ENHANCING NATIVE FORB ESTABLISHMENT AND PERSISTANCE USING A RICH SEED MIXTURE

by

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April 16, 2004

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ABSTRACT

Introducing and establishing desirable competitive forbs is crucial for successful invasive plant management and the re-establishment of a desirable plant community. The objectives of this study were: 1) To measure germination, dormancy, and viability of six native forbs, 2) To determine whether increasing forb seeding rate will yield an increase in forb establishment, 3) To examine the effects of a species mixture versus a single species on establishment and survival of desired species, and 4) To determine whether a mixture or a monoculture of forbs is more competitive with spotted knapweed. We hypothesized that high forb densities will occur at the highest seeding rate. We also hypothesized that seeding a species rich mixture of forbs will establish average densities of all the species seeded as monocultures, regardless of water frequency. Monocultures of purple coneflower, arrowleaf balsamroot, annual sunflower, dotted gayfeather, western white yarrow, sticky geranium and a mixture of all forbs were used to test emergence at two seeding densities, two watering frequencies and all were seeded with a background density of spotted knapweed. The highest seeding rate produced the highest plant densities, regardless of water frequency. The mixture yield about average of the individual plant densities and about doubled in response to the highest seeding rate, but was not influenced by watering frequency. Our final hypothesis stated that a mixture of forbs will be more competitive with spotted knapweed than a monoculture of purple coneflower. Spotted knapweed, purple coneflower, and a mixture of associated forbs were used as a model system to test our hypothesis. Multiple linear regressions predicting biomass was calculated using initial and final densities of both species. The forb mixture was 7 times more competitive with spotted knapweed than purple coneflower alone when using initial density. This study suggests using a mixture of forbs, rather than a single species, will enhance the likelihood of establishment in various and unpredictable environments because the group possesses a variety of traits that may match year to year and site to site environments, and once established the mixture may have a greater chance of persisting than a monoculture.

CHAPTER 1

ENHANCING NATIVE FORB ESTABLISHLMENT AND PERSISTENCE USING A RICH SEED MIXTURE

Introduction

Non-native invasive plants threaten the diversity, function, and utility of rangelands throughout the western United States and Canada (Sheley and Petroff 1999). Nonnative invasive plants continue to spread and dominate millions of hectares of range and wild land, costing several billion dollars each year (Pimentel 2002). In addition to controlling invasive weeds, a general objective for invasive plant management is to establish and/or maintain a healthy plant community that is relatively weed resistant, while meeting other land use objectives (Sheley et al. 1996). Invasive plant dominated rangelands are often void of desirable competitive species. Many weed control procedures open niches where desirable species are not available to occupy (Kedzie-Webb et al. 2002, Jacobs et al. 1998, James 1992). Introducing and establishing desirable competitive plants is crucial for successful invasive plant management and the re-establishment of a desirable plant community (Bottoms and Whitson 1998, Laufenberg 2003).

Species richness and diversity have been recognized as valuable contributors to ecosystem function and invasion resistance (McNaughton 1993, Vitousek and Hooper 1993, Naeem et al. 1994, McNaughton 1977, Chapin and Shaver 1985, Rejmanek 1989, Woods 1993, Naeem and Li 1997, Tilman et al. 1997). In Minnesota, greater plant diversity of grasslands increased the uptake of limited soil nitrogen (N) and decreased nitrate leaching (Tilman et al. 1996). A grassland community with high species richness produced 143% more biomass than the average biomass of all grassland monocultures (Spehn et al. 2000). Disturbed plant communities with high species diversity tend to resist negative impacts relative to communities with lower species diversity (Crawley 1987, Lawton and Brown 1993, Naeem 1998) and high species richness and diversity create plant communities important in early successional dynamics following disturbance (Elton 1958, Sheley et al. 1996). Frank and McNaughton (1991) found that grassland community composition with greater plant diversity was more stable in response to drought among eight grassland communities in Yellowstone National Park.

A number of researchers have suggested that the probability of plant invasion decreases as indigenous species diversity increases (McGrady-Steele et al. 1997, Tilman 1997), while others maintain that species-poor communities resist invasion more than diverse plant communities (Robinson et al. 1995, Levine and D'Antonio 1999, Stohlgren et al. 1999). Increasing species richness with niche differentiated species will increase resource use and increase niche occupation (Carpinelli 2004). The studies that actually quantified niche differentiation found invasion of spotted knapweed (*Centaurea maculosa* Lam.) decreased when species richness and niche occupation increased (Jacobs and Sheley 1999, Carpinelli 2000, Carpinelli 2004). More recently, Pokorny et al. (2004) found that the forb functional group was critical to invasion resistance of species with similar life history traits, such as spotted knapweed. Other research have found that increasing functional group diversity increased resource use (Hooper and Vitousek 1998) and increasing functional group diversity increase above ground biomass (Tilman et al. 1997). Establishing indigenous forbs during restoration of native plant communities is central to achieving diverse systems that function sustainably and resist invasion (Pokorny et al. 2004).

In most cases, revegetation or restoration is not included in weed management because of the high cost and risk of failure (Jacobs et al. 1998). Failures occur because of poor germination and emergence (Rose et al. 2003). Where revegetation or restoration is attempted, forbs are usually not included because propagation techniques are highly variable and largely unknown. In many cases, environmental conditions do not coincide with ecological requirements of individual species selected for establishment. For example, Wirth and Pyke (2003) found that only 8% of the seeds of *Astragalus purshii* Dougl. Ex Hook. emerged, while 38% of two *Crepis* species emerged across various site preparation treatments in a sagebrush-grassland habitat.

One method for enhancing forb establishment may be increasing the availability of seeds to increase the probability that they reach a safe site. Jacobs et al. (1996) found that bluebunch wheatgrass (*Pseudoroegenia spicata* (Pursh.) Love) was four times more competitive than spotted knapweed seedlings at densities of 1,000-5,000 plants/m^2, higher than normal for rangeland restoration. Because species differ in traits and ranges of tolerances, greater species richness may increase the likelihood of containing a species within a functional group that will germinate and emerge under varying and unpredictable environmental conditions (Tilman 1994). In addition, establishing multiple species mixtures may maximize resource uptake (Brown 1998, Carpinelli 2000, Pokorny et al. 2004).

Enduring invasive plant management requires the development of ecological strategies for establishing forbs during revegetation and restoration of invasive plant dominated rangeland. Our objectives were to determine: 1) germination, dormancy, and seed viability of seven species, 2) whether increasing forb seeding rate will yield an increase in forb establishment, 3) to examine the effects of a species mixture versus a single species on establishment and survival of desired species, and 4) whether a mixture of forbs is more competitive with spotted knapweed than a forb monoculture. Our overall hypotheses were: 1) high forb densities will occur at the highest seeding rate, 2) seeding a species rich mixture of forbs will establish average densities of all the species seeded as monocultures, regardless of watering frequency, because species with varying traits will average their response in varying moisture regimes, and 3) a mixture of forbs will be more competitive with spotted knapweed than a monoculture of purple coneflower (*Echinacea angustifolia* L.).

