

Establishment of Native Species in Soils From Russian Knapweed (*Acroptilon repens*) Invasions

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Abstract

Russian knapweed (*Acroptilon repens* [L.] DC.), an exotic perennial forb, has invaded many native ecosystems in western North America. Russian knapweed's success is attributed to allelopathy, extensive tap rooting, zinc accumulation in soils, and a lack of North American predators. Revegetation following chemical control slows exotic reestablishment, but the impacts of Russian knapweed-invaded soils on the establishment of native forbs and shrubs have not been determined. In a greenhouse experiment, we monitored the establishment of two native forbs, Indian blanketflower (*Gaillardia aristata* Pursh) and purple prairie clover (*Dalea purpurea* Vent.) and two native shrubs, winterfat (*Krascheninnikovia lanata* [Pursh] A.D.J. Meeuse & Smit syn. *Ceratoides lanata*) and Wyoming big sagebrush (*Artemisia tridentata* Nutt. subsp. *wyomingensis* [Hook.] Nutt.) in soils obtained from three Russian knapweed invasions and adjacent noninvaded areas. We analyzed soils collected near Greybull and Riverton, Wyoming, and Greeley, Colorado, for cation exchange capacity, organic matter, electroconductivity, pH, and total nitrogen, carbon, and plant-available potassium, zinc, manganese, copper, and phosphate. We documented seedling emergence of the four natives and Russian knapweed every two days for 14–17 weeks, harvested seedlings biweekly to assess their growth, and determined their zinc accumulation. All species established in invaded soil and seedlings were larger in invaded than in noninvaded soils. Invaded rangeland soils had greater organic matter (8.6% and 1.1% in invaded vs. 2.5% and 0.4% in noninvaded soils) and lower pH (7.4 in invaded versus 8.0 noninvaded soils). Zinc concentrations in invaded soils (from 0.15 to 6.56 mg · kg⁻¹) were not high enough to limit plant growth. Reports that Russian knapweed is a hyper-accumulator of zinc are not supported by our seedling data, which suggests that previously invaded soils may not limit native seedlings.

Resumen

“Russian knapweed” (*Acroptilon repens* [L.] DC.), una hierba perenne introducida, ha invadido muchos ecosistemas nativos del oeste de Norte América. El éxito del “Russian knapweed” es atribuido a alelopatía, su sistema radical pivotante extensivo, la acumulación de zinc en los suelos y la falta de predadores para esta especie en Norte América. La revegetación posterior al control químico hace más lento el reestablecimiento de especies exóticas, pero los impactos de los suelos invadidos por “Russian knapweed” sobre el establecimiento de herbáceas y arbustos nativos no ha sido determinado. En un experimento en invernadero monitoreamos el establecimiento de dos herbáceas nativas “Indian blanketflower” (*Gaillardia aristata* Pursh) y “Purple prairie clover” (*Dalea purpurea* Vent.) y dos arbustos nativos “Winterfat” (*Krascheninnikovia lanata* [Pursh] A.D.J. Meeuse & Smit syn. *Ceratoides lanata*) y “Wyoming big sagebrush” (*Artemisia tridentata* Nutt. subsp. *wyomingensis* [Hook.] Nutt.) en suelos provenientes de tres sitios invadidos por “Russian knapweed” y tres sitios adyacentes no invadidos. Analizamos suelos colectados cerca de Greybull y Riverton, Wyoming y Greeley, Colorado para determinar la capacidad de intercambio catiónico, contenido de materia orgánica, conductividad eléctrica, pH, nitrógeno total, carbón, potasio disponible para la planta, zinc, manganeso, cobre y fósforo. Documentamos la emergencia de plántulas de las cuatro especies nativas y del “Russian knapweed” cada dos días durante 14 a 17 semanas, cosechamos plántulas cada dos semanas para evaluar su crecimiento y determinar su acumulación de zinc. Todas las especies se establecieron en suelos los invadidos por “Russian knapweed” y las plántulas fueron más largas que las de los suelos no invadidos. Los suelos de pastizal invadidos por “Russian knapweed” tuvieron más materia orgánica (8.6% y 1.1% en suelos invadidos vs. 2.5% y 0.4% en suelos no invadidos) y menor pH (7.4 en suelos invadidos versus 8.0 en los no invadidos). Las concentraciones de zinc en los suelos invadidos (0.15 a 6.56 mg · kg⁻¹) no fueron lo suficientemente altos para limitar el crecimiento de las plantas. Reportes respecto a que “Russian knapweed” es un hiperacumulador de zinc no son soportados por nuestros datos de plántulas, sugiriendo que los suelos previamente invadidos pueden no limitar las plántulas nativas.

Key Words: allelopathy, *Artemisia tridentata* subsp. *wyomingensis*, *Dalea purpurea*, exotic weed, *Gaillardia aristata*, *Krascheninnikovia lanata*, organic matter, zinc toxicity

Research was funded by USDA-NRI 2003-35320-13437 and the Department of Renewable Resources, University of Wyoming, Laramie, WY.

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Manuscript received 2 November 2006; manuscript accepted 1 July 2007.

INTRODUCTION

Russian knapweed (*Acroptilon repens* [L.] DC.), an invasive perennial, currently occupies over 607 000 ha in western North America (Duncan 2001) and is an extremely effective competitor with native vegetation (Grant et al. 2003). This exotic species has no known natural enemies in North America (Watson 1980) and is usually not grazed, which gives it greater competitive advantage within rangelands. The impact of the species on rangeland soils and restoration seedings is not well detailed.

