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Rehabilitating Downy Brome (*Bromus tectorum*)–Invaded Shrublands Using Imazapic and Seeding with Native Shrubs

Suzanne M. Owen, Carolyn Hull Sieg, and Catherine A. Gehring*

Rehabilitation of downy brome–infested shrublands is challenging once this invasive grass dominates native communities. The effectiveness of imazapic herbicide in reducing downy brome cover has been variable, and there is uncertainty about the impacts of imazapic on native species. We used a before-after-control-impact (BACI) field experiment and greenhouse studies to (1) determine if imazapic herbicide applied at 132 g ai ha^{-1} (8 oz/ac^{-1}) and seeding with two native shrub species (Wyoming big sagebrush [*Artemisia tridentata*] and Mexican cliffrose [*Purshia mexicana*]) reduced downy brome cover and promoted shrub establishment, (2) assess potential effects of imazapic on nontarget plant species and plant community composition, and (3) determine if imazapic affected downy brome or seeded shrub species when applied at different developmental stages. Seeding shrubs, alone, or in combination with imazapic application, did not significantly increase shrub density, possibly because of droughty conditions. In the field, imazapic reduced downy brome cover by 20% and nontarget forb cover by 25% and altered plant community composition the first year after treatment. Imazapic was lethal to downy brome at all growth stages in the greenhouse and reduced shrub germination by 50 to 80%, but older shrub seedlings were more tolerant of the herbicide. We conclude that a one-time application of imazapic combined with seeding shrubs was only slightly effective in rehabilitating areas with high downy brome and thatch cover and resulted in short-term impacts to nontarget species. These results highlight the need to treat downy brome infestations before they become too large. Also, removing thatch prior to treating with imazapic, although likely lethal to the native shrubs we studied, could increase the effectiveness of imazapic.

Nomenclature: Imazapic; downy brome, *Bromus tectorum* L.; Mexican cliffrose, *Purshia mexicana* (D. Don) Henrickson; Wyoming big sagebrush, *Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young. Nomenclature of all plants follows the USDA-NRCS PLANTS database (http://plants.usda.gov/), and common names of invasive plant species follow the WSSA database (http://www.wssa.net/Weeds/ID/WeedNames/namesearch.php).

Key words: Exotic species management, herbicide, nontarget species, rangeland seeding.

One of the most invasive and widespread exotic plants in North America is downy brome (*Bromus tectorum* L.) (Chambers et al. 2007; Young et al. 1987). Downy brome has invaded over 22 million ha in the western United States (Duncan et al. 2004). As an annual grass, it can use moisture and nutrients before perennial plant growth initiates in the spring, limiting native plant survival (Williams et al. 2002; Young et al. 1987). Downy brome can reduce soil nutrient availability (Evans et al. 2001) and alter soil community composition (Belnap and Philips 2001). Downy brome expansion has increased fire frequencies in some rangelands from a historical frequency of 30 to 100 yr to $<$ 5 yr because it provides a continuous cover of fine fuel that dries early in the growing season (Whisenant 1990). In particular, big sagebrush (*Artemisia tridentata* Nutt.) and pinyon–juniper (*Pinus–Juniperus*) ecosystems are experiencing more frequent and severe fires related to downy brome invasions (Keane et al. 2008). Downy brome is able to persist under this more-frequent fire regime, but other species, such as big sagebrush, can be eliminated with frequent fires. Many big sagebrush communities are being converted to downy brome–dominated grasslands (Eiswerth et al. 2009). Ungulates

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Interpretive Summary

Downy brome is a highly invasive annual grass that can outcompete native plant species and increase the frequency of fires. We examined if treatment with imazapic herbicide and/or seeding with native shrubs was effective in rehabilitating shrublands highly invaded by downy brome. We also determined the effects of imazapic on different growth stages of both downy brome and three shrub species in the greenhouse. Imazapic was lethal to downy brome at all growth stages in the greenhouse, yet only reduced downy brome cover by 20% for one season in the field, likely due to high thatch cover and the large amount of available downy brome seeds. Imazapic also altered plant community composition and reduced forb cover by 25% in the field the first year after treatment. Shrub establishment was low in all seeding treatments, probably because of droughty conditions. Shrub germination was reduced in the greenhouse by up to 80% by imazapic, yet remnant shrubs in the field and 4-mo-old shrubs in the greenhouse were tolerant of the herbicide, leading us to recommend that imazapic be applied after shrubs are established instead of at the time of seeding. We conclude that a one-time imazapic application and seeding treatment was minimally effective in reducing downy brome or increasing shrub density, but fortunately, the disturbances associated with seeding did not increase downy brome populations. Removing thatch before treating with imazapic, although likely lethal to the native shrubs we studied, could increase the effectiveness of imazapic. Because imazapic can alter native plant communities, we recommend further research on the unintended consequences of this herbicide and the consideration of other strategies for downy brome control, such as seeding native plant barriers and using herbicides that selectively reduce downy brome seed viability.

and other species dependent on mixed sagebrush communities are at risk because of the loss of forage and habitat (Knick et al. 2003; Lambert et al. 2005).

