each treatment: sandy clay loam, loamy sand, and sand (Table 1). Soils were constructed using a combination of topsoil, potting soil, and sand to achieve the desired textures. The hydrometer method (Day 1965) was used to determine the percentage sand, silt, and clay of each soil. We found that the carbon:nitrogen ratio (C:N), an index of nitrogen availability, increased as sand content increased (Table 1).

Native perennial grass species were selected that commonly occur in communities that are susceptible to *A. repens* invasion in Colorado. Grasses included two C_4 species (*Bouteloua gracilis* [H.B.K.] Lag. ex Griffiths [blue grama]; *Sporobolus cryptandrus* [Torr.] Gray [sandropseed]), and two C_3 species (*Agropyron smithii* Rydb. [western wheatgrass]; *Koelaria cristata* Pers. [prairie junegrass]) (nomenclature follows The Great Plains Flora Association 1986). Seeds were obtained from a local commercial seed source with a tested viability and average seed weight of 84% and 5.9 mg for *B. gracilis*, 86% and 3.1 mg for *K. cristata*, 86% and 1.4 mg for *S. cryptandrus*, and 78% and 54.8 mg for *A. smithii*.

For the root addition treatment, *A. repens* roots with adventitious buds were collected from the Mead field site (see site description below) in December (1997) and February (1998). Root sections (7–10 cm)

Table 1. Properties of soils used in greenhouse studies that were collected from inside and outside stands of *A. repens* for three sites.

Soil Origin	C:N[1]	%			Texture class
		Sand	Silt	Clay	
Greenhouse					
Fine	19.5	54	25	21	Sandy Clay Loam
Intermediate	33.6	84	9	7	Loamy Sand
Coarse	39.9	100	0	0	Sand
Field sites					
Mead					
Inside stands	10.8 (0.3)	41	37	22	Loam
Outside stands	13.5 (2.6)	41	34	25	Loam
Ordway					
Inside stands	11.2 (0.5)	34	23	43	Clay
Outside stands	14.2 (1.4)	37	21	42	Clay
Brown's Park					
Inside stands	18.3 (4.0)	60	22	18	Sandy Loam
Outside stands	15.8 (0.4)	44	31	25	Loam

[1] Carbon: nitrogen ratio; mean (standard deviation)

containing buds were grown in a greenhouse for two weeks and then transplanted into 8 liter pots containing the same amount of soil. *A. repens* plants were allowed to grow in the pots for 6 weeks to allow allelochemicals to accumulate in the soil. At that time, aboveground material was clipped, and bioassay seeds were added 1–2 cm below the soil surface depending on seed size, with one species per pot. Twenty seeds per pot were used for *B. gracilis* and *A. smithii*, while more seeds were used for species with very small seeds where higher mortality was expected (40 *K. cristata*; 150 *S. cryptandrus*). Live *A. repens* sprouts re-emerging from the soil were removed weekly to eliminate both light competition and the possibility of allelopathy from foliar chemicals.

For the litter addition treatment, *A. repens* standing dead material (senesced leaves and stems) was collected at the Mead field site in February 1998. Bioassay seeds were sown into pots of each soil texture followed by the addition of 28 g of litter material manually broken-up and spread evenly across the soil surface to a depth of 1 cm. This amount of material provided similar litter depth to that observed in the field. Plant material was not chopped or ground to limit the potential leaching of organic compounds that would not be released due to natural processes under field conditions (Inderjit and Dakshini 1994). Nutrients were not added to the experimental pots to avoid changing the soil chemistry.

All pots were watered 250 ml per day for the first week, followed by 500 ml per pot every other day until the end of the experiment. Emergence and initial survival data were recorded every fourth day beginning 8 days after seeds were added. Survival was calculated as a percentage of the control seedlings counted on the same date. Seedlings were harvested after 28 days. Total, shoot, and root weights were obtained separately for *A. repens* and for the grasses in each pot; plant material was oven dried at 60 °C for 3 days. Data were transformed to ARCSIN squareroot to stabilize variance, where appropriate. Analysis of variance (ANOVA) was performed separately for each species and time trial using the General Linear Model procedure (PROC GLM) in [SAS] Statistical Analysis System (1996). Means were separated using least significant difference (LSD, $p \leq 0.05$).