Exotic invaders often change the soil chemistry on invaded sites (Duda et al. 2003; Ehrenfeld 2003; Blank and Young 2004). Higher zinc (Zn) concentrations occur in soils from Russian knapweed invasions compared to concentrations in adjacent grassland soils (Bottoms 2001). Hyper-accumulation of Zn in Russian knapweed litter deposits on invaded sites may increase soil Zn concentrations to levels too high for native plant establishment. However, impact of intentionally elevated soil Zn content has little impact on grass seedlings (Morris 2005; Morris et al. 2006).

Russian knapweed may also produce allelopathic chemicals (Fletcher and Renney 1963; Stevens 1982, 1986; Stermitz et al. 2003), consequently reducing native species establishment. Incorporation of activated carbon (C) into soil mixtures to absorb organic root exudates of a related species, diffuse knapweed (*Centaurea diffusa* Lam.), limits its dominance over native grasses (Callaway and Aschehoug 2000), and additions of activated C also release growth of Idaho fescue (*Festuca idahoensis* Elmer) growing with spotted knapweed (*Centaurea maculosa* Lam.; Ridenour and Callaway 2001). Knowing the effects of Russian knapweed on soils is especially critical to reseeding efforts to prevent return of the invader following its removal.

Reseeding with competitive native grass species can limit the return of exotics (Bottoms and Whitson 1998; Ferrell et al. 1998). Because the use of native nongraminoid species may be cost-prohibitive or limited by seed availability (Monsen and Shaw 2001), careful selection of native shrub and forb species for their potential in restoration seedings of Russian knapweed infestations is warranted. Select North American species such as German chamomile (*Matricaria recutita* L.) and blanketflower (*Gaillardia grandiflora* Van Houtte) can be highly resistant to (-)-catechin (Weir et al. 2003; Perry et al. 2005), and Indian blanketflower (*Gaillardia aristata* Pursh) appears to be resistant to the Russian knapweed root exudate, 7,8-benzoflavone (Stermitz et al. 2003). These studies suggest we should expect emergence and establishment of some native forb and shrub species to be reduced in soils from Russian knapweed invasions, while others may not.

We examine the emergence and growth of four native nongraminoid species in soils obtained from within and outside of Russian knapweed invasions to ascertain if inclusion of nongraminoid native seed will enhance the success of revegetation of Russian knapweed infestations. We document seedling emergence and growth, plant Zn concentrations of two commercially available native forbs, Indian blanketflower and purple prairie clover, and two native shrubs, winterfat and Wyoming big sagebrush, sown in Russian knapweed-invaded and noninvaded soils. We also detail physical and chemical

properties of soils from the two positions relative to Russian knapweed invasion. We hypothesized that 1) soil physical and chemical properties within Russian knapweed invasions would differ from soil properties in adjacent noninvaded soils, and that as a consequence, 2) fewer forb and shrub species would emerge and establish in soils invaded by Russian knapweed than in soils taken from adjacent noninvaded areas, 3) growth of established native species would be reduced in soils from Russian knapweed invasions relative to soils from adjacent noninvaded areas, and 4) Zn concentrations of species grown in soils from Russian knapweed invasions would be greater than Zn concentrations of the same species grown in soils from adjacent noninvaded areas.

MATERIALS AND METHODS

Study Sites

In May 2004 we selected three noncultivated sites invaded by Russian knapweed for more than 25 years (Mealor et al. 2004) that were adjacent to comparable noninvaded soils (<100 m away). Two sites were native rangelands near Greybull (44°27'N, 108°02'W) and Riverton (43°17'N, 108°14'W), Wyoming, and one site was an abandoned pasture near Greeley (40°23'N, 104°54'W), Colorado.

The Riverton site was on a flat below a south facing slope lying at 1457 m elevation (Western Regional Climate Center [WRCC] 2005). Precipitation at Riverton in 2004 was 244 mm, and long-term mean annual precipitation for Riverton is 224 mm (WRCC 2005). Litter in the invaded area was 3101 kg · ha⁻¹ and 287 kg · ha⁻¹ in the noninvaded area. Russian knapweed shoot density was 31.6 · m⁻². Vegetation other than knapweed included winterfat, Indian ricegrass (*Achnatherum hymenoides* [Roemer & J. A. Schultes] Barkworth), fringed sagebrush (*Artemisia frigida* Willd.), Russian thistle (*Salsola iberica* [Sennen & Pau] Botsch. ex Czerepanov), pricklypear cactus (*Opuntia polyacantha* Haw.), and an *Astragalus* sp. L.

The Greybull site was located west of the Big Horn River in the Big Horn Basin. Russian knapweed shoot density was 76.4 · m⁻². Annual precipitation at Greybull in 2004 was 123 mm (WRCC 2005), and elevation is 1158 m. Mean annual precipitation for Greybull is 176 mm. Litter in the invaded area was 3952 kg · ha⁻¹ and 462 kg · ha⁻¹ in the noninvaded area. Vegetation other than Russian knapweed at this site included alkali sacaton (*Sporobolus airoides* L.), Russian olive (*Elaeagnus angustifolia* L.), green rabbitbrush (*Chrysothamnus viscidiflorus* [Hook.] Nutt.), greasewood (*Sarcobatus vermiculatus* [Hook.] Torr.), and basin big sagebrush (*Artemisia tridentata* Nutt. subsp. *tridentata* [Hook.] Nutt.).