Rehabilitation of downy brome–infested shrublands is challenging because seeding shrubs may not be effective, and efforts to control downy brome may damage remnant shrubs and other native species. Success of shrub seeding often depends on precipitation patterns, but can be enhanced by using locally collected seeds (Lysne 2005; McKay et al. 2005) and by reducing competing exotic plant cover (Eisworth et al. 2009; Morris et al. 2009; Shaw et al. 2005). Although downy brome can be temporarily set back using prescribed fire, mowing, and grazing, these strategies rarely control populations and can result in high mortality of some native species that managers aim to restore (Lambert 2005). Imazapic herbicide has been used with varying success for treating downy brome infestations (Baker et al. 2009; Davidson et al. 2007; Morris et al. 2009). Imazapic is an imidazolinone chemical herbicide that inhibits the enzyme acetohydroxyacid synthase (AHAS), which is needed for cell growth (Shaner 1991). Imazapic temporarily reduced downy brome cover and facilitated establishment of seeded grass species in one study (Morris et al. 2009). However, there is less certainty about the rehabilitation success of using imazapic and seeding with locally collected shrub species in shrublands highly invaded with downy brome. Vollmer and Vollmer (2008) and Davidson and Smith (2007) found that imazapic did not harm established sagebrush species, but little is known about the effect of imazapic on shrub germination. Retention of remnant foundation shrub species (Prevéy et al. 2010) and maintenance of native plant species biodiversity are increasingly recognized as key objectives of restoration.

Studies on the effects of imazapic on nontarget species and on the timing and pretreatment conditions necessary for imazapic to be effective against downy brome have produced mixed results. Imazapic suppressed invasive species without affecting native plant species in some studies (Davidson and Smith 2007; Kirby et al. 2003), whereas others found imazapic negatively affected some native species (Baker et al. 2009; Shinn and Thill 2004). If imazapic is used in the fall, it may be selective for actively growing plants with minimal harm to perennial plants (Davidson and Smith 2007; Vollmer and Vollmer 2008). Imazapic has been used as both a preemergent and postemergent herbicide (Davidson and Smith 2007; Kyser et al. 2007; Shinn and Thill 2004) with varying success in controlling downy brome. There is also uncertainty as to whether imazapic was most successful when applied before downy brome germination or while it was actively growing and whether newly emerging plants were more susceptible than more mature plants. Finally, some studies have had success in suppressing exotics by removing standing thatch (with mowing or burning) before imazapic applications in the field (Kyser et al. 2007; Morris et al. 2009). However, techniques such as mowing or burning to remove standing vegetation and litter before imazapic application may be lethal to sagebrush and could reduce seed production by remnant shrubs (Lambert 2005).

The objectives of this study were to determine if imazapic application (1) reduced downy brome cover and promoted shrub establishment when combined with seeding of two native shrub species, (2) harmed nontarget plant species and/or altered plant community composition, and (3) influenced downy brome or seeded shrub species' survival or growth when applied at different developmental stages. We used a field experiment and complementary studies in the greenhouse to reach these objectives. The field experiment was conducted in two needle pinyon–Utah juniper [*Pinus edulis* Engelm–*Juniperus osteosperma* (Torr.) Little] and mixed shrub communities, in areas highly degraded by a large wildfire in 1996 and subsequent invasion by downy brome. This was an ideal study system for the field experiment because pinyon–juniper and mixed shrub communities represent a significant woodland in the southwestern United States, covering $>$ 20 million ha ($>$ 49 million ac; Miller and Wigand 1994). Also, many southwestern shrublands have had, or are at risk of experiencing, large wildfires (Floyd et al. 2004) with subsequent downy brome invasion (Chambers et al. 2007). Before the wildfire and downy brome invasion, much of the study area was critical winter range for mule deer (*Odocoileus hemionus* Raf.). The native browse species, Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young.) and Mexican cliffrose [*Purshia mexicana* (D. Don) Henrickson] have been slow to recover since the fire and are at risk of being permanently displaced by downy brome, drastically reducing mule deer winter range.

Methods

Field Sites and Experimental Design. The field experiment was located on the west side of the Kaibab Plateau on the Kaibab National Forest (36°37′N, 112°26′W) in northern Arizona, USA. The study area was in a mixed shrubland community, within areas burned in the 1996 Bridger–Knoll Complex wildfire. The Bridger–Knoll fire burned more than 20,000 ha of pinyon–juniper and mixed shrub communities (USDA Burned-Area Report 1996). Common native plants included Mexican cliffrose, Wyoming big sagebrush, Mormon tea (*Ephedra viridis* Coville), two needle pinyon, Utah juniper, prairie junegrass [*Koeleria macrantha* (Ledeb.) Schult.], and smallflower globemallow (*Sphaeralcea parvifolia* A. Nelson). The most common exotic plants were downy brome and redstem filaree [*Erodium cicutarium* (L.) L'Hér. ex Ait.]. Long-term mean annual precipitation (MAP) averaged 379 mm (15 in), and the mean annual temperature was 12.4 C (54.3 F; years 1976 to 2009; WRCC 2009). Most precipitation occurs in late summer (August to October) and in the winter (January to March), and droughts are common. The MAP was 27 to 43% lower than average for the 3 yr of the study (217 to 276 mm). The average elevation was 1,940 m (6,365 ft). The soils were classified as Mellenthin series, derived from Kaibab limestone and sandstone, a mix of loamy-skeletal, mixed, superactive, mesic Lithic Ustic Haplocalcids (USDA-NRCS Web Soil Survey 2009).