Field studies

Field sites were selected in three semiarid grasslands located throughout Colorado, USA based on unique precipitation regimes. Locations consisted of well-defined patches of *A. repens* surrounded by shortgrass-dominated vegetation. One field site was located near Mead (40°10′ N, 105°04′ W) along the east slope of the Rocky Mountains. Elevation is 1500 m with a 30 year average annual precipitation of 36 cm and an average daily temperature of 9.3 °C. Most precipitation occurs in April, May, and June. The plant community is dominated by a mixture of *Bromus inermis* Leyss. (smooth brome) and native grasses, including *A. smithii*. The second site was near Ordway (38°13′ N, 103°45′ W) in the Arkansas Valley of southeastern Colorado. Elevation is 1305 m with an average annual precipitation of 28 cm. Most precipitation occurs from May through August with an average daily temperature of 11.2 °C. Previously, the plant community was Conservation Reserve Program (CRP) land that was planted with a mixture of grasses, including *B. gracilis* and *A. smithii*, over ten years ago. The third site was at the Brown's Park National Wildlife Refuge (40°48′ N, 108°55′ W) located on the west slope of the Rocky Mountains. Elevation is 1620 m with an average annual precipitation of 22 cm that has a bimodal distribution with one peak in March and another in October. The average daily temperature is 7.4 °C. The plant community at the Brown's Park site is dominated by a mixture of native C_3 grasses such as *A. smithii*, with some *Artemisia tridentata* Nutt. (big sagebrush).

At each site, patches of vegetation dominated by *A. repens* were randomly selected within a 2 km^2 area containing a mosaic of *A. repens* patches within a matrix of perennial grass-dominated vegetation. Dense patches of *A. repens* were selected that had 200–300 shoots/m^2 uniformly distributed throughout the patch. Bioassay species were used to compare grass survival and growth inside *A. repens* dominated patches to grass dominated areas. The experiment was a randomized complete block design with treatments replicated four times at each site. Transects were randomly located from 4 to 10 m apart across the boundary of each *A. repens* patch with grass-dominated vegetation. Vegetation of all plants surrounding each transect was clipped in a circle with a radius of 15 cm to a height of 1 cm to reduce light competition. Small furrows (5 cm wide, 1–2 cm deep) were used to provide a microsite for seeds and seedlings. Care was used in creating furrows to minimize soil and surface litter disturbance, and reduce the volatile losses of allelochemicals. Each transect was divided into five 60 cm sections. On four of these transects, the same number of seeds per pot for each species in the greenhouse experiment was sown into a section of a transect with one species per section. On the other four transects, ten four-week-old seedlings were planted with one species randomly assigned to each section. Gravity-driven irrigation systems were set up at each site to facilitate germination and prevent dessication. Transects were irrigated at a rate of 16 liters/day at Mead, and 8 liters/day at the other two sites based on long-term precipitation received at each site.

Soil samples collected to a depth of 15 cm were used to characterize the carbon, nitrogen, and textural properties at each site. Two sets of two samples collected from inside *A. repens* patches were combined, and two sets of two samples collected from adjacent grass-dominated communities were combined for the analyses. Samples were analyzed for carbon and nitrogen using a carbon-hydrogen-nitrogen (CHN) analyzer (Nelson and Sommers 1996) and for texture using the hydrometer method (Day 1965). Soils ranged from clay loam at Ordway to loam (Mead) and sandy loam/loam at Brown's Park (Table 1). The C:N ratio was higher inside than outside the *A. repens* stand at Brown's Park, but was lower inside than outside for stands at Ordway and Mead. Soil texture differences were minimal inside and outside stands at all three sites.

Vegetation response data were collected beginning in July and August (1998), and continued at weekly intervals for seven weeks. Seedling emergence was counted, and seedling survival data were recorded weekly beginning one week after seeds were sown or seedlings were planted. Response variables measured for transplanted seedlings included: height of tallest tiller, and number of tillers per plant. Analysis of variance (ANOVA) was performed using the General Linear Model procedure (PROC GLM) in [SAS] Statistical Analysis System (1996). Mean separations are reported for least significant difference (LSD, p ≤ 0.05). Only comparisons identified *a priori* were tested. Effects of *A. repens* were consistent across all soil textures since there was no treatment by texture interaction. Thus, results for plant responses were averaged over all soil textures within each treatment. The influence of *A. repens* on seedling survival was consistent at all field sites, and no site by treatment interactions occurred in the statistical model. Therefore, results for seed and seedling responses were averaged over all three sites.

Results

Greenhouse: seedling survival

Results from the two greenhouse trials were significantly different for *B. gracilis*, *K. cristata*, and *S. cryptandrus*; thus these trials were analyzed separately. Because the results for *A. smithii* were similar for both trials, the data were averaged across trials for the analysis. Although the results of the two trials were consistent, seedling survival when *A. repens* roots were present was generally less in trial 1 than 2 for all species (Table 2). Differences between results of the two trials probably occurred because average *A. repens* root biomass was substantially greater in trial 1 (3.77 g/pot) than trial 2 (1.85 g/pot), and concentrations of root allelochemicals in the soil may be proportional to root biomass. This difference in biomass probably occurred because of different times of *A. repens* root collection.