The Greeley site was located in a drainage in an abandoned pasture surrounded by cropland. Russian knapweed shoot density was 20 shoots · m⁻². Annual precipitation in 2004 for this area was 302 mm (WRCC 2005), and elevation is 1469 m. Mean annual precipitation for Greeley is 295 mm. Vegetation other than knapweed included smooth brome (*Bromus inermis* Leyss.), field bindweed (*Convolvulus arvensis* L.), wildrye (*Elymus* L. spp.), and showy milkweed (*Asclepias speciosa* L.) This area was grazed by cattle prior to 1980. Yearly fertilizer amendments are added to the surrounding croplands on a bean

and corn rotation. Amendments for beans are $23 \text{ kg} \cdot \text{ha}^{-1}$ nitrogen (N), $1.3 \text{ kg} \cdot \text{ha}^{-1}$ phosphorus, and trace amounts of Zn ($0.04 \text{ kg} \cdot \text{ha}^{-1}$), and corn amendments are $82 \text{ kg} \cdot \text{ha}^{-1}$ N and trace amounts of Zn.

Russian knapweed shoot density was determined by counting live stems in a 0.25 m^2 quadrat placed randomly 10 times within the invasion area. In October 2005 we returned to the two rangeland sites to estimate litter accumulation in the invaded and noninvaded areas for site descriptions. A 0.25-m^2 quadrat was randomly placed eight times in the invaded and noninvaded areas, and all standing litter within the quadrat was clipped, placed into paper bags, dried at 65°C for 48 hours, and weighed. At Greeley the smooth brome elevated litter and OM throughout the site, regardless of Russian knapweed presence. Because this site had been plowed to incorporate and homogenize the soils in the past, the impacts of Russian knapweed alone on organic matter (OM) could not be separated from the impacts of the grass. For this reason we did not collect litter at the Greeley site.

Soil Collection and Handling

In May 2004 we collected approximately 150 L of soil from inside (invaded) and outside (noninvaded) Russian knapweed invasions at each of the three sites. We collected soil from the top 10 cm of the soil profile from more than 10 random locations from both inside and outside each invasion. We included any litter present on the soil surface with soil collections, although litter was removed when soils were sieved in the lab. Soils were transported to the University of Wyoming Plant Science Greenhouses in Laramie, Wyoming ($41^\circ19'\text{N}$, $105^\circ33'\text{W}$) after collection and prior to establishing the greenhouse experiments. We sieved soils with a 1-cm^2 sieve to eliminate leaf litter and then placed soils into foamed styrene plastic containers.

Native Species Selected for Greenhouse Study

We selected two forb and two shrub species for our study based on desirable characteristics. Indian blanketflower appears to be resistant to the Russian knapweed root exudate, 7,8-benzoflavone (Stermitz et al. 2003). Purple prairie clover is relatively unaffected by the herbicide imazapic (Beran et al. 1999), which can be used to control Russian knapweed (Vollmer and Vollmer 2001). We chose the shrubs Wyoming big sagebrush and winterfat because they are an integral component of sagebrush-steppe communities and because of their common and successful use in revegetation seedings (Carey 1995; Howard 1999). Both shrub species were present in at least one of the soil collection sites, and all four species naturally occur in Wyoming and northern Colorado (USDA, NRCS 2004). All native seeds were obtained from commercial seed producers.

Wild collections of Russian knapweed seed were also used in this study to assess its growth in both invaded and noninvaded soils. We collected Russian knapweed seeds near Mud Lake, Idaho, on 11 June 2003. We did not use seed from the soil collection sites, so potential local adaptation by Russian knapweed would not provide an advantage in any of the soils used in this study. The results of a previous seed bank study eliminated speculation that Russian knapweed seeds were

present in our noninvaded soils at Riverton and Greybull (data not shown). Because of a dense understory of smooth brome, seed bank samples were not taken from Greeley.

To determine germination rates of the five species we germinated a randomly selected subset of each species in growth chambers at the University of Wyoming Shrubland Ecology Laboratory. We placed 50 seeds of each species on moist filter paper in five replicate Petri dishes. Distilled water was added as needed to keep the filter paper moist. Covered Petri dishes were placed into an environmental growth chamber under conditions of 27°C for 16 hours (day) and 20°C for 8 hours (night) to mimic corresponding greenhouse conditions. Mean number of germinated seeds was determined after a 30-day incubation period following recommendation of Baskin and Baskin (1998). We used the resulting germination percentages for each species to determine the appropriate number of seeds needed to attain at least one seedling per cell in each container in our greenhouse experiment.

Greenhouse Growth Experiment

Three foamed styrene plastic containers were filled with either invaded or noninvaded soil from one of the three collection sites (three invaded containers and three noninvaded containers from each of three sites, 18 in all). The containers used in the experiment consisted of 144 cells per container (9 rows of 16 cells per row); each cell was 11 cm deep, with a volume of 90 mL. So that seeding and harvest times could be staggered, we repeated the seeding on four seeding dates (4, 8, 11, and 24 June 2004), resulting in 72 containers in the study. To control for variation in growing conditions (i.e., longer days, or higher temperatures) within the greenhouse associated with different seeding dates, we considered each seeding date a block, and each of the three containers assigned identical treatments within a seeding date constituted subsamples.