We used a before–after–control–impact (BACI) design, with six replications of four treatments (including an untreated control). This experimental approach includes random assignment of treatments and untreated controls and sampling both before and after treatments are applied. Such experimental approaches are useful in controlling confounding factors, so that changes observed are likely due to the treatments (Underwood 1994). We established six 60- by 80-m study sites in July to August 2007 and collected pretreatment plant data, including downy brome cover, other understory plant canopy cover, and plant community composition. Each study site was divided into four 25- by 30-m plots, with 10- to 20-m buffer zones between plots within a site. Four treatments—(1) seed + herbicide, (2) herbicide only, (3) seed only, and (4) a nontreated control—were randomly assigned to each site (for a total of six replications for each treatment). Study sites were a minimum of 200 m, but up to 2 km (1.2 mi), apart from each other.

Seeding Treatments. Seeding treatments were conducted in October 2007 with a rangeland drill pulled by a rubber-tired tractor. The drill was 2.5 m long with 14 double discs spaced at 15 cm (6 in). Discs were set to seed at approximately 3.8 cm deep, and seeds were covered with soil by dragging a 1-m metal chain with a 15 by 3-cm flat nail attached to the end. The drill made seven passes in each seeded plot, and the seeding rate was 5.6 pure live seed (PLS) kg ha^{-1} (5 PLS lb ac^{-1}) of cliffrose and 2.7 PLS kg ha^{-1} of big sagebrush. Big sagebrush and cliffrose seeds were constantly mixed in the seed box to help with even distribution (Monson et al. 2004). All seed was collected locally from the area surrounding the wildfire.

Herbicide Treatments. Herbicide treatments were conducted in November 2007. A rubber-tired tractor pulled a trailer-mounted tank of herbicide. A Teejet[1] 844-E computerized flow meter regulated the application rate by the speed of the tractor. Imazapic[2] herbicide was applied at a rate of 560 ml ha^{-1} (8 oz ac^{-1}), or 132 g ai ha^{-1}, mixed with methylated seed oil[3] (MSO) surfactant at 105 ml ha^{-1} and a blue inert dye at 280 ml ha^{-1} (to show the area being treated). All of this was mixed with water and distributed at approximately 234 L ha^{-1} (25 gal ac^{-1}). In a previous study, this rate of imazapic application temporarily reduced downy brome cover in a salt desert shrub plant community (Morris et al. 2009), and when applied with MSO, it provided greater exotic plant control (Markle and Lym 2001). The tractor made three passes per treated plot, turning around in the buffer zones with spray valves shut off. The spraying unit was 9 m wide with 24 spray valves. We placed a piece of white felt on the ground 1 m on the outside of the last spray valve and the absence of herbicide (dyed blue) on this felt indicated that our buffer zones were sufficient to protect untreated areas from herbicide drift.

Downy Brome and Shrub Sampling. To determine if imazapic herbicide affected downy brome and if seeding treatments increased shrub species abundance, we measured pretreatment downy brome cover and density of seeded shrub species in August 2007, before herbicide and seeding treatments. These measurements were taken again after treatment in August of 2008 and 2009. In our posttreatment evaluations, downy brome was generally mature and in flower. We also measured downy brome biomass during both posttreatment years. We permanently marked a 15- by 20-m area in the center of each plot that included four 15-m transects for vegetation sampling. Downy brome cover was measured in 20 by 50-cm quadrats spaced at 1-m intervals along each transect, for a total of 56 quadrats per plot (Daubenmire 1959). Cover-class midpoints were used to calculate average downy brome cover based on seven classes: 0 = < 1%, 1 = 1 to 5%, 2 = 5 to 25%, 3 = 25 to 50%, 4 = 50 to 75%, 5 = 75 to 95%, and 6 = 95 to 100%. We measured posttreatment downy brome biomass in each plot by clipping all downy brome plants at ground level in seven randomly located 20- by 50-cm quadrats on the opposite side of transects used for estimating plant cover data, for a total of 28 quadrats per plot. We randomly chose 28 different locations to clip biomass in the fall of 2008 and 2009. The biomass samples were dried in an oven at 70 C for 3 d and weighed to the nearest 0.1 g. To determine the success of native shrub seeding, pretreatment and posttreatment density of the seeded species was determined by counting the number of living shrubs in each plot.