Materials and Methods

Germination, Dormancy and Viability Test

A germination test predicts the maximum performance of a seed if grown under ideal conditions. Four replications of 100 seeds per petri dish of each species were used to test the relative germination and dormancy of each species: spotted knapweed, purple coneflower, arrowleaf balsamroot (*Balsamorhiza sagittata* [Pursh] Nutt), annual sunflower (*Helianthus annuus* L.), dotted gayfeather (*Liatris punctata* Hook), western white yarrow (*Achillea millefolium* L.) and sticky geranium (*Geranium viscosissimum* Fisch). Each group of 100 seeds were placed on two sheets of pre-soaked Whatman No. 1 filter paper and spread evenly in 100 mm by 15 mm petri dish, the dishes were then covered with parafilm. For the germination test, seeds were sown on November 20, 2002 and counted between December 3, 2002 and December 4, 2002. Germination occurred in an alternate controlled environment set at 15º C at night and 25º C during the day in 16 hours in dark and 8 hours in light. Emergence of the radical was the indication of seedling emergence. After 2 weeks of germination, the germinated seeds were counted and removed from each petri dish. The remaining non-germinated seeds were then used in the dormancy test. Dormancy is a situation where a seed embryo fails to grow because of physiological or environmental limitations (Rees 1986). Seeds were placed in 100 mm by 15 mm petri dish lined with Kimpak® material, which was soaked in a 1% tetrazolium chloride solution. Seeds were incubated for 24 hours, respectively until red pigmentation of the embryo. Red pigmentation of the seeds was counted as dormant but viable seeds (AOSA 2000).

Seed viability was tested separately using the same procedure as described before with the seed dormancy test. Straight tetrazolium chloride was used to test seed viability. The test comprised of two replications of 100 seeds of each species. Seeds were placed in 100 mm by 15 mm petri dish lined with Kimpak® material, which was soaked in a 1% tetrazolium chloride solution. After seeds incubated for 24 hours, respectively red pigmentation of the embryo was recorded as viable seeds (AOSA 2000).

Data Analysis. Germination, dormancy and viability data was converted to percent. Difference among percent germination, dormancy, and viability data were tested using analysis of variance (ANOVA). Means was separated using Fisher's protected LSD comparisons at the 0.05 level of significance (Peterson 1985).

Emergence Study

This experiment was conducted in a plant growth room at the USDA-Agricultural Research Station in Burns, Oregon. Monocultures of purple coneflower, arrowleaf balsamroot, annual sunflower, dotted gayfeather, western white yarrow and sticky geranium and a mixture of all the forbs was used to test emergence of each individual species and the combination of forbs at two densities and under two watering frequencies. Seeding densities were 800 seeds/m^2 or 2000 seeds/m^2. Pots were misted with about 50 ml, respectively, of water twice (Tuesday and Friday) and three times (Sunday, Tuesday, Friday) per week beginning on December 12, 2003 and ending on February 11, 2004. This study included 28 treatments (2 seeding rates, 2 misting frequencies, 6 monocultures and 1 mixture). Pots were arranged in split-split plot design with seeding density and watering as whole-plots and monocultures or mixtures as subplots. The experiment was replicated 10 times.

Procedures. Pots were filled with "A" horizon soil from an Idaho fescue and bluebunch wheatgrass habitat type located at Placidea Butte about 58 km west of Burns, Oregon. Soils are classified as Madeline- Decantel Variant complex, which is a sandy loam (Lentz and Simonson 1986). The soil was saturated with water and allowed to equilibrate to pot capacity. Since invasive plants possess large and persistent seed-banks (Davis 1990), we seeded a background density 2000 seed/m^2 of spotted knapweed in all pots. Seeds of all species were manually broadcasted in plastic pots, each with a 1,540 mm^2 soil surface area and 111 mm depth. About 2 mm depth of soil was used to cover the surface of the soil. Room temperature was set at a constant 22° C. Plants were allowed to emerge and grow for 62 days.

Sampling. Species density was recorded by counting seedlings of all species in each pot. Species richness was determined for each mixture pot and total density was determined for each monoculture pot. Species richness was measured as the total number of species (purple coneflower, arrowleaf balsamroot, annual sunflower, dotted gayfeather, western white yarrow and sticky geranium) per experimental mixture pot. Total density was measured as the number plants that emerged in each monoculture pots.

Data Analysis. Seedling density data were analyzed using ANOVA for a split-split plot procedures described by Cody and Smith (1997). For the species richness data, the whole-plots were tested using their replication*main effect interaction as the error term. The interaction of seed density and watering was tested using the replication*seed rate*watering frequency as the error term. All other main effects or interactions were tested using the error term for the overall model. For total density, the whole-plots were tested using replication*seed rate effect interactions as the error term. For both species richness and total density the means were separated using Fisher's protected LSD comparisons at the 0.05 level of significance (Peterson 1985).

Survivorship

We used spotted knapweed, purple coneflower, and a mixture of purple coneflower and associated forbs (arrowleaf balsamroot, annual sunflower, dotted gayfeather, western white yarrow and sticky geranium) as a model system to test our hypotheses that a mixture would be more competitive than a monoculture against spotted knapweed. Varying densities and proportions of spotted knapweed and purple coneflower were arranged to provide one addition series matrix (Radosevich 1987). In another matrix, spotted knapweed was seeded with the mixture of forbs in the same densities and proportions. Densities of both matrices were factorially arranged 0:0, 0:100, 0:400, 0:700, 0:1000, 100:0, 100:100, 100:400, 100:700, 100:1000, 400:0, 400:100, 400:400, 400:700, 400:1000, 700:0, 700:100, 700:400, 700:700, 700:1000, 1000:0, 1000:400, 1000:700, 1000:1000 seeds per pot based on pure live seeds. Initial density ranged from 0 to 1,200 plants/m^2, while final density ranged from 0 to about 1,000 plants/m^2. Each matrix and density combination was replicated four times, and pots were completely randomized and placed in a growth room.

Procedures. Seeds were sown in 1,824 mm^2 (surface area) x 400 mm (depth) polyvinyl chloride tubes from November 7, 2002 to November 12, 2002. Tubes were filled non-pasteurized soil that had been sieved through a 2 mm screen. The soil was a Calcidic arguistoll, which is a Varny clay loam (consisting only of A horizon soil) from Red Bluff Research Ranch, located near Norris, Montana. The soil was saturated with water and allowed to equilibrate to capacity. Seeds were broadcast on the soil surface and manually arranged until a uniform distribution was achieved. A small amount (< 2 mm depth) of dry soil was used to cover the seeds. Soil was evenly misted on alternate days until emergence. Tubes were placed randomly in an environmental chamber (10° C, 12-hour day length, 500µE/m^2/s, spectral light). Conditions of this study were within the range of those found during establishment of forbs within a bluebunch wheatgrass/Idaho fescue habitat type (Mueggler and Stewart 1980).