We randomly assigned each plant species (four natives and Russian knapweed) to one of five interior rows in each container. Seeds of each species were individually sown into container rows in June 2004 using the number of seeds established by the germination study. The four outside rows of each container were not seeded and contained only soil to serve as a temperature buffer for seeded rows. To ensure germination across light and temperature variations in the greenhouse we placed blocks in random locations on greenhouse benches and rerandomized periodically. We lacked enough soil to fill one Greybull-invaded container in block four, and so the fourth block contained 17 containers. Day length in Laramie, Wyoming, during the time of this study ranged from 11 to 14 hours per day (QPAIS 2005). Greenhouse temperatures varied throughout the experiment but the mean high temperature was 29°C , and low was 17°C . No artificial lighting was used in this experiment. Containers were hand watered daily with enough water to adequately moisten soil without leaching of soil material (approximately 900 mL of water per container each time).

Germination was determined by emergence of cotyledons and was monitored on alternate days beginning on 12 June 2004, when the cotyledons of the first seedlings were fully emerged. Cumulative mean emergence was determined for each 2-day period after initial seeding until harvesting began. To

ensure that a seedling would be established in each cell, we delayed thinning until there were at least two true leaves (not cotyledons) on at least one plant in each cell. Seedlings were thinned to leave the largest plant (greatest number of true leaves) in the cell. Indian blanketflower and winterfat were thinned 6 weeks after seeding. Purple prairie clover, Russian knapweed, and Wyoming big sagebrush were thinned at 7, 8, and 9 weeks respectively. Harvest dates were determined individually for each species when germination began to slow. A random subset of cells containing each species was harvested on four dates within the 14-week growing period. Harvesting dates were 14 days apart at 8, 10, 12, and 14 weeks after seeding. Because Russian knapweed and Wyoming big sagebrush were slow to germinate, Russian knapweed was harvested at 9, 11, 13, and 15 weeks and Wyoming big sagebrush at 11, 13, 15, and 17 weeks after seeding.

Root biomass, shoot biomass, leaf area, and number of leaves for each seedling were determined on each harvesting date. When harvested, all live true leaves were counted, and leaf area (cm^2) was determined using a leaf area meter. Plants were washed on a 2-mm sieve, divided into roots and shoots, and dried at 65°C for 36 hours to obtain root and shoot biomass. Root and shoot biomass were summed to give total biomass.

Zinc Concentrations in Plant Shoot Material

Because previous studies observed greater Zn concentrations in soils beneath Russian knapweed invasions (Bottoms 2001; Morris 2005), Zn concentrations were determined in shoot material from plants grown in the greenhouse experiment. We used only shoot material to follow procedures of Bottoms (2001) and ensure that we noted only values for seedling uptake. All harvested above-ground plant material was combined within position (invaded or noninvaded) by block and species. Shoot material was hand-ground using a mortar and pestle. Shoot Zn concentrations were determined using the dry ash method (Gavlak et al. 2003).

Soil Analyses

In addition to collecting soil for the greenhouse experiment, we also collected five replicate soil samples (0–10 cm + surface litter) randomly from each site and position (invaded and noninvaded) for analysis of basic soil properties. Samples were placed into unsealed plastic bags and air dried. We sieved (2-mm mesh) and hand-ground soils with a mortar and pestle prior to analysis. Soils which were subsampled for N and C analyses were roller-ground for 24–48 hours.

Soil texture was determined on two (of the five) randomly selected soil samples from each position (invaded and noninvaded) from each site using a modified version of the hydrometer method (Gee and Bauder 1986). Electrical conductivity (EC), pH, OM, cation exchange capacity (CEC), and concentrations of total C and N, and plant-available phosphate (PO_4), iron (Fe), potassium (K), manganese (Mn), copper (Cu), and extractable Zn were determined on duplicate samples of each of the five replicate samples. EC and pH were determined by saturation paste method (Gavlak et al. 2003). OM was obtained using the weight loss on ignition method (Storer 1984). Ammonium bicarbonate-DTPA (diethylenetriaminepen-

taacetic acid) was used to extract Zn, Fe, Mn, Cu, and K (Soltanpour and Schwab 1977), while the sodium bicarbonate method was used to obtain extractable PO_4 (Olsen and Sommers 1982). CEC was determined using the ammonium replacement method (Gavlak et al. 2003). Samples were analyzed for total N and C by micro-Dumas combustion (Fison 1108 Elemental Analyzer) at the University of Wyoming Stable Isotope Facility 14 months after soil collection. Electrical conductivity, pH, OM, CEC, and concentrations of total C, and N, and plant-available PO_4 , Fe, K, Mn, Cu, and extractable Zn were determined with the assistance of the University of Wyoming Soil Testing Laboratory within one year after collection. Because Bottoms (2001) presented Russian knapweed as a hyper-accumulator of Zn based on ratios of soil available to plant Zn content, we also calculated these ratios for all five species. Ratios were calculated by dividing the soil test Zn by the plant shoot Zn content of seedlings grown in each soil.

Experimental Design and Data Analysis

The experimental design for the establishment study was a randomized block design using position (invaded and noninvaded) as treatments. Blocks (seeding date) were initiated 3–20 days apart (4–24 June) in the greenhouse. Monitoring data from the three containers treated alike within a block (subsamples assigned identical site and position within a species) were averaged prior to data analysis. Each species and site was analyzed separately. Differences in number of true leaves, leaf area, root, shoot, and total biomass were analyzed using a randomized block design analysis of variance (ANOVA; (Steel and Torrie 1980). We included harvest week as repeated measures for analysis of biomass, leaf area, and number of leaves. When the harvest date (week) by position (invaded versus noninvaded) interaction was significant ($P < 0.05$), the LSD mean separations were conducted using error terms that were specific to the contrast (Milliken and Johnson 1992). We compared percentage total emergence for each species grown in invaded and noninvaded soils using a two-group t test ($P \leq 0.05$; SAS Institute 1999). Species and sites were analyzed separately.