Responses were species-specific with *B. gracilis* and *K. cristata* consistently showing reduced survival in the presence of *A. repens* roots in both trials (Table 2). Largest variation in survival was observed for *S. cryptandrus*. Response to the treatment consisting of a combination of *A. repens* roots in the soil and *A. repens* litter on the soil surface was similar to the in-

Table 2. Seedling survival of *B. gracilis, K. cristata, S. cryptandrus, and A. smithii* as a percentage of control as influenced by the presence of *A. repens* roots, litter, and roots + litter over time in two greenhouse trials.

Grass species	*A. repens* treatment	Trial 1							Trial 2						
		Day of Collection							Day of Collection						
		8	12	16	20	24	28	Mean	8	12	16	20*	24	28	Mean
B. gracilis	Roots	44a	64a	68a	65a	69a	86	**66**	34a	78a	83a	86	77a	73a	**72**
	Litter	82	90	87	89	87	87	**87**	86	95	73a	73	73a	70a	**78**
	Roots + Litter	42a	54a	66a	59a	59a	67a	**58**	26a	63a	68a	73	78a	62a	**68**
K. cristata	Roots	35a	74a	69	69a	68a	78	**67**	78	54a	86	54	52a	59a	64
	Litter	93	120	97	97	89	80	**97**	124	118	130	104	79	104	**110**
	Roots + Litter	48a	106	111	93	99	70a	**88**	68	101	108	104	103	75	**93**
S. cryptandrus	Roots	49	9a	68	19a	17a	52a	**36**	19	63	91	115	101	66	**76**
	Litter	42a	120	102	80	70	61	**79**	66	92	80	50	30a	23a	**57**
	Roots + Litter	08a	03a	40a	16a	08a	51a	**21**	101a	25a	94	40	40a	48a	**41**
A. smithii	Roots	103	88	91	87	79a	77a	**87**	127	106	117	107	104	97	**110**
	Litter	88	104	97	96	91	95	**95**	94	99	104	101	99	104	**100**
	Roots + Litter	56a	69a	73a	74a	66a	68a	**68**	48	98	90	91	84	87	**83**

Means followed by the letter a differ from controls (LSD, 0.05). Compare means only within a species, trial, and column.
*Control data were missing at day 20 in Trial 2, so no mean separations were performed.

fluence of *A. repens* roots alone for *B. gracilis*, but varied for the other species. Seedling survival of *B. gracilis* was decreased 14 to 56% by the presence of *A. repens* roots. *B. gracilis* survival was reduced 5 to 30% by the presence of *A. repens* litter on the soil surface in trial 2, but was similar to controls in trial 1. *K. cristata* seedling survival was decreased 14 to 65% by the presence of *A. repens* roots, but was not influenced by the presence of *A. repens* litter on the soil surface. Survival of *S. cryptandrus* seedlings was reduced by *A. repens* roots 32 to 91% in trial 1, but was similar to controls in trial 2. *A. smithii* was generally unaffected by roots or litter. When averaged over time and trials, seedling survival was decreased 9% (*A. smithii*), 34% (*K. cristata*), 31% (*B. gracilis*), and 44% (*S. cryptandrus*).

Greenhouse: seedling growth

In general, height of the tallest tiller was stimulated by *A. repens* litter and reduced by its roots. Height was stimulated by the presence of *A. repens* litter on the soil surface for all species at 16 days (*B. gracilis* 48%, *K. cristata* 51%, *S. cryptandrus* 28%, *A. smithii* 42%) and all species except *B. gracilis* after 28 days in both trials (*K. cristata* 55%, *S. cryptandrus* 75%, *A. smithii* 23%). Average plant heights for *B. gracilis* and *K. cristata* were reduced by *A. repens* roots 14 and 45%, respectively, 16 days after seed was sown

in both greenhouse trials, but were similar to controls after 28 days.

In most cases, biomass and number of tillers were significantly larger in the presence of *A. repens* litter than in treatments containing roots; controls and roots + litter treatments had intermediate values (Table 3). Species-specific variation in sensitivity to *A repens* was indicated by differences in response. Based on frequency of significance across trials and response variables, *B. gracilis* was the most sensitive followed by *K. cristata, S. cryptandrus,* and *A. smithii.* Biomass and number of tillers of *B. gracilis* were significantly greater in litter treatments and significantly less in root treatments and controls for all responses except root weights in Trial 2. For *K. cristata,* biomass was less in the root treatments than in controls. However, only shoot weight (Trial 1), total weight (Trial 2), and number of tillers (Trial 1) were significantly greater in litter than controls. Response by *S. cryptandrus* was infrequently affected by *A. repens* root or litter.