Sampling. Three weeks after initial seeding, density by species was counted in each pot to determine initial emergence density.

Data Analysis. Data were incorporated into simple linear regression models using initial density to predict final density for purple coneflower and the mixture at each spotted knapweed density (0, 100, 400, 700, 1000 plants/m^2). Differences in intercepts ($ß_0$) and survivorship rates ($ß_i$) were determined by calculating regression coefficients for each replication (n = 4) and comparing Beta coefficients for purple coneflower with that of the mixture using ANOVA (Neter et al. 1989).

Competition

After collecting initial density data from the survivorship experiment, we continued to let the seedlings grow for another 64 days. No further misting or watering occurred after emergence. Plants were allowed to grow for 85 days after seeding.

Sampling. Density was also counted at the end of the experiment (final density). Plants were harvested at ground level and separated by species on day 85. Plant material were then dried for 1-week at 60º C and weighed (g).

Data Analysis. Data were incorporated into multiple linear regression models using initial and final density of each species to predict their biomass (Spitters 1983). Regressions predicted the biomass of an isolated individual of purple coneflower, forb mixture and spotted knapweed as dependent variables using initial and final densities of purple coneflower, forb mixture and spotted knapweed as independent variables. The predictor variable was the measured densities, and the response variable was biomass per plant. R^2 from the three individual regressions were evaluated to determine the most suitable model (Spitters 1983). The regressions were of the form:

$$y_s = \beta_{0s} + \beta_{sp}N_p + \beta_{sm}N_m + \beta_{ss}N_s$$

$$y_m = \beta_{0m} + \beta_{mp}N_p + \beta_{mm}N_m + \beta_{ms}N_s$$

$$y_p = \beta_{0p} + \beta_{pp}N_p + \beta_{pm}N_m + \beta_{ps}N_s$$

where y_s, y_m and y_p are the response of each species (average weight or biomass of spotted knapweed ,forb mixture and purple coneflower), β_{0s}, β_{0m}, and β_{0p} is the y-intercept (intercept as the weight of individuals in a pot), $\beta_{ss}N_s$, $\beta_{mm}N_m$, and $\beta_{pp}N_p$ is the product of the coefficient of intraspecific competition of species *s, m,* and *p* and its density (N_s, N_m, and N_p), and β_{sp}, β_{sm},β_{pm}, β_{ps}, β_{mp}, and β_{ms} and its density (N_s, N_m, and N_p). Since the data was not transformed, a positive response denotes positive interference, and a negative response denotes negative interference. The relative competitive ability of each species is calculated as:

$$RC_s = \beta_{ss}/\beta_{pm}$$

$$RC_m = \beta_{mm}/\beta_{ms}$$

$$RC_p = \beta_{pp}/\beta_{ps}$$

where RC_s, RC_m and RC_p are the relative competitive abilities of all the species on species *s*, *m* and *p*. Relative competitive abilities of each species are used to calculated niche differentiation (Spitters 1983):

$$ND_{pvs.m} = RC_p / RC_m$$

$$ND_{mvs.s} = RC_m / RC_s$$

$$ND_{pvs.s} = RC_p / RC_s$$

where ND = niche differentiation. Niche differentiation increases as ND departs from unity; that is, species *s, m* and *p* are decreasingly limited by the same resources. Non-significant competition coefficients indicate complete niche differentiation in which there is no interaction between species.

Results

Germination

There were significant differences between species in percent germination, with a Fisher's $LSD_{(0.05)}$ of 3.62 (Figure 1). Arrowleaf balsamroot had the lowest germination, which was about 2%, followed by sticky geranium seeds, with a 14% germination rate. Annual sunflower germination was about 22%. Purple coneflower and dotted gayfeather seeds had 47% and 60% germination, respectively. Western white yarrow and spotted knapweed seeds had the highest germination rates, at 95% and 98%, respectively.

Dormancy

Seeds that did not germinate were used to quantify dormancy (Figure 2). Spotted knapweed had zero dormancy: 98% of the seeds germinated and about 2% of the seeds contained empty seed coat. The same was true for western white yarrow seeds, which had 95% germination, 1% of the seeds were dormant and about 4% contained empty seed coat. Of the non-germinated purple coneflower, only 23% of the seeds were dormant. Annual sunflower, dotted gayfeather, and sticky geranium all averaged about 33% dormancy of the non-germinated seeds. Of the un-germinated arrowleaf balsamroot seeds, about 77% were dormant.