Nontarget Vegetation Sampling. To determine if imazapic herbicide affected nontarget plant species, we measured total plant cover, species richness, and individual species cover both before treatment and for 2 yr after treatment. We estimated total plant canopy cover in the same 20 by 50-cm quadrats used for downy brome cover, spaced at 1-m intervals along each of the four (15-m) transects, for a total of 56 quadrats per plot. Total plant, litter (thatch), bare ground, functional group (forbs, graminoids, and shrubs), exotic species, and individual species cover were estimated by the same seven cover classes described above for downy brome cover, and cover-class midpoints were used to calculate average cover estimates. Species richness was estimated by tallying each species in the entire area of each plot, and plants were identified to species in the field or collected and identified to species at the Deaver Herbarium at Northern Arizona University, or at the U.S. Department of Agriculture (USDA) Rocky Mountain Research Station. Scientific nomenclature and nativity follows the USDA PLANTS Database (2010) and the Weed Science Society of America Composite List of Weeds (2010).

Greenhouse Experiments: Downy Brome and Shrub Sensitivity to Imazapic. We designed a greenhouse experiment to determine if the effect of imazapic on downy brome, the target species, varied with developmental stage. This experiment was important because downy brome cohorts of different ages, and potentially different sensitivities to herbicide application, can be present in the field simultaneously. For example, we observed two cohorts of downy brome in the field just before the herbicide treatments. The first and most common cohort had 2 to 4 leaves; and the older, less common cohort had 5 to 10 leaves. After surface-sterilizing locally collected downy brome seeds in a 10% bleach solution for 10 min, we planted five seeds in a mixture of topsoil and sand in 13- by 15-cm pots and used herbicide or a deionized (DI) water control as controls. We mixed herbicide, MSO, and blue dye in a 1-L container of water as directed by the BASF Corporation (2006) to mimic the amount used in the field (described above). We used 20 replicated pots of three growth stages: (1) seeds (pregermination), (2) plants with 2 to 4 leaves, and (3) plants with 5 to 10 leaves. We sprayed the soil or plants and soil with the herbicide mixture while paired controls were sprayed with DI water. Each pot received approximately 1 ml of either herbicide mixture or DI water to mimic the volume used in the field. We recorded the percentage of seeds that germinated, plant mortality, height of living plants, and proportion of plants that flowered.

Greenhouse experiments were conducted to determine both seed and seedling sensitivity to imazapic for each of the seeded shrub species and an additional species, fourwing saltbush [*Atriplex canescens* (Pursh) Nutt.], that was seeded by land managers near our field study. We surface-sterilized locally collected seeds of each shrub species by soaking them in a 10% bleach solution for 10 min. To determine seed sensitivity to imazapic we planted 10 seeds of each shrub species together in a 25 by 50-cm flat of topsoil mixed with sand and hand-sprayed the planted seeds to evenly cover the topsoil of five flats, each flat receiving approximately 2 g of the herbicide mixture ($n = 5$). Five control flats were set up the same way, but sprayed with an equal amount of distilled DI water instead of herbicide mixture. Seed germination was monitored and germinants were counted 4 wk and 12 wk after treatment.

To determine shrub-seedling sensitivity to imazapic, we applied the same herbicide mixture as described above to 25 seedlings of each shrub species. An equal number of seedlings were sprayed with DI water and used as controls. The seedlings were 4 mo old and were grown in 3.8-L (1 gallon) pots in a mixture of sand and topsoil. Before treating with herbicide, we measured the height and number of branches and leaves for each species and found no significant pretreatment differences ($P > 0.05$) in any of the three species for any of the response variables. The shrubs received five sprays per pot of herbicide, or DI water if they were control seedlings, to evenly cover leaves, stems, and topsoil, representing the desired goal of field applications (560 ml ha^{-1}). Seedling height and number of branches and leaves were monitored both 2 and 10 wk after treatment. At the end of the 10 wk, we separated the roots and shoots, dried them in an oven at 70 C for 3 d, and weighed them to the nearest 0.1 g.

Data Analysis. For the field experiments, we used repeated-measures ANOVA or analysis of covariance (ANCOVA) if assumptions of normality and homogeneous variances were met, and comparable nonparametric tests, if they were not. We compared average plant cover values among treatments and years for total cover, litter, bare ground, forbs, graminoids, shrubs, all exotic species, and major species, including downy brome, sand dropseed [*Sporobolus cryptandrus* (Torr.) Gray], squirreltail [*Elymus elymoides* (Rafin.) Swezey], globemallow, and redstem filaree. We tested residuals to determine if they met assumptions of normality and homogeneous variances with the Shapiro-Wilks and Levene's tests (Devlin 2004). If we found significant pretreatment differences, we added a pretreatment covariate to account for those differences. If we had significant treatment-by-year interactions, we analyzed each year separately with one-way ANOVAs. We used the nonparametric Kruskal-Wallis (K-W) test to examine differences in downy brome biomass among treatments in 2008 and 2009. We ran these analyses in JMP for Windows 8.0[4] with $\alpha = 0.05$.