Only Russian knapweed and Indian blanketflower produced enough plant material to allow for sufficient replication for statistical analysis of Zn concentrations. We compared Zn concentrations of Russian knapweed and Indian blanketflower plants grown in invaded and noninvaded areas using a two-group t test ($P \leq 0.05$; SAS Institute 1999). Species and sites were analyzed separately. Because seedlings of purple prairie clover, Wyoming big sagebrush, and winterfat were small, we combined shoot material from the four blocks for one replication within each species and position (invaded and noninvaded) for each study site. Mean Zn concentrations for purple prairie clover, Wyoming big sagebrush, and winterfat are presented without statistical analysis.

Soil physical and chemical properties from each site were analyzed independently using a two-group t test ($P \leq 0.05$) (SAS Institute 1999) to compare invaded and noninvaded positions ($n = 5$ invaded, $n = 5$ noninvaded). Duplicate samples could not be analyzed for Riverton noninvaded total N data because N concentrations were below detectable limits for one of the two subsamples. Nitrogen values for this set of data were

Table 1. Emergence (%), standard errors, and *t* test probabilities of four native species and Russian knapweed grown in soils from Russian knapweed invasions (Inv.) and from adjacent noninvaded soils (Non.) near Greybull and Riverton, Wyoming, and Greeley, Colorado. Within a site, bold values indicate significant differences between invaded and noninvaded positions (two group *t* test, $n = 4$, $\alpha = 0.05$).

	Greeley abandoned pasture				Greybull rangeland				Riverton rangeland			
	Inv. (%)	Non. (%)	SE	<i>P</i> value	Inv. (%)	Non. (%)	SE	<i>P</i> value	Inv. (%)	Non. (%)	SE	<i>P</i> value
Indian blanketflower	77	79	5.9	0.76	58	45	17.3	0.49	60	70	8.7	0.31
Purple prairie clover	70	65	15.4	0.81	42	13	18.6	0.17	51	47	13.8	0.81
Wyoming big sagebrush	41	40	11.8	0.96	14	6	2.6	0.02	38	38	12.3	0.98
Winterfat	16	13	5.5	0.67	7	3	4.8	0.48	10	10	4.4	0.94
Russian knapweed	38	34	6.4	0.61	32	28	11.1	0.74	37	39	7.6	0.81

interpolated using the regression equation $y = -0.002x^2 + 0.093 - 0.011$ ($r^2 = 0.99$), where $y = N$ (%) and $x = C$ (%). This regression equation was determined by regressing all the *C* and *N* values determined by micro-Dumas combustion.

RESULTS

Seedling Emergence

Wyoming big sagebrush seedling emergence (%) was greater in invaded than noninvaded soils from the Greybull site (Table 1). Seedling emergence did not differ between position (invaded versus noninvaded) at any site for any other species tested.

Growth of Native Herbaceous Species

Indian blanketflower shoot and total biomass were greater for plants grown in invaded soils from Greybull, and root biomass was greater in invaded soils in weeks 10 and 14 (Table 2). Biomass (shoot, root, and total) did not differ

between positions when plants were grown in Riverton or Greeley soils. Leaf area and leaf number were larger for plants when grown in invaded soils from Greeley. In Greybull and Riverton soils, leaf area and leaf number did not differ between positions.

Purple prairie clover shoot biomass was greater when plants were grown in Greeley soils invaded by Russian knapweed, and root biomass was greater in invaded soils at weeks 10, 12, and 14 (Table 2). Total biomass and leaf area of purple prairie clover did not differ between positions when grown in soils from any of the three sites. Purple prairie clover grown in soils from the invasions at Greybull and Greeley had more leaves, but leaf numbers did not differ between positions when grown in Riverton soils.

Growth of Shrub Species

Wyoming big sagebrush seedlings had greater shoot, root, and total biomass when grown in soils from inside the invasion at Greeley (Table 2). Biomass did not differ between positions for

Table 2. Growth parameters of four native species and Russian knapweed (across four harvest dates) when grown in soils from within the Russian knapweed invasion (Inv.) and from adjacent noninvaded areas (Non.) from Greeley, Colorado, USA, and Greybull and Riverton, Wyoming. Position means within a site for the same growth parameter in bold differ ($\alpha = 0.05$).