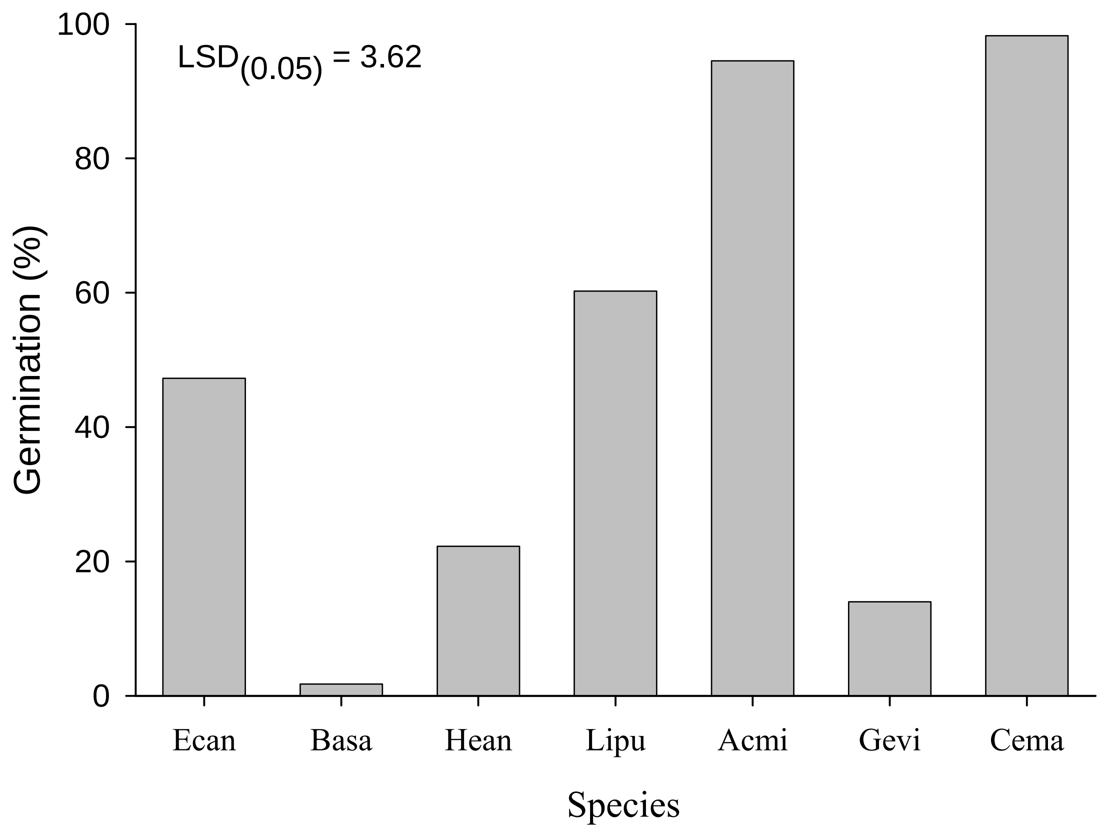


Figure 1. Comparison of the percent germination of purple coneflower (Ecan), arrowleaf balsamroot (Basa), annual sunflower (Hean), dotted gayfeather (Lipu), western white yarrow (Acmi), sticky geranium (Gevi), and spotted knapweed (Cema).

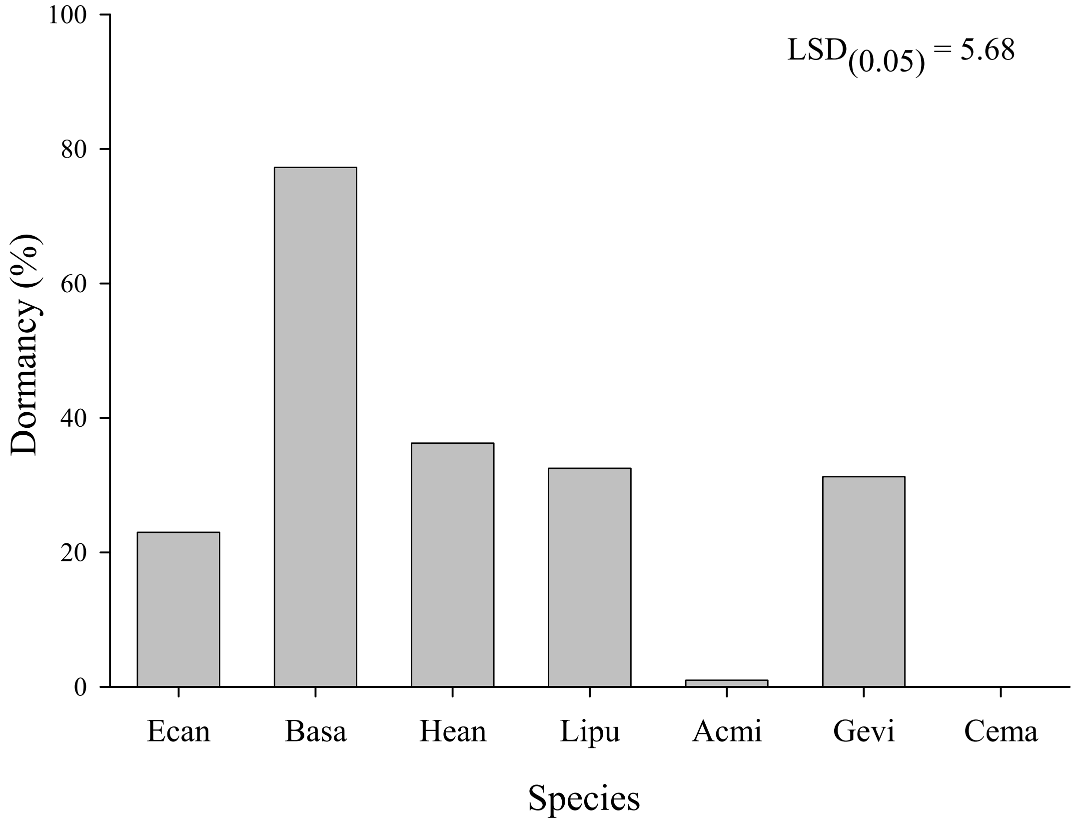


Figure 2. Comparison of the percent dormancy of purple coneflower (Ecan), arrowleaf balsamroot (Basa), annual sunflower (Hean), dotted gayfeather (Lipu), western white yarrow (Acmi), sticky geranium (Gevi), and spotted knapweed (Cema).

<u>Viability</u>

Species also varied in seed viability (Figure 3). The species with the least viable seed percentages were arrowleaf balsamroot (18%), sticky geranium (30%) and annual sunflower (49%). Dotted gayfeather and purple coneflower both had moderately viable seeds, which was 70 and 71 percent, respectively. Western white yarrow and spotted knapweed seeds were 94 and 97 percent viable, respectively.