We used PC-ORD[5] to examine community-level differences among treatments and controls. Multi-response permutation procedures (MRPP) were used to determine if plant communities differed among treatments. The "A" statistic in MRPP is the difference in within-group homogeneity compared to random occurrence (McCune and Grace 2002). An A statistic for ecological data near 0.1 with a P value < 0.05 can indicate differences among groups (McCune and Grace 2002). If no pretreatment community differences were found, we tested each posttreatment year separately. If significant differences were found, indicator-species analysis was used to identify species associated with each treatment. We considered indicator species to be significant if they had an indicator value (IV; *frequency × relative abundance*) > 25 and a P < 0.05.

We used a combination of ANOVA and repeated-measures multivariate analysis of variance (MANOVA) to analyze data from the greenhouse experiments. We tested residuals for normality and homogeneous variances, as described above, and if variables failed to meet assumptions, log-transformations were applied. A one-way ANOVA was used for each species to test for differences in shrub germination among treatments and untreated controls and the post hoc Tukey-Kramer Honestly Significant Differences test was used for subsequent pair-wise comparisons. We used repeated-measures MANOVAs for each of the older shrub species because we took various measurements from the same plant over time.

Results and Discussion

Downy Brome Cover. Downy brome cover decreased on herbicide treatments the first growing season after treatment, but recovered by the second year. We found a significant treatment-by-time interaction with downy brome cover (*Trt × Time F* = 11.88, P = 0.001), and subsequent one-way ANOVAs indicated that downy brome cover was lower in the herbicide only and seed + herbicide treatments in the first year after treatment (*F* = 3.61, P = 0.045) (Figure 1A). No differences in downy brome cover were found among treatments the following year in 2009 (*F* = 0.56, P = 0.64) (Figure 1A). There was a strong trend for average downy brome biomass to be lower in the herbicide only and seed + herbicide treatments in 2008 (K-W $\chi^2 = 7.21$, P = 0.074), although this trend was not observed in 2009 (K-W $\chi^2 = 0.96$, P = 0.81) (Figure 1B). Others have found that a one-time application of herbicide in areas with high levels of downy brome only temporarily reduced its cover (Baker et al. 2009; Morris et al. 2009). Because our field sites had approximately 60% thatch cover in all plots, the herbicide effectiveness could have been reduced because of thatch blocking the herbicide contact (Kyser et al. 2007). The rapid recovery of downy brome in the second growing season indicates the difficulty of rehabilitating high-density infestations because of the large amount of viable downy brome seeds and its ability to outcompete native species and to recover after disturbances (Chambers et al. 2007). The stage of exotic invasion and lack of native foundation species as competitors can affect the outcome of management treatments over time (Bradford and Lauenroth 2006; Jessop and Anderson 2007; Prevéy et al. 2010; Sakai et al. 2001).

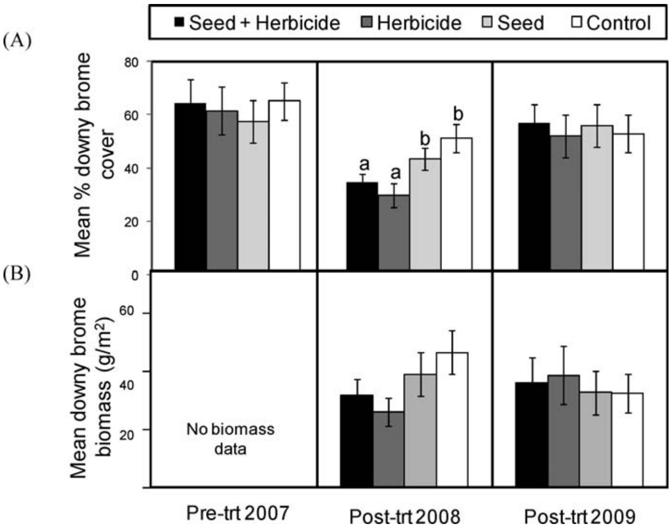

Figure 1. (A) Mean percentage (± 1 SE) of downy brome cover and (B) biomass. Different letters indicate significant differences between treatment means (Tukey-Kramer Honestly Significant Differences P < 0.05).

Native Shrub Density. The seeding treatments did not increase Mexican cliffrose or Wyoming big sagebrush densities, although big sagebrush densities increased over time. We found significant pretreatment differences in the