For *A. smithii,* total and shoot weight were significantly greater in the presence of *A. repens* litter and significantly less in the presence of *A. repens* roots compared to controls (Table 4). Root weights were not affected by any of the treatments. Number of tillers was greater in litter treatments than the remaining treatments and the control.

Table 3. Influence of *A. repens* roots, litter, and roots + litter on biomass and tillering of *B. gracilis, K. cristata* and *S. cryptandrus* after 28 days from two greenhouse trials. Mean (standard error).

Species	A. repens treatment	Trial 1: Biomass (mg)			Tillers	Trial 2: Biomass (mg)			Tillers
		Total	Shoots	Roots	(number)	Total	Shoots	Roots	(number)
B. gracilis	Roots	6.3a	2.5a	3.8a	1.1a	20.8a	13.1a	7.7a	1.3a
		(1.10)	(0.47)	(0.90)	(0.05)	(2.52)	(1.67)	(1.18)	(0.07)
	Litter	49.2c	23.5d	25.7c	2.0c	45.1c	25.7c	19.4b	2.5c
		(6.37)	(2.37)	(4.99)	(0.07)	(4.69)	(2.62)	(2.66)	(0.22)
	Roots + Litter	7.9a	3.2a	4.7a	1.0a	34.6bc	22.3bc	12.3ab	1.8b
		(1.68)	(0.51)	(1.47)	(0.02)	(4.84)	(2.76)	(2.53)	(0.12)
	Control	25.7b	11.7c	14.0b	1.4b	33.5ab	16.1ab	17.4b	1.9b
		(4.03)	(2.09)	(2.13)	(0.14)	(4.45)	(2.04)	(2.77)	(0.15)
K. cristata	Roots	2.8a	1.2a	1.6a	1.0a	3.2a	1.9a	1.3a	1.0a
		(0.29)	(0.13)	(0.27)	(0.00)	(0.70)	(0.47)	(0.24)	(0.11)
	Litter	13.4b	6.2d	7.2c	1.6b	8.3c	4.6b	3.7c	1.2b
		(1.70)	(0.38)	(1.76)	(0.08)	(0.64)	(057)	(0.29)	(0.04)
	Roots + Litter	6.0a	2.6b	3.4ab	1.1a	5.5b	3.4b	2.1b	1.1ab
		(1.12)	(0.30)	(0.97)	(0.05)	(0.54)	(0.32)	(0.23)	(0.04)
	Control	10.0b	4.0c	6.0bc	1.1a	6.5b	3.4b	3.1c	1.1ab
		(2.33)	(0.70)	(1.81)	(0.04)	(0.67)	(0.46)	(0.29)	(0.02)
S. cryptandrus	Roots	3.1a	1.3a	1.8ab	1.0a	8.3a	5.9a	2.4ab	1.0a
		(0.96)	(0.91)	(0.60)	(0.00)	(1.63)	(1.15)	(0.54)	(0.03)
	Litter	9.9b	7.2b	2.7ab	1.1a	27.6b	14.1b	13.5c	1.8b
		(1.66)	(1.27)	(0.56)	(0.09)	(4.45)	(2.43)	(2.43)	(0.19)
	Roots + Litter	2.9a	1.5a	1.4a	1.0a	6.6a	5.1a	1.5a	1.0a
		(0.80)	(0.49)	(0.35)	(0.02)	(1.73)	(1.37)	(0.38)	(0.20)
	Control	9.7b	5.9b	3.8b	1.3a	16.9ab	8.1ab	8.8bc	1.1a
		(2.48)	(1.67)	(0.86)	(0.23)	(5.21)	(3.06)	(2.38)	(0.07)

Compare means only within a species, trial, and among treatments. Means followed by the same letter are not different (LSD, 0.05).

Table 4. Influence of the presence of *A. repens* roots, litter, and roots + litter on biomass and tillering of *A. smithii* from two greenhouse trials combined. Mean (standard error).[1]

A repens treatment	Biomass (mg)			Tillers
	Total	Shoot	Root	(number)
Roots	32.2a	14.3a	17.9a	1.2a
	(5.11)	(1.90)	(4.28)	(0.05)
Litter	62.1c	34.8c	27.3a	1.6b
	(5.16)	(2.70)	(2.79)	(0.07)
Roots + Litter	42.8a	19.9ab	22.9a	1.3a
	(5.32)	(3.05)	(5.01)	(0.08)
None (Controls)	46.1b	23.9b	22.2a	1.4a
	(5.46)	(2.78)	(2.78)	(0.11)

[1] Data averaged over greenhouse trials 1 and 2. Compare means only within a column. Means followed by the same letter are not significantly different (LSD, 0.05).