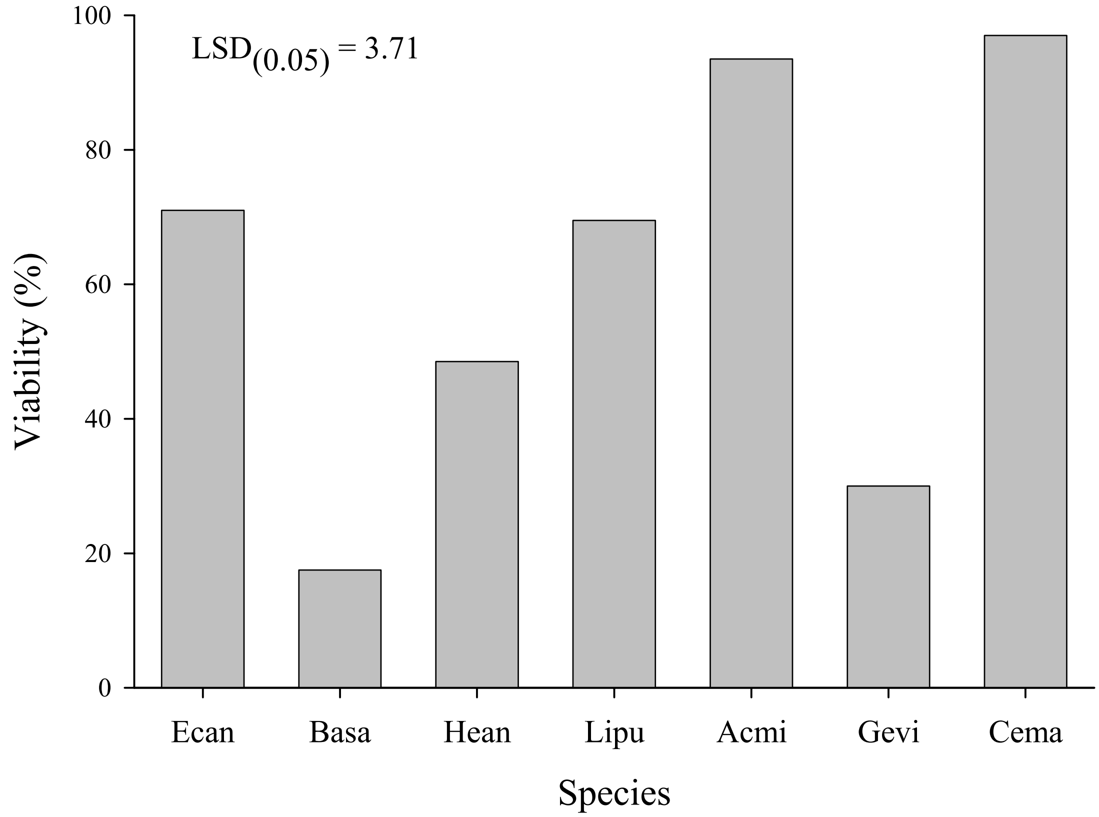


Figure 3. Comparison of the viability based on tetrazolium chloride test of purple coneflower (Ecan), arrowleaf balsamroot (Basa), annual sunflower (Hean), dotted gayfeather (Lipu), western white yarrow (Acmi), sticky geranium (Gevi), and spotted knapweed (Cema).

Emergence

Total Density. The influence of seeding rate on density depended upon the watering frequency (Table 1). Seeding at 800 seeds/m^2, while watering twice produced about 0.5 seedlings per pot (Figure 4). Increasing water from two to three times per week increased seedling density to 2.2 plants/pot at the same seeding rate. The highest seeding rate produced the highest seedling densities, which averaged 4.35 plants per pot, regardless of water frequency.

Table 1. Significant p-values from ANOVA for total density.

Dependent Variable	df	Pr > [t]
Seed rate*Water frequency	1	0.005
Seed rate*Species (mixture)	6	< 0.0001
Water frequency*Species (mixture)	6	0.064

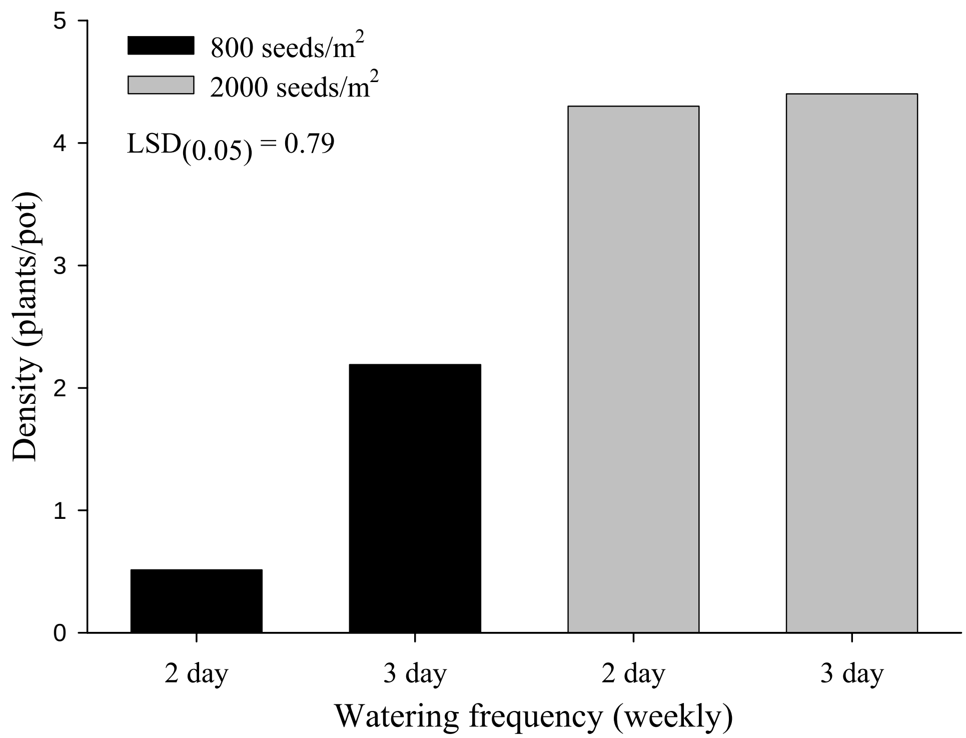


Figure 4. The influence of watering frequency and seeding rate on density across all species, except spotted knapweed.

The effects of seeding rate or watering frequency on plant density depended upon the particular species or mixture (Table 1). Seeding at 2000 seeds/m^2, increased the seedling density of most species by three- or four-fold over those pots seeded with 800 seeds/pot (Figure 5). The exceptions were arrowleaf balsamroot, which did not establish, and sticky geranium, which did not show a significant increase in establishment with a higher seeding rate. The mixture yielded about average of the individual plant density and about doubled in response to the highest seeding rate.

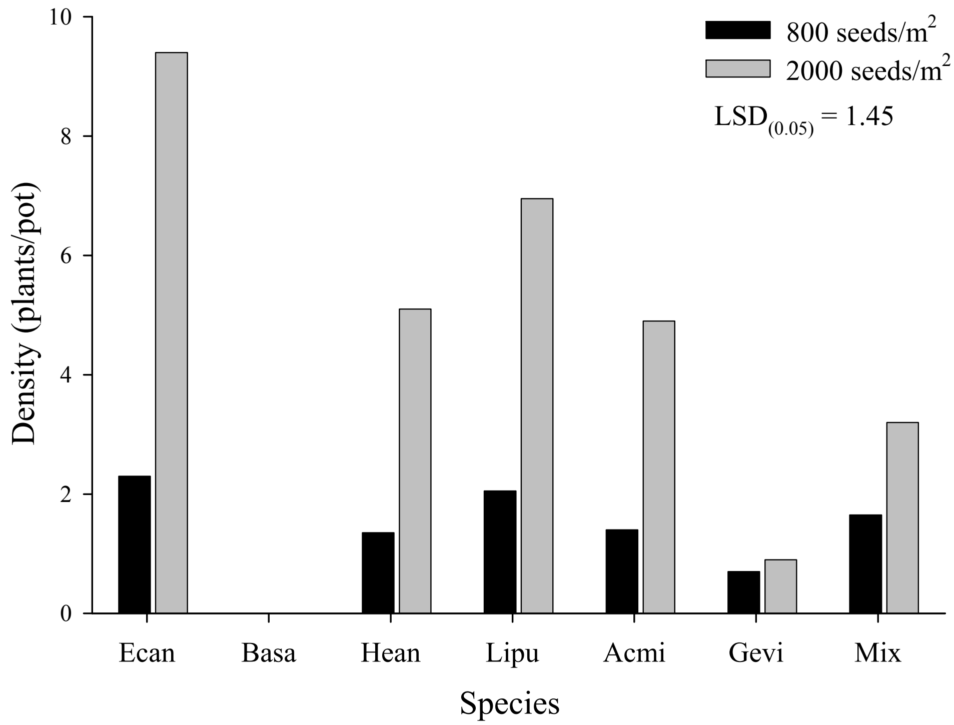


Figure 5. The influence of seeding rate on purple coneflower (Ecan), arrowleaf balsamroot (Basa), annual sunflower (Hean), dotted gayfeather (Lipu), western white yarrow (Acmi), sticky geranium (Gevi), spotted knapweed (Cema), and the mixture on desired forbs density.