Species	Site	Shoot biomass (g)		Root biomass (g)		Total biomass (g)		Leaf area (cm ²)		Leaf no.	
		Inv.	Non.	Inv.	Non.	Inv.	Non.	Inv.	Non.	Inv.	Non.
Indian blanketflower	Greeley	0.11	0.06 ¹	0.05	0.03 ²	0.15	0.09 ³	4.9	4.7	6.48	3.98
	Greybull	0.10	0.05	0.05	0.05	0.15	0.10	5.1	5.0	6.65	6.21
	Riverton	0.08	0.07	0.03	0.02	0.11	0.09	4.4	3.9	4.55	2.57
Purple prairie clover	Greeley	0.03	0.02	0.08	0.04 ⁴	0.11	0.06	28.4	24.0	1.84	1.06
	Greybull	0.02	0.01	0.07	0.04	0.09	0.05	21.6	15.1	1.43	0.66
	Riverton	0.02	0.01	0.05	0.03	0.07	0.04	21.2	18.5	1.10	0.66
Wyoming big sagebrush	Greeley	0.05	0.03	0.06	0.03	0.11	0.06	51.5	37.0	2.86	2.27
	Greybull	0.06	0.07	0.05	0.04	0.11	0.10	51.1	50.2	3.32	3.34
	Riverton	0.03	0.02	0.04	0.03	0.07	0.04	40.3	26.3	2.01	0.97
Winterfat	Greeley	0.06	0.04	0.10	0.06	0.15	0.10	54.9	38.3	4.13	2.86
	Greybull	0.06	0.03	0.10	0.09	0.16	0.12	51.6	41.2	4.34	3.72
	Riverton	0.04	0.03	0.07	0.05	0.11	0.07	43.4	28.1	2.69	1.63
Russian knapweed	Greeley	0.11	0.07	0.05	0.04	0.16	0.10	6.6	6.7	6.51	5.06
	Greybull	0.11	0.09	0.05	0.05	0.16	0.14	6.8	6.2	7.13	6.74
	Riverton	0.07	0.06	0.03	0.02	0.10	0.07	6.2	5.0	4.96	2.38 ⁵

¹Means differed at week 14, Inv.—0.18, Non.—0.08.

²Means differed at week 10, Inv.—0.05, Non.—0.03; week 14, Inv.—0.06, Non.—0.04.

³Means differed at week 14, Inv.—0.24, Non.—0.11.

⁴Means differed at week 10, Inv.—0.06, Non.—0.03; week 12, Inv.—0.09, Non.—0.05; week 14, Inv.—0.13, Non.—0.07.

⁵Means differed at week 15, Inv.—6.78, Non.—2.61.

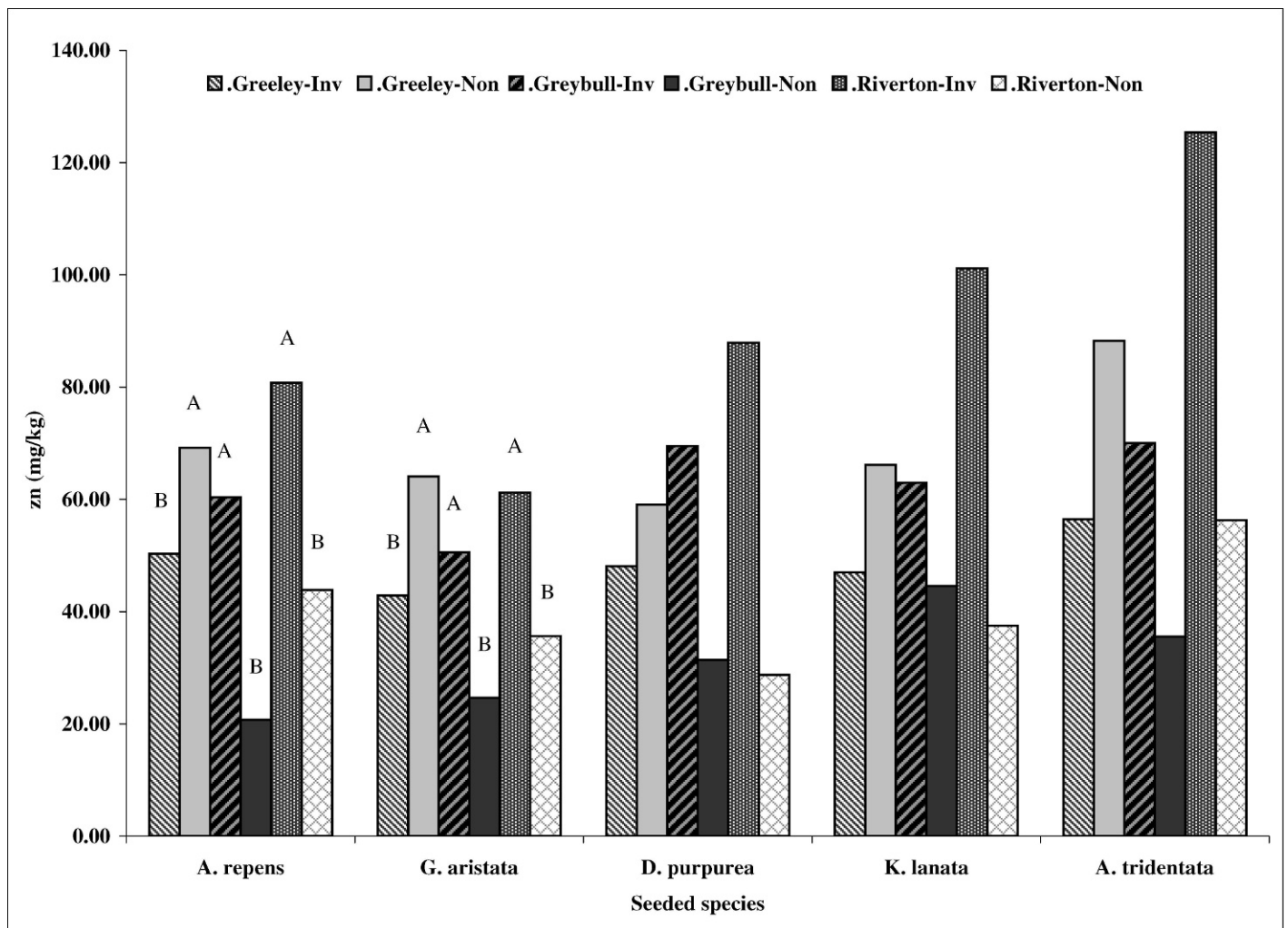


Figure 1. Zinc concentrations ($\text{mg} \cdot \text{kg}^{-1}$) of Russian knapweed and four native species grown in soils from Russian knapweed-invaded and adjacent noninvaded positions. Soil collection sites were two rangelands near Greeley and Riverton, Wyoming, and an abandoned pasture near Greeley, Colorado. Three species did not grow large enough to allow statistical comparison. Within each site, position means (invaded [Inv] versus noninvaded [Non]) with the same letter do not differ (two-group t test, $\alpha = 0.05$).