Field studies

Results varied somewhat between field and greenhouse bioassays. Field data supported greenhouse findings that *B. gracilis* seedling survival is suppressed by the presence of *A. repens*. Survival averaged over 7 weeks at the 3 sites was only 68% of controls (Table 5). Survival was less in the presence of *A. repens* at the drier sites than at the most mesic site in the summer of 1998 (Ordway). *K. cristata* initial survival was also reduced by the presence of *A. repens* in the field. Average seedling survival over seven weeks was reduced 57%. Average survival of *S. cryptandrus* seedlings over 7 weeks was only 64% of controls. *A. smithii* was not influenced by the presence of *A. repens*. Average seedling survival of this species inside *A. repens* stands at the 3 sites over 7 weeks was 95% of controls.

Bioassay species grown from seedlings inside *A. repens* stands were similar to seedlings growing in surrounding shortgrass vegetation for all measured

Table 5. Seedling survival as a percentage of controls of *B. gracilis, K. cristata, S. cryptandrus,* and *A. smithii* grown inside *A. repens* stands for three sites[1].

Grass Species	Site			Average
	Brown's Park	Mead	Ordway	
B. gracilis	58	48	97	**68**
K. cristata	43	17	69	**43**
S. cryptandrus	63	26	102	**64**
A. smithii	86	125	75	**95**

[1] Data averaged over 5 to 7 weeks at each site.

variables in the field after 14 and 21 days. Differences between seedlings inside and outside stands became apparent after 28 days. Seedlings were generally less sensitive to *A. repens* than plants grown from seed, and responses were not consistent through time. Only significant differences inside and outside stands are included here. After 28 days, *K. cristata* seedling height was decreased 40%, and number of tillers was decreased 26% compared to adjacent control plots at all sites (data not shown). Number of tillers of *K. cristata* was decreased 35% at day 42. *B. gracilis* height was stimulated 21% after 49 days inside *A. repens* stands at all sites. Number of tillers of *S. cryptandrus* was decreased 35%, and height was decreased 24% after 42 days at Mead. Number of tillers of *A. smithii* was decreased at all sites by 30% after 28 days, and height of this species was stimulated 28% after 42 days at Mead.

Discussion

Responses of four native grasses to the invasive weed, *A. repens,* were variable within and between species. Although no effort was made to separate resource competition and allelopathy, some species were more sensitive to the presence of *A. repens.* Species-specific response by grasses have also been reported for another knapweed, *Centaurea diffusa* (Callaway and Aschehoug 2000). This variability between species means that some plant communities are more susceptible to knapweed invasion than other communities. Transplanted seedlings showed less sensitivity to *A. repens* than plants growing from seed, likely as a result of their larger biomass. *A. smithii* had the largest seed and seedling biomass of any of the test species and was least affected by *A. repens.* Thus, differences in seed and seedling biomass of different species may correspond to differences in sensitivity.

In general for all species, *A. repens* litter increased seedling growth and roots decreased seedling survival and growth compared to controls or litter + roots. *K. cristata* was the most consistently sensitive to *A. repens,* while *A. smithii* was least affected. These results show that interactions between *A. repens* and native grasses are complex, with important implications for grass revegetation following *A. repens* invasion and for the success of *A. repens* in different grass communities.

Quantification of negative influences of *A. repens* on survival and growth of different grass species may help determine the selection of species and seeding rates for seeding trials. Since native grass seed availability and precipitation are low and variable in space and time in the shortgrass steppe (McGinnies et al. 1988; Coffin and Lauenroth 1989), repeated seeding efforts may be necessary for successful establishment. Artificial seeding with grasses following herbicide application may be the most effective way to control *A. repens* (Benz et al. 1999). By decreasing survival of native species, *A. repens* stands resist re-invasion by native grasses. Foliar allelochemicals may stimulate germination of some species that will subsequently perish when the seedlings are exposed to *A. repens* roots, which would decrease soil seed reserves (Grant et al. 1998).

The consistent decrease in biomass and survival of *K. cristata* indicates that this species is highly susceptible to the negative influences of *A. repens.* *K. cristata,* a C_3 species, is found over a wide range of elevations in Colorado and frequently occurs in shortgrass communities, but rarely dominates large areas (Harrington 1954; Monson et al. 1983, 1986). Although *K. cristata* recovers after disturbance through seedling establishment, recovery following *A. repens* invasion is unlikely based on our studies.

The decrease in biomass and number of tillers by *B. gracilis* in the presence of *A. repens* roots has important implications for dominance patterns in the shortgrass steppe. This species dominates these communities despite infrequent recruitment by seed and slow tillering rates. A restrictive set of microenvironmental conditions is required for seed germination and seedling establishment (Hyder et al. 1971; Briske and Wilson 1977, 1978; Lauenroth et al. 1994). *B. gracilis* also has low and variable seed production, and few seeds stored in the soil (Coffin and Lauenroth 1989, 1992). Competition between adult *B. gracilis* plants and conspecific seedlings also negatively affects establishment (Aguilera and Lauenroth 1993).