Watering frequency had no effect on arrowleaf balsamroot (0 plants/pot) and sticky geranium (0.8 plants/pot), both of which produced the lowest plant densities and both densities of the plants were lower than the pots seeded with the mixture (Figure 6). Annual sunflower and western white yarrow produced intermediate emergence densities, averaged across watering frequency, ranging from 2.4 to 3.9 plants/pot. Plant densities of the species were lower than those of purple coneflower when watered twice weekly. In the most frequent watering regime, purple coneflower yielded the highest density, which was 7.3 plants/pot. The mixture produced 2.3 and 2.6 plants/pot when watered 2 or 3 days per week, respectively.

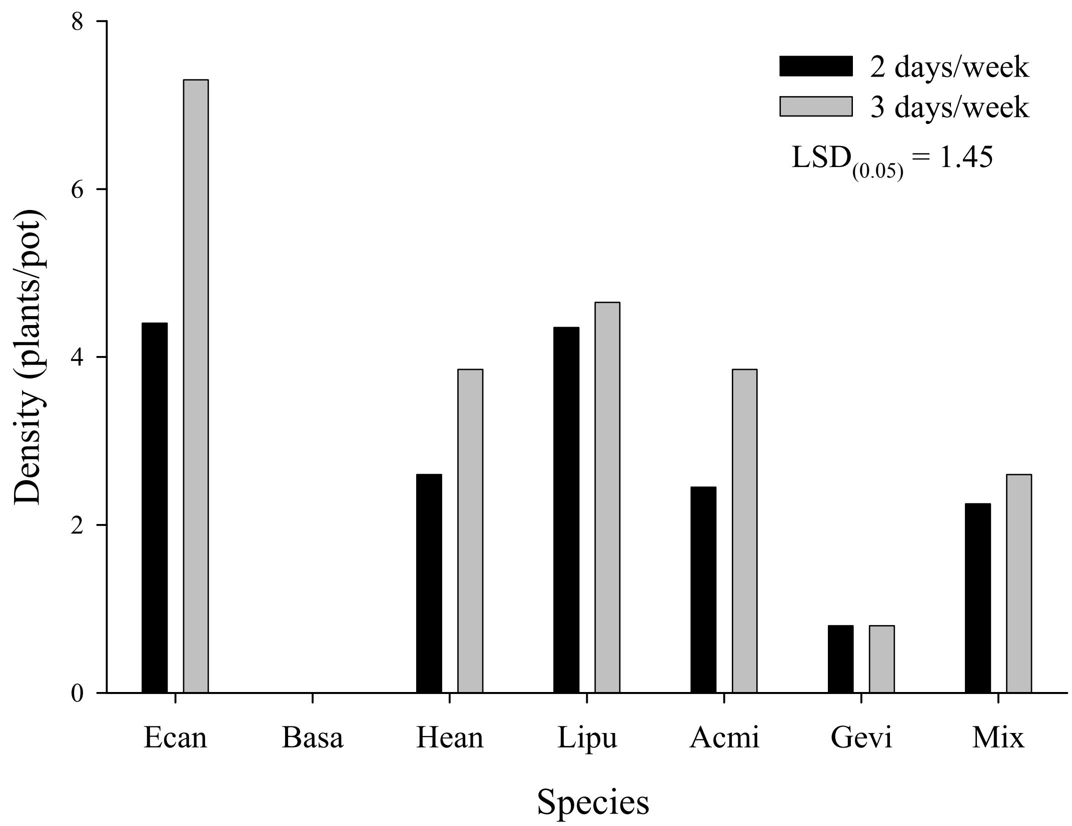


Figure 6. The influence of watering frequency on purple coneflower (Ecan), arrowleaf balsamroot (Basa), annual sunflower (Hean), dotted gayfeather (Lipu), western white yarrow (Acmi), and sticky geranium (Gevi), on desired forbs density.

Species Richness. Seeding rate affected species richness, with seeding at 800 seeds/m^2 producing 1.1 plant/pot, and seeding at 2000 seeds/m^2 producing 2.1 plants/pot (Figure 7). The p-value for the seed rate effect on species richness was 0.002 with 1 degree of freedom.

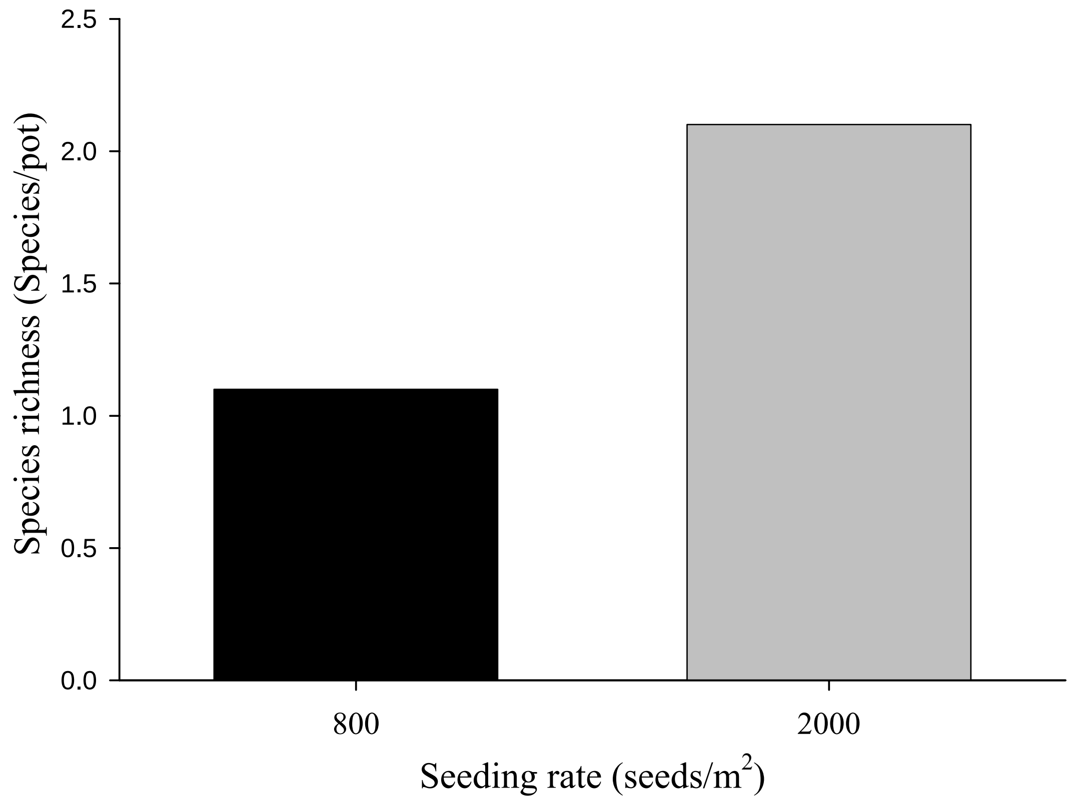


Figure 7. The influence of seeding rate on species richness.