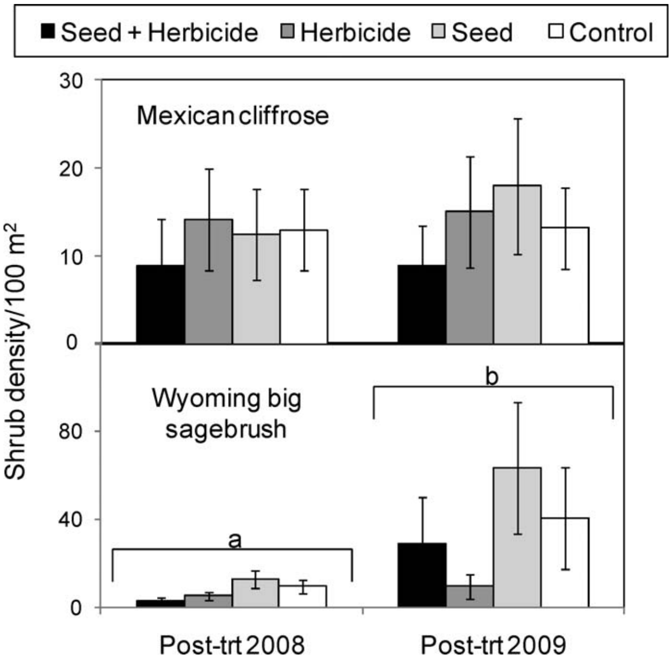

Figure 2. Mean shrub density of Mexican cliffrose and Wyoming big sagebrush. Adjusted means are shown because pretreatment data were used as a covariate. Different letters indicate significant differences in sagebrush cover between 2008 and 2009 (Tukey-Kramer Honestly Significant Differences P < 0.05).

density of these seeded species among our designated treatments (P < 0.05); therefore, we used pretreatment 2007 shrub density as a covariate in our analyses. No differences in the density of Mexican cliffrose were found through time or among treatments (*Trt* $F = 0.26$, P $= 0.85$; *Time* $F = 1.33$, P $= 0.29$; *Trt* × *Time* $F = 2.16$, P $= 0.07$) (Figure 2). Wyoming big sagebrush density increased from 2008 to 2009, but did not differ among treatments (*Trt* $F = 1.56$, P $= 0.25$; *Time* $F = 6.59$, P $= 0.024$; *Trt* × *Time* $F = 1.59$, P $= 0.26$) (Figure 2). We observed very few new shrub seedlings in 2008 but many more in 2009. Over 95% of the new big sagebrush seedlings observed in 2009 were found growing 0.3 to 0.6 m from the base of an already established adult shrub (sometimes > 100 seedlings). Because sagebrush seedlings naturally occur within 2 m from an established adult (Eisworth et al. 2009; Monson et al. 2004), these seedlings were probably not the result of our seeding treatments.

We attributed the poor success of the seeding treatment mostly to dry conditions during the study; but inadequate soil organisms and limited time since seeding may also be important. Drought can be a major factor limiting the success of shrub seedling establishment (Maier et al. 2001; Shaw et al. 2005), and sagebrush recruitment pulses are often associated with high precipitation events (Lysne 2005). In addition to adequate soil moisture, successful shrub establishment may be dependent upon soil organisms. Sagebrush is associated with symbiotic arbuscular mycorrhizal fungi (Lambert 2005), and some species of cliffrose are known to form associations with actinorhizal bacteria, which fix atmospheric nitrogen (Busse et al. 2007; Monson et al. 2004). Fire may have altered the abundance and species composition of these associates, limiting shrub establishment (e.g., Owen et al. 2009). Additional time may be required to observe significant increases in shrub forage resources (Lambert 2005; Williams et al. 2002). Eisworth et al. (2009) found that postfire seeding with big sagebrush increased its density with time since fire, up to 5 yr after seeding. The seeding treatments could eventually be more successful over time, with additional precipitation.

Nontarget Impacts. We found short-term effects of imazapic herbicide on overall plant cover. Average total plant cover was relatively high, with approximately 65 to 85% cover across all treatments through time. We found a significant treatment-by-time interaction for mean plant cover (Appendix 1A), and succeeding one-way ANOVAs showed that total plant cover was lower the first year after treatment in the seed + herbicide and herbicide only treatments compared to the seed only treatment and control ($F = 3.74$, P $= 0.028$). Mean plant cover was similar across all treatments the following year in 2009 ($F = 0.17$, P $= 0.79$). Thatch cover was high, averaging approximately 60% cover, whereas bare ground cover was fairly low, averaging about 15%. Both thatch cover and bare ground fluctuated over time but did not differ significantly among treatments or through time (Appendix 1A).

Plant functional groups responded differently to imazapic treatments. Graminoids were the most abundant, averaging approximately 65% cover across time and treatments. Mean graminoid cover was lower the first year post-treatment (2008) than in the second year (2009; Appendix 1B). The majority of graminoid cover consisted of exotic downy brome. We found a treatment-by-time interaction for exotic plant cover and found it was lower the first year post treatment in the herbicide only and seed + herbicide treatments compared to the seed only treatment and controls ($F = 2.26$, P $= 0.011$). No differences were found in exotic plant cover, including downy brome, the following year in 2009 ($F = 0.499$, P $= 0.76$; Appendix 1B). Shrubs were the least abundant of the functional groups, averaging $< 1\%$ cover across all treatments and over the 3 yr of sampling (Appendix 1B). We found a significant treatment-by-time interaction for forb cover (*Trt* × *Time* $F = 9.9$, P $= 0.003$). Forb cover was lower in the herbicide only and seed + herbicide treatments compared to the seed only treatment and controls the first year post treatment in 2008 ($F = 11.78$, P < 0.001; Figure 3A) and had a trend of staying lower in