Findings from the current study suggest another limitation to seedling establishment of this species following invasion by *A. repens*. Reduced tillering in the presence of *A. repens* roots indicates that *B. gracilis* vegetative expansion into *A. repens*-dominated patches may also be very slow.

Low germination by *S. cryptandrus* seeds likely resulted from the lack of pretreatments to eliminate primary dormancy (Sayers 1968; Sabo 1979). Under field conditions where dormancy is broken, *S. cryptandrus* establishes quickly from seed following disturbance with high seed availability in the soil (2340 seeds/m^2; Karl et al. (1999)). The low and inconsistent sensitivity of *S. cryptandrus* to *A. repens* shows that natural recovery of this species may be possible after invasion.

The stimulatory influence of *A. repens* litter and no effect by roots on *A. smithii* may allow this species to survive and grow with *A. repens* in the field where both roots and litter are present. This supports the findings by Hanson (1991) that *A. smithii* is not adversely influenced by the presence of *A. repens*. *A. smithii* is a C$_3$ grass that dominates northern mixed grass prairie and can codominate with *B. gracilis* on fine textured soils in the shortgrass steppe (Monson et al. 1986). Because *A. repens* is also commonly found on fine textured soils (Roché and Roché 1991), *A. smithii* has a high probability of successful revegetation following invasion by *A. repens*.

Contrary to our hypothesis, soil texture did not influence allelopathic expression since species responses to *A. repens* were not related to clay content in the greenhouse. Field observations of more complete dominance by *A. repens* on fine- than coarse-textured soils may require explanations other than allelopathy (K.G. Beck personal observation). Alternatively, the greenhouse conditions did not represent the full set of field conditions under which *A. repens* dominates.

Stimulatory effects of *A. repens* litter on the soil surface may result from low concentrations of foliar alleochemicals. Lab studies using sesquiterpene lactones present in the shoot material of *A. repens* showed decreased growth of lettuce at high allelochemical concentrations, but stimulated lettuce growth at lower levels (Stevens and Merrill 1985). However, *A. repens* forms a dense litter layer once established which may increase soil moisture levels and promote its growth in a positive feedback, or promote the growth of other species. In addition, a deep layer of litter may decrease chances of seedling emergence after gemination.

Although our results support work by others that identify *A. repens* as allelopathic, growth responses cannot be solely attributed to interference from allelopathy because some degree of resource competition also occurred. However, competition for soil resources between native grasses may be more intense than competition with *A. repens* because of a shared rooting zone for grasses with roots concentrated in the top 20 cm of the soil profile (Bartos and Sims 1974; Milchunas et al. 1992); *A. repens* roots are also found in shallow layers, but can penetrate deep into the soil to depths of 7 m (Rogers 1928; Ivanova 1966). Water is the most frequently limiting resource in semi-arid regions (Lauenroth et al. 1978), and the ability of plant functional types to exploit different soil water resources is dependent on their root structure (Aguiar et al. 1996; Dodd and Lauenroth 1997). It may be unrealistic to attempt to completely separate direct competition from allelopathy (Inderjit and Del Moral 1997; Inderjit and Dakshini 1999), especially in experiments that attempt to be ecologically realistic. The important relationship from an ecological standpoint is not whether the source of inhibition is from resource competition or allelopathy, but rather how severely one species influences the growth of its neighbors.

Generalizations cannot be made about the influence of *A. repens* allelopathy on native grasses because responses were species specific. These results may explain variation in susceptibility of different plant communities to invasion and in the ability of different species to recover following invasion. *B. gracilis* and *K. cristata* were very sensitive to *A. repens* and consistently showed decreased survival in its presence. *A. smithii* was not influenced by the presence of *A. repens* and may therefore be a good candidate for revegetation. While *A. repens* roots generally had a negative influence on survival and growth, responses of native grasses were not attributable to either resource competition or allelopathy alone. Complex interactions between these processes are likely to occur under natural field conditions. Colonization of grass populations inside dense *A. repens* stands may be unlikely, although artificial reseeding would improve the chances of successful recruitment. The invasion and persistence of *A. repens* stands is related to its ability to reduce initial survival and growth of some native grass species.

Acknowledgements

References

Aguiar M.R., Paruelo J.M., Sala O.E. and Lauenroth W.K. 1996. Ecosystem responses to changes in plant functional type composition: An example from the Patagonion steppe. Journal of Vegetation Science 7: 381–390.

Aguilera M.O. and Lauenroth W.K. 1993. Seedling establishment in adult neighborhoods: Intraspecific constraints in the regeneration of the bunchgrass *Bouteloua gracilis*. Journal of Ecology 81: 253–261.