Wyoming big sagebrush grown in Greybull soils. In Riverton soils, Wyoming big sagebrush shoot biomass was greater when grown in soils from inside the invasion, and root and total biomass did not differ between positions. Sagebrush seedlings had greater leaf area when grown in invaded soils from Greeley, but leaf area did not differ between positions for seedlings grown in soils from Greybull and Riverton. Leaf number did not differ between positions for seedlings grown in soils from any of the three sites. Winterfat biomass, leaf area, and number of true leaves did not differ between positions when plants were grown in soils from any of the three sites (Table 2).

Russian Knapweed Growth in Invaded and Noninvaded Soils

In Greeley, Riverton, and Riverton soils, Russian knapweed seedling biomass and leaf area did not differ between positions (Table 2). Leaf number did not differ between positions for Russian knapweed grown in soils from Greeley and Greybull but was greater in Riverton invaded soils on the last harvest date.

Shoot Zinc Concentrations

Russian knapweed and Indian blanketflower shoot material contained greater concentrations of Zn when grown in invaded soils from the two rangeland sites (Greybull and Riverton; Fig. 1). Zinc concentrations in shoot material of seedlings grown in Greeley soils were lower in invaded areas. Shoot Zn concentrations of Wyoming big sagebrush, winterfat, and purple prairie clover followed the same trends as Russian knapweed and Indian blanketflower (not statistically analyzed). In general, plant Zn content of Russian knapweed was not especially high when compared to other species and in all species, plant Zn content was quite varied, depending on the soils in which seedlings were grown.

Soil Texture and Chemical Analyses

Soil texture of Greeley invaded and noninvaded soil was a loam, and soil from both positions at Riverton was sandy. Greybull invaded soil was a sandy clay loam whereas noninvaded soil was a silt loam. At Greeley, CEC, OM, N, C, Zn, and Fe were greater, EC and pH were lower in the invaded soil, and K, PO_4

Table 3. Chemical and physical characteristics of soils invaded by Russian knapweed and adjacent non-invaded soils near Greybull and Riverton, Wyoming, and Greeley, Colorado. Bold values indicate significant differences between invaded and non-invaded positions for a given site (two-group *t* test, $\alpha = 0.05$). EC indicates electrical conductivity; CEC, cation exchange capacity; and OM, organic matter.

	pH	EC dS · m ⁻¹	CEC cmol · kg ⁻¹	Clay	OM	N	C	C:N	K	Zn	Fe	Mn	Cu	PO ₄
				%						mg · kg ⁻¹				
Greeley														
Invaded	7.2	1.4	20.80	22.0	4.89	0.27	3.30	12.4	494.40	1.48	10.33	5.74	1.58	12.08
Noninvaded	7.8	1.8	25.37	22.5	5.56	0.33	3.95	12.2	411.60	1.60	8.33	7.33	1.57	9.93
Greybull														
Invaded	7.5	3.6	20.74	27.0 ¹	8.61	0.48	6.23	12.8	905.20	6.56	21.05	11.53	2.54	19.68
Noninvaded	8.1	18.2	14.25	21.0	2.53	0.16	1.88	12.5	777.60	0.61	6.50	6.30	1.20	26.54
Riverton														
Invaded	7.6	4.2	6.13	6.0	1.15	0.07	0.78	12.1	267.50	0.15	1.42	2.57	0.23	8.86
Noninvaded	8.1	0.6	4.79	6.5	0.44	0.02 ²	0.36	12.0 ²	95.50	0.06	1.95	0.79	0.28	3.52

¹Only two samples within each position (invaded and noninvaded) from each site were used to determine percent clay. No statistical analysis was performed on this data.

²One set of subsamples of Riverton noninvaded nitrogen data was interpolated from the regression equation $y = -0.002x^2 + 0.093 - 0.011$ ($r^2 = 0.99$).

and Mn did not differ between positions (Table 3). At Riverton EC, CEC, OM, N, C, K, Zn, Mn, and PO₄ were greater in invaded soils, and pH, Fe, and Cu were greater in noninvaded soils. There were no differences in soil physical properties and nutrients between invaded and noninvaded positions in Greeley soils except N and C were both greater in noninvaded soils. The C:N ratio was not different between positions at any of the three sites. Ratios of soil available:plant tissue Zn content were highly variable. Russian knapweed ratios ranged from 1:9 to 1:730. Native species were equally as variable: Wyoming big sagebrush ratios ranged from 1:10 to 1:938, and values for Indian blanket flower ranged widely (from 1:7 to 1:594), with purple prairie clover (1:11 to 1:585) and winterfat (1:10 to 1:625).

DISCUSSION

Because Russian knapweed is thought to exude chemicals that negatively affect growth of other species (Stevens 1986; Stermitz et al. 2003) we were surprised to observe similar or greater root and shoot biomass when seedlings were grown in invaded soils. This suggests that soils from Russian knapweed invasions were conducive to native seedling emergence and establishment.