Survivorship

Regression and ANOVA indicated that purple coneflower and the mixture had similar intercepts, regardless of the background density of spotted knapweed (Figure 8). The only exception was that purple coneflower had a higher intercept than the mixture where spotted knapweed was not seeded. All intercepts approached zero, except purple coneflower in pots not seeded with spotted knapweed, where the final density was about 200 plants/m^2.

Based on the Beta coefficient, there was a positive relationship between initial and final density for purple coneflower and the mixture in all cases (Figure 8).

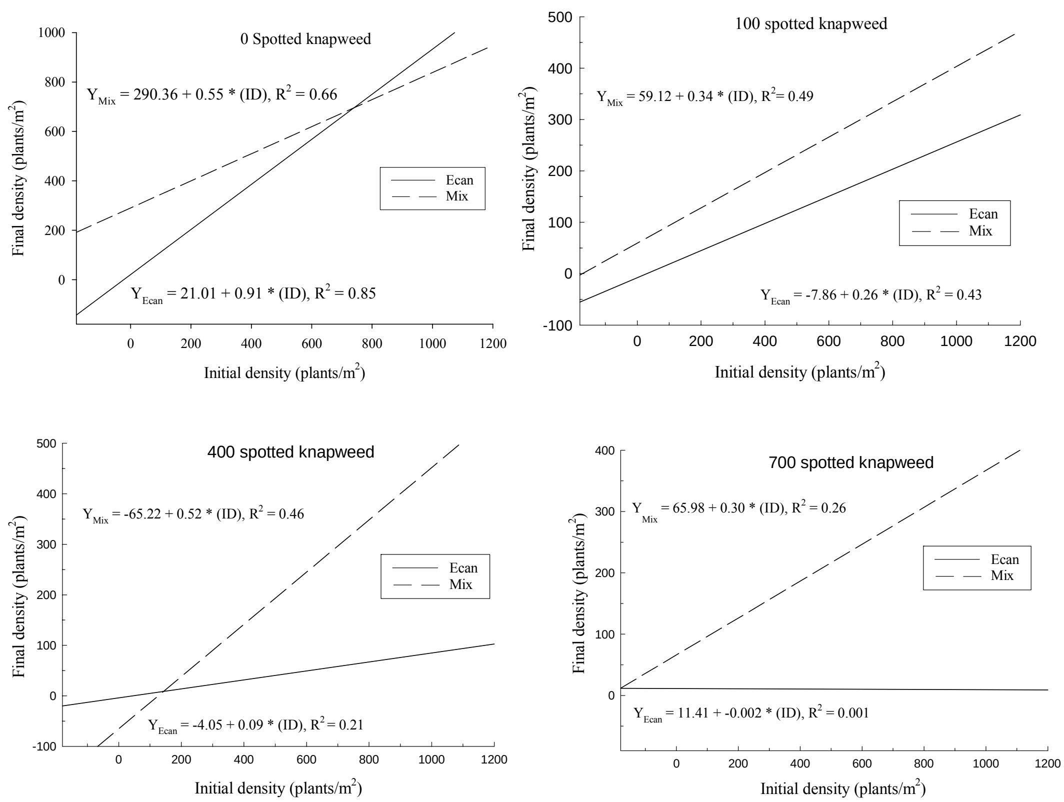
Survivorship of purple coneflower and the mixture did not significantly differ where either zero or 100 seeds per pot of spotted knapweed were seeded as a background. In pots where spotted knapweed was seeded with 400, 700, or 1000 seeds, survivorship was higher for the mixture than for purple coneflower. For example, at 400 spotted knapweed seeds per pot, it required 6 (Beta= 0.09) initial plants of purple coneflower for a single plant at the final count, whereas a single surviving plant resulted from two plants of the mixture (Beta=0.52).

Competition

Initial Density vs. Biomass. The maximum predicted biomass of an isolated individual was 183.5, 12.2, and 46.7 g/plant for spotted knapweed, purple coneflower, and the forb mixture (treated as an isolated individual for the analysis), respectively (Table 2). Adding a single purple coneflower reduced spotted knapweed biomass by 0.0038 g/plant. A single increase in the forb mixture reduced spotted knapweed by 0.0295 g/plant. Adding an individual spotted knapweed plant increased its own biomass by 0.0118 g/plant. Adding a purple coneflower plant increased its own biomass by 0.0366 g/plant. A single increase in the forb mixture reduced purple coneflower by 0.0057 g/plant. Adding an individual spotted knapweed decreased purple coneflower by 0.0015 g/plant. Adding an individual purple coneflower decreased the forb mixture by 0.0455 g/plant. A single increase of a forb mixture increased the forb mixture by 0.066 g/plant. Adding an individual spotted knapweed plant reduced the forb mixture by 0.005 g/plant.

Table 2. Multiple regression analysis for the prediction of biomass (g/plant) using their initial density.

Dependent Variable Biomass (g/plant)	Intercept $ß_0$	Purple coneflower $ß_1$	Forb mixture $ß_2$	Spotted knapweed $ß_3$	R^2
Spotted knapweed	183.5	-0.0038	-0.0295	0.0118	0.38
SE	(12.09)	(0.02)	(0.008)	(0.001)	
Purple coneflower	12.20	0.0366	-0.0057	-0.0015	0.28
SE	(3.47)	(0.005)	(0.002)	(0.0004)	
Forb mixture	46.70	-0.0455	0.066	-0.005	0.48
SE	(8.75)	(0.01)	(0.006)	(0.0009)	



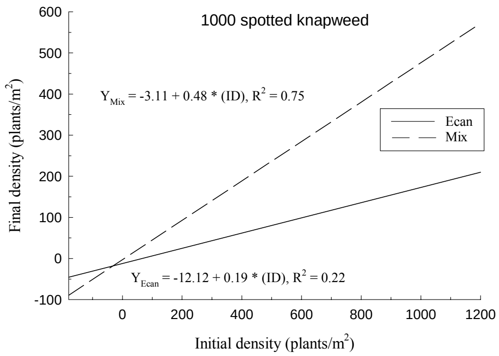


Figure 8. Regressions comparing survivorship of purple coneflower (Ecan) and a mixture of forbs (Mix). Regression coefficients by the same letter indicate they are not significantly difference at $\alpha = 0.05$.