Bartos D.L. and Sims P.L. 1974. Root dynamics of a shortgrass ecosystem. Journal of Range Management 27: 33–36.

Benz L.J., Beck K.G., Whitson T.D. and Koch D.W. 1999. Reclaiming Russian knapweed infested Rangeland. Journal of Range Management 52: 351–356.

Bottoms R.M. and Whitson T.D. 1998. A systems approach for the management of Russian knapweed (*Centaurea repens*). Weed Technology 12: 363–366.

Bottoms R.M., Whitson T.D. and Koch D.W. 1996. Chemical and biological control techniques for Russian knapweed. Proceedings Western Society of Weed Science 49: 56–60.

Briske D.D. and Wilson A.M. 1977. Temperature effects on adventitious root development in blue grama seedlings. Journal of Range Management 30: 276–280.

Briske D.D. and Wilson A.M. 1978. Moisture and temperature requirements for adventitious root development in blue grama seedlings. Journal of Range Management 31: 174–178.

Callaway R.M. and Aschehoug E.T. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290: 521–523.

Coffin D.P. and Lauenroth W.K. 1989. Spatial and temporal variation in the seed bank of a semiarid grassland. American Journal of Botany 76: 53–58.

Coffin D.P. and Lauenroth W.K. 1992. Spatial variability in seed production of the perennial bunchgrass *Bouteloua gracilis* (Gramineae). American Journal of Botany 79: 347–353.

Cronk Q. and Fuller J.L. 1995. Plant Invaders. Chapman & Hall, London.

Day P.R. 1965. Particle fractionation and particle-size analysis. In: Black C.A., Evans D.D., Ensminger L.E., White J.L. and Clark F.E. (eds), Methods of Soil Analysis. Part I. Soil Science Society of America Inc, Madison, WI, USA, pp. 545–567.

Del Moral R. and Muller C.H. 1970. Allelopathic effects of *Eucalyptus camaldulensis*. American Midland Naturalist 83: 254–282.

Dodd M.B. and Lauenroth W.K. 1997. The influence of soil texture on the soil water dynamics and vegetation structure of a shortgrass steppe ecosystem. Plant Ecology 133: 13–28.

Fletcher R.A. and Renney A.J. 1963. A growth inhibitor found in *Centaurea* spp. Can. J. Plant Sci. 43: 475–481.

Gentle C.B. and Duggin J.A. 1997. Allelopathy as a competitive strategy in persistent thickets of *Lanata camara* L. in three Australian forest communities. Plant Ecology 132: 85–95.

Grant D.W., Beck K.G. and Coffin D.P. 1998. Perennial grass survival response to Russian knapweed soil allelochemicals. Proceedings Western Society of Weed Science 51: 65–66.

Hanson D.E. 1991. Russian knapweed interference with corn VA mycorrhiza, western wheatgrass, and smooth brome. MSc Dissertation, Colorado State University, Fort Collins, USA.

Harrington 1954. Manual of the Plants of Colorado. Sage Books, Denver, CO, USA, p. 666.

Hyder D.N., Everson A.C. and Bement R.E. 1971. Seedling morphology and seedling failures with blue grama. Journal of Range Management 24: 287–292.

Inderjit and Dakshini K. 1994. Allelopathic effect of Pluchea lanceolata (Asteraceae) on characteristics of four soils and tomato and mustard growth. American Journal of Botany 81: 799–804.

Inderjit and Dakshini K. 1999. Allelopathy: One component in a multifaceted approach to ecology. In: Inderjit, Dakshini K. and Foy C.L. (eds), Principles and Practices in Plant Ecology: Allelochemical Interactions. CRC Press, Boca Raton, FL, USA, pp. 3–14.

Inderjit and Del Moral R. 1997. Is separating resource competition from allelopathy realistic? The Botanical Review 63: 221–230.

Ivanova T.S. 1966. Biological control of mountain bluet (*Acroptilon picris* C.A.M.) [in Russian]. Izv. Acad. Nauk. Tadzhik. SSR. (Otel Biol. Nauk.) 2: 51–63.

James L.F., Evans J.O., Ralphs M.H. and Child R.D. 1991. Preface. In: James L.F., Evans J.O., Ralphs M.H. and Child R.D. (eds), Noxious Range Weeds. Westview Press, Boulder, CO, USA.

Karl M.G., Heitschmidt R.K. and Haferkamp M.R. 1999. Vegetation biomass dynamics and patterns of sexual reproduction in a northern mixed-grass praire. American Midland Naturalist 141: 227–237.