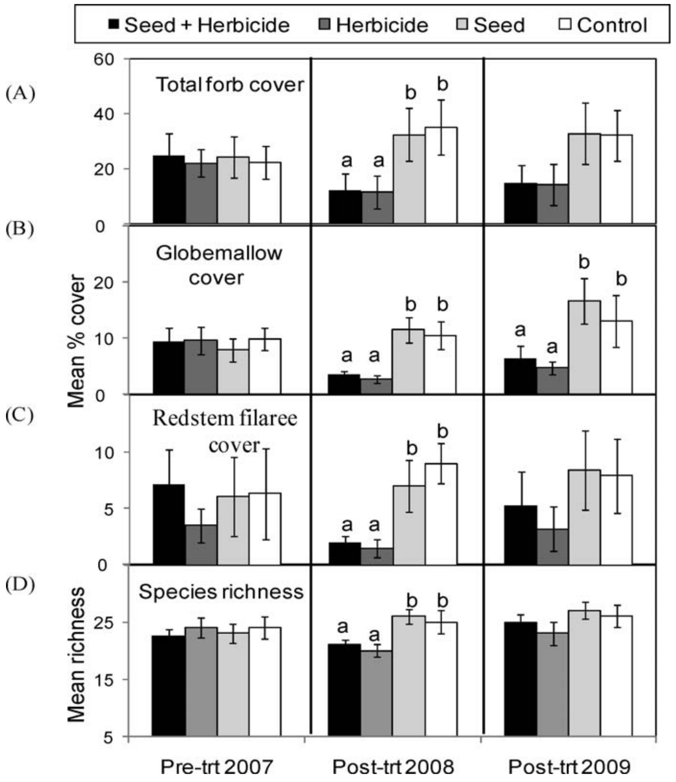

Figure 3. Mean percentage (± 1 SE) of cover by (A) total forbs, (B) globemallow, and (C) redstem filaree; and (D) total species richness. Different letters indicate significant treatment differences in 2008 (Tukey-Kramer Honestly Significant Differences P < 0.05).

these two treatments in 2009 (F = 2.59, P = 0.064; Figure 3A). Baker et al. (2009) found that high applications of imazapic (175 g ai ha^{-1}) in a sagebrush community reduced nontarget forb cover by 80 to 84%.

Two abundant forbs contributed to the decreased forb cover in the herbicide treatments: a native, globemallow and an exotic, redstem filaree. These species collectively made up more than half of the overall forb cover. There was a significant treatment effect on globemallow, but there was no effect of time or time-by-treatment interaction (*Trt F* = 5.86, P = 0.005, *Time F* = 3.09, P = 0.09, *Trt* × *Time F* = 0.46, P = 0.71). Both herbicide only and seed + herbicide treatments had less than half of the average globemallow cover compared to the seed only treatment and controls both years after treatment (F = 9.25, P $<$ 0.0001) (Figure 3B). It was thought that if applied in the fall, imazapic would mostly affect annual plants that were actively growing and have little effect on perennials that had largely completed their life cycle (Vollmer and Vollmer 2008). However, in our study, globemallow, a dominant native perennial, was significantly reduced for 2 yr after herbicide treatments. Baker et al. (2009) also found that imazapic reduced globemallow cover in a big sagebrush community. We detected a treatment-by-time interaction for cover of redstem filaree (*Trt* × *Time F* = 5.38, P = 0.03). Average filaree cover was lower in both herbicide treatments in 2008 (F = 3.94; P = 0.023), but not in 2009 (F = 0.87, P = 0.47) (Figure 3B). Davidson et al. (2007) also documented a reduction in redstem filaree biomass compared to controls the first year after treatment, and similar to our cover results, filaree biomass in their study did not differ between treatments and controls the second year after treatment.

We speculate that imazapic tolerance is related to plant photosynthetic pathways. Forbs that were reduced in the herbicide treatments (globemallow and filaree) were C$_3$ plants, whereas sand dropseed, a C$_4$ graminoid species, had a strong trend of increasing following herbicide treatments, at the same time that downy brome decreased. Thus, sand dropseed could be more tolerant of imazapic and may increase in response to reduced downy brome cover. The average plant canopy cover of three other C$_4$ graminoids on our plots—Indian ricegrass [*Achnatherum hymenoides* (Roemer & J.A. Schultes) Barkworth], purple threeawn (*Aristida purpurea* Nutt. var. *purpurea*), and blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths]—were not significantly altered by the herbicide treatments. Research on imazapic tolerance related to plant photosynthetic pathways could add to our understanding of imazapic effects on nontarget plants.