However, we must note that disturbance in handling our soils may have duplicated tillage effects and reduced potential allelopathic impacts of Russian knapweed. No soil physical or chemical data were available for our field sites prior to the invasion of Russian knapweed. We did not test directly for allelopathic chemicals in the soil, and our soils were stored in the greenhouse for 3 weeks, sieved, and mixed. It is likely that these actions affected the phyto-activity of any chemicals produced by Russian knapweed. Consequently our soil-handling limits extrapolation of our results to field restoration. If we assume noninvaded areas were comparable to invaded areas prior to Russian knapweed introduction, our results at the rangeland sites (Greybull and Riverton) support the hypothesis that soils from Russian knapweed invasions have developed soil physical and chemical properties that differ from

adjacent noninvaded soils. In our study greater seedling growth was probably favored by lower pH and generally greater micronutrient concentrations within invaded soils. Although not specifically tested in this study, it appears that as Russian knapweed litter degrades, organic acids are produced that decrease the pH of the soil. Callaway et al. (2004) observed a similar reduction in pH in spotted knapweed invasions. High pH in arid soils may restrict Fe, Mn, An, and Cu availability to plants (Brady and Weil 2002), which may explain the higher plant-available concentrations observed within the invaded soils where the pH was reduced. Russian knapweed produces more litter than surrounding native grasslands which increases soil OM and C concentrations. At the rangeland sites, increased OM in the top 10 cm of the soil profile within Russian knapweed invasions agrees with Bottoms (2001).

Fewer differences occurred between treatments at the Greeley site, possibly because of lower Russian knapweed densities than at the rangeland sites. Soil collection areas in Greeley were located in a drainage surrounded by land farmed using a bean and corn rotation that may have been subject to fertilizer runoff from surrounding croplands. Finally, smooth brome on the site may have competed with Russian knapweed and contributed litter to the soils in both positions, further limiting position differences.

We did observe greater Zn concentrations within Russian knapweed invasions at both rangeland sites corresponding with results found on a study of Russian knapweed-invaded soils at a single site in Utah (Morris 2005; Morris et al. 2006). Our results agree well with Morris et al. (2006) and refute recent speculation that increased Zn concentrations beneath Russian knapweed plants inhibits the growth of other species (Bottoms 2001). Although higher Zn concentrations were present within soils invaded by Russian knapweed at both native rangeland sites and in seedlings grown in these soils, Zn concentrations did not attain toxic levels in either soil or shoot material. Our soil Zn concentrations inside naturally occurring field invasions were lower than those of Morris (2005) and were well below toxic levels documented elsewhere (Zhang et al. 1997). Available literature does not include Zn concentration data for Indian blanketflower and Russian knapweed, although

other herbaceous species have demonstrated tolerance at higher concentrations (20–200 mg · kg⁻¹; Jones et al. 1991) than observed in our seedlings (50–81 mg · kg⁻¹). Bottoms's (2001) characterization of Russian knapweed as a Zn hyper-accumulator is not supported by our study. Widely variable Zn soil-available to plant tissue content ratios were primarily driven by the hundred-fold range in soil-available Zn in our soils, demonstrating that this ratio is not a useful measure of hyper-accumulation of micronutrients in plants.

MANAGEMENT IMPLICATIONS

Timing of germination may be crucial to the success of native seedlings against the reinvasion of Russian knapweed. Indian blanketflower germinates and establishes before Russian knapweed seedlings (in our study, within the first week versus three weeks; Tyrer 2005), which suggests the native plants may obtain a slight competitive advantage with early seeding. Early germination and establishment of Indian blanketflower seedlings in our study and the species' resistance to Russian knapweed allelopathic chemicals (Stermitz et al. 2003) suggest its competitive potential. The species is also competitive with spotted knapweed (Callaway et al. 2004). Because purple prairie clover, Wyoming big sagebrush, and winterfat all incorporated more resources into root biomass than shoot biomass, common for some legume and shrub species (Lory et al. 1992; Jackson et al. 1996; Khan et al. 2002), these allocations to root growth may allow Russian knapweed seedlings with extensive shoots to shade out slower-growing seedlings of these native species. However, our study examines seedlings grown alone in soils rather than in direct competitive interactions with Russian knapweed, and so the competitive ability of these native species requires more direct study.

We are optimistic that revegetation of Russian knapweed-infested areas with native forbs is feasible. An integrated weed management program of Russian knapweed removal, and seeding with early-emerging, competitive native species, should increase restoration success. Mixing of soils (tilling) may disperse allelopathic chemicals (if present), distribute Russian knapweed leaf litter through the profile, and improve establishment of seedlings. However, tilling is not required to disperse toxic Zn concentrations in dense invasions. Where native species remain within invasions, tilling may disrupt soil structure and favor Russian knapweed return. Early emergence, rapid above-ground canopy development, and demonstrated resistance to the presence of Russian knapweed are important criteria for native species selection.

ACKNOWLEDGMENTS

We thank Drs S. F. Enloe, S. D. Miller, and L. J. Ingram for assistance on the project and suggestions on an earlier version of this manuscript. We appreciate assistance from numerous technicians and graduate students in the UW Shrubland Ecology Laboratory. We express special thanks to Dr D. E. Legg for statistical consultation and the land owners and managers for access to soil collection sites. This study was funded by grants from the US Department of Defense, Sustainable Environmental Research and Development Program, Sustainable Infrastructure SI-1389, and the National Research Initiative USDA-CSREES grant 2003-35320-13473.

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