Lauenroth W.K., Dodd J.L. and Sims P.L. 1978. The effects of water- and nitrogen-induced stresses on plant community structure in a semiarid grassland. Oecologia (Berlin) 36: 211–222.

Lauenroth W.K., Sala O.E., Coffin D.P. and Kirchner T.B. 1994. The importance of soil water in the recruitment of *Bouteloua gracilis* in the shortgrass steppe. Ecological Applications 4: 741–749.

Mack R.N., Simberloff D., Londsdale W.M., Evans H., Clout M. and Bazzaz F.A. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10: 689–710.

McGinnies W.J., Laycock W.A., Tsuchiya T., Yonker C.M. and Edmunds D.A. 1988. Variability within a native stand of blue grama. Journal of Range Management 41: 391–395.

Milchunas D.G., Lee C.A., Lauenroth W.K. and Coffin D.P. 1992. A comparison of ^{14}C, ^{86}Rb, and total excavation for determination of root distribution of individual plants. Plant and Soil 144: 125–132.

Monson R.K., Littlejohn R.O. and Willimas G.J. 1983. Photosynthetic adaptation to temperature in four species from the Colorado shortgrass steppe: a physiological model for coexistence. Oecologia 58: 43–51.

Monson R.K., Sackschewsky M.R. and Williams G.J. 1986. Field measurements of photosynthesis, water-use efficiency and growth in *Agropyron smithii* (C_3) and *Bouteloua gracilis* (C_4) in the Colorado shortgrass steppe. Oecologia 68: 400–409.

Mooney H.A. and Drake J.A. (eds) 1986. Ecology of Biological Invasions of North America. Springer-Verlag, New York.

Moore R.J. and Frankton C. 1974. The thistles of Canada. Can. Dept. Agric. Monogr. No. 10. Information Canada, Ottawa.

Muller C.H. and Del Moral R. 1966. Soils toxicity induced by terpenes from *Salvia leucophylla*. Bulletin Torrey Botanical Club 93: 130–137.

Nelson D.W. and Sommers L.E. 1996. Total carbon, organic carbon, and organic matter. In: Sparks D.L. (ed.), Methods of Soil Analysis. Part III. Soil Science Society of America, Inc., Madison, WI, USA, pp. 961–1010.

Ridenour W.M. and Callaway R.M. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia 126: 444–450.

Roché B.F. and Roché C.T. 1991. Identification introduction distribution ecology and economics of *Centaurea* species. In: James L.F., Evans J.O., Ralphs M.H. and Child R.D. (eds), Noxious Range Weeds. Westview Press, Boulder, CO, pp. 274–291.

Rogers C.F. 1928. Canada Thistle and Russian Knapweed. Colorado Experiment Station Bulletin 348.

Sabo D.G. 1979. Germination requirements of 19 species of arid land plants. USDA-Forest Service, Rocky Mountain Forest and Range Experiment Station Research Paper. USDA Forest Service, USA, RM-210.

[SAS] Statistical Analysis System 1996. General Linear Model Procedure (PROC GLM). SAS/STAT Software: changes and enhancements through release 6.11. SAS Institute, Cary, NC, USA.

Sayers R.L. 1968. Germination requirements of sand dropseed (*Sporobolus cryptandrus*) and sand lovegrass (*Eragrostis trichodes*). PhD Dissertation, Colorado State University, Fort Collins, USA.

Sheley R.L., Petroff J.K. and Borman M.M. 1999. Introduction. In: Sheley R.L. and Petroff J.K. (eds), Biology and Management of Noxious Rangeland Weeds. Oregon State University Press, Corvalis, OR, USA, pp. 1–3.

Sheley R.L., Svejlar T.J. and Maxwell B.D. 1996. A theoretical framework for developing successional weed management strategies on rangeland. Weed Technology 10: 766–773.

Stevens K.L. 1986. Allelopathic polyacetlyenes from *Centaurea repens* (Russian knapweed). Journal of Chemical Ecology 12: 1205–1211.

Stevens K.L. and Merrill G.B. 1985. Sesquiterpene lactones and allelochemicals from *Centaurea* species. In: Thompson A.C. (ed.), The Chemistry of Allelopathy: Biochemical Interactions Among Plants. American Chemical Society, Washington, DC, USA, pp. 83–98.

The Great Plains Flora Association 1986. Flora of the Great Plains. University Press of Kansas, Lawrence, KS, USA.

Tyser W.T. and Key C.H. 1988. Spotted knapweed in natural area fescue grasslands: An ecological assessment. Northwest Science 62: 151–160.

Usher M.B. 1988. Biological invasions of nature reserves: a search for generalizations. Biological Conservation 44: 119–135.

Vitousek P.M. 1990. Biological invasions and ecosystem processes: Towards an integration of population biology and ecosystem studies. Oikos 57: 7–13.