<u>Final Density vs. Biomass</u>. The maximum predicted biomass of an isolated individual was 176, 31.2, and 4.3 g/plant for spotted knapweed, forb mixture (treated as an isolated individual for the analysis), and purple coneflower, respectively (Table 3). Adding an individual purple coneflower plant decreased spotted knapweed biomass by 0.06 g/plant. A single increase in the forb mixture reduced spotted knapweed by 0.07 g/plant. Adding an individual spotted knapweed plant increased spotted knapweed biomass by 0.04 g/plant. Adding a purple coneflower plant increased purple coneflower biomass by 0.1 g/plant. A single increase of the forb mixture reduced purple coneflower by 0.004 g/plant. Adding an individual spotted knapweed decreased purple coneflower by 0.001 g/plant. Adding an individual purple coneflower decreased the forb mixture by 0.06 g/plant. A single increase of a forb mixture increased the forb mixture by 0.14 g/plant. Adding an individual spotted knapweed plant reduced the forb mixture by 0.01 g/plant.

Table 3. Multiple regression analysis for the prediction of biomass (g/plant) using their final density.

Dependent Variable Biomass (g/plant)	Intercept $ß_0$	Purple coneflower $ß_1$	Forb mixture $ß_2$	Spotted knapweed $ß_3$	R^2
Spotted knapweed	176.03	-0.06	-0.07	0.04	0.64
SE	(10.58)	(0.02)	(0.01)	(0.003)	
Purple coneflower	4.26	0.10	-0.004	-0.001	0.79
SE	(2.15)	(0.004)	(0.002)	(0.0006)	
Forb mixture	31.22	-0.06	0.14	-0.01	0.74
SE	(7.11)	(0.01)	(0.007)	(0.002)	

Niche Differentiation. The double ratio (niche differentiation) analysis indicates that resource partitioning occurred with respect to initial and final density to the total biomass of purple coneflower vs. mixture, mixture vs. spotted knapweed and purple coneflower vs. spotted knapweed (Table 4 and 5). Ratio values ranged from 5.3 to 75.8.

Table 4. Double ratio assessing niche differentiation for initial density. The greater the distance from unity, the greater the differentiation.

Dependent Variable	Niche Differentiation
Purple coneflower vs. Forb mixture	-9.3
Forb mixture vs. Spotted knapweed	5.3
Purple coneflower vs. Spotted knapweed	75.8

Table 5. Double ratio assessing niche differentiation for final density. The greater the distance from unity, the greater the differentiation.

Dependent Variable	Niche Differentiation
Purple coneflower vs. Forb mixture	66.7
Forb mixture vs. Spotted knapweed	8
Purple coneflower vs. Spotted knapweed	58.1

Discussion

Traditionally, species choice for seeding during revegetation has focused on establishing a single species, typically grasses for livestock production (Mueggler and Stewart 1980). Where mixtures are recommended, they often only include grasses (Borman et al. 1991). Sustainable rehabilitation and restoration must include forbs because they play important roles in nutrient cycling and energy flow (Pokorny et al. 2004), and they provide resistance to invasion (Carpinelli 2000, Pokorny 2002). However, establishing forbs is very difficult because of their individual propagation characteristics (Rose et al. 2003, Stringer 2003).

We found evidence supporting our hypothesis that seeding a rich mixture of species would provide average, but consistent seedling establishment. In our study, two species had lower establishment than the mixture, two species had similar density as the mixture, and two species had higher density than the mixture. Although some species had lower emergence in the driest regime, the establishment within the mixture was not influence by watering. This suggests that without *a priori* knowledge of species germination and establishment characteristics, or stable weather patterns, a mixture may provide consistent forb establishment. Seeding success using a single species relies upon the quality of the knowledge about the propagation of that species. A mixture may possess a variety of requirements for germination and emergence so at least one or two species traits will match current year's conditions.

Increasing either water frequency or seeding rate increased establishment. Although their study only considered grasses, Sheley et al. (1999) found that increasing seeding rates from 500 seeds/m^2 where no establishment occurred to 2,500 and 12,500 seeds/m^2 increased tiller density to 80 and 140 plants/m^2, respective at one site and 158 and 710 plants/m^2, respectively, at another site two years after seeding. In our study, increasing the seeding rate about 2.5 times, increased emergence of four species 3- to 4-fold. Increasing the seeding rate of the mixture doubled the density of forbs. It appears that increasing the seeding rate enhances the likelihood that a seed reaches a safe site (Sheley et al. in press). Conversely, we believe that increasing watering frequency increased the availability of safe sites for germination and emergence.

Data on germination, dormancy and viability are often provided to restorationists in hopes of allowing the prediction of emergence of each species (Sheley et al. 1993). However, other studies focusing on forbs have found little relationship between germination or viability of each species and their emergence (Wirth and Pyke 2003). In our study, low germination of arrowleaf balsamroot and sticky geranium indicated poor establishment, which occurred. Conversely, high germination and/or viability did not necessarily translate directly to higher establishment. Therefore, we believe the ability to predict forb emergence from germination, dormancy, and viability data is unlikely (Rose 2003).

Comparison of survival rates of the mixture versus purple coneflower revealed that seeding a mixture had substantial benefits for enhancing the final density of forbs, especially if high densities of invasive weeds are present in the seed bank (Davis 1990).

In this case, we believe that as competition became more intense, the ability to acquire resources from multiple niches within the forb functional group allowed them to survive, similar to that found by Carpinelli (2000).

Seeding a diverse mixture of species, including alfalfa, spotted knapweed was more competitive than the desired species during the first two years of establishment (Carpinelli et al. 2004), but the desired mixture ultimately dominated the site 7 years later (Sheley et al. in press). We found partial evidence that a mixture of species would be more competitive with spotted knapweed during establishment. The influence of the forb mixture on spotted knapweed biomass was 7 times that of purple coneflower alone using initial density to predict biomass. However, using final density to predict biomass the two coefficients were nearly the same. In both analyses, the mixture had greater niche overlap than purple coneflower alone, suggesting that the mixture was using resources more similarly to spotted knapweed.

It is critical that forbs are part of species mixtures, if revegetation is to be sustainable (Pokorny 2002). However, forbs are often not included in seed mixtures, despite their importance, because of their difficulty of establishment. We believe that using a mixture of forbs, rather than a single species will enhance the likelihood of establishment in various and unpredictable environments because a group of forbs may possess a range of traits that may match year-year and site-site environments. Furthermore, once a higher richness of forbs is established, they may be more competitive with invasive weeds, and therefore, persist longer.

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