Plant Species Richness and Community Composition. Imazapic had a short-term effect on plant species richness. Average species richness was approximately 23 species plot^{-1} in 2007, with little variation among treatments (Figure 3D). After treatment, we found a time-by-treatment interaction for mean plant richness (*Trt* × *Time F* = 3.48, P = 0.045). Average plant richness was lower in both herbicide only and herbicide + seed treatments in 2008 (F = 6.22, P = 0.016), although no differences were found in 2009 (F = 2.54, P = 0.09) (Figure 3D). We found a total of 51 plant species, including 33 forbs, 12 graminoids, 4 shrubs, and 2 cacti (Appendix 2). Forbs were the most species-rich group, with globemallow and redstem filaree having the most cover. Dominant native graminoids were sand dropseed and squirreltail. Squirreltail averaged approximately 11% cover, with no significant changes in cover among treatments or through time (P $>$ 0.05). Sand dropseed averaged about 25% cover among pretreatment plots, but interestingly, it increased from 2008 and 2009 to approximately 32 to 38% cover, with a trend of higher cover in both herbicide-treated plots compared with the seed only and control plots (*Trt F* = 2.46, P = 0.09, *Time F* = 38.9, P $<$ 0.001, *Trt* × *Time F* = 1.17, P = 0.34). All of the shrubs from our census were native. The most abundant were Mexican cliffrose, Wyoming big sagebrush, and Mormon tea (Appendix 1), although they still averaged approximately 1% combined cover. We found nine exotic plant species (Appendix 2). We also found an additional exotic species in 2009, bur buttercup [*Ceratocephala testiculata* (Crantz) Roth], which was not found in previous plant censuses (Appendix 2). This species was found only in the seed and seed + herbicide treatments, and we suspect that the seeding equipment we used on our plots may have helped to spread this species. Bur buttercup has small achenes that are surrounded by sharp spikes that attach readily to tires, and therefore, it is a difficult species to detect and remove from equipment.

Imazapic herbicide had a short-term impact on plant community composition. Pretreatment plant composition did not differ among assigned treatments in the fall of 2007 ($A = 0.005$, $P = 0.59$) or the second year after treatment in 2009 ($A = 0.03$, $P = 0.16$). However, in 2008, plant-community composition differed among treatments ($A = 0.16$, $P = 0.001$), and a posteriori multiple-group comparisons showed that the composition of seed + herbicide and herbicide only treatments both differed from the seed only treatment and control ($P = 0.041$), but the seed only treatment and controls were not different from each other ($P = 0.79$). Indicator species for the seed only and control areas were downy brome (IV = 29 for seed and 39 for control, $P = 0.02$ and globemallow (IV = 46 for seed and 45 for control, $P = 0.047$). There was a trend for redstem filaree ($P = 0.064$) and prairie junegrass ($P = 0.065$) to be indicator species for the seed only treatment as well as the control areas. There were no indicator species for the herbicide or seed + herbicide treatments.

More research is needed to determine whether nontarget plant communities can recover from the shifts in species richness and composition caused by imazapic. The timing and rate of imazapic application, as well as knowledge of the growth stage of the associated plant species at the time of application could all play a role in reducing impacts to nontarget plants (Kyser et al. 2007; Morris et al. 2009). Because imazapic has a half-life of 120 d, after a fall application, plants that develop later in the following summer will be exposed to less herbicide than those growing in the spring. Also, imazapic could have an indirect effect on plant communities because other organisms, such as bacteria and some fungi, have the enzyme acetohydroxy acid synthase (AHAS), which is inhibited by imazapic (McCourt and Duggleby 2006). The microbes potentially affected by imazapic could play important roles in soil processes vital for plant growth.

Downy Brome and Native Shrub Sensitivity to Imazapic. Our greenhouse experiments indicated that imazapic was lethal to downy brome, regardless of growth stage (Table 1A). Downy brome had between 90 and 100% mortality after treatment with imazapic herbicide, and surviving plants had stunted growth and did not flower, whether herbicide was applied before germination, when plants had 2 to 4 leaves, or when plants had 5 or more leaves; in contrast control plants were taller and flowered (Table 1A). Contradictory field and greenhouse results suggest that other confounding factors, besides the rate of herbicide application and whether it was used as a preemergent or postemergent, reduced effectiveness of imazapic in the field. Imazapic effectiveness could have been reduced in the field because of high thatch cover and propagule pressure (Kyser et al. 2007; Morris et al. 2009).

In the greenhouse, germinating shrub seeds and established 4-mo-old seedlings responded differently to imazapic herbicide (Tables 1B and 1C). Herbicide application reduced seed germination by nearly 50% in all shrub species relative to controls (Table 1B). In contrast, herbicide application had no significant effect on 4-mo-old shrubs 10 wk after treatment (Table 1C). The repeated-measures results showed that all species had greater height and number of leaves over time ($P < 0.001$), and the treatment-by-time interactions ($P > 0.05$) and treatment effects on leaves, height, and biomass were all non-significant (Table 1C). Collectively, our greenhouse experiments showed that the native, perennial shrubs we tested are susceptible to imazapic during germination, but tolerant of it as older seedlings. The trend towards lower shrub density in herbicide treatments observed in the field may reflect the sensitivity of shrub seeds to imazapic.

Conclusion

In this study, a one-time herbicide treatment temporarily reduced a dense infestation of downy brome. Although the scale of our experiment limits the inference of the results to similar areas, by using a BACI design, we were able to control a number of confounding factors, and thus elucidate actual treatment effects. Although long-term rehabilitation goals were not achieved, we did not find evidence that disturbances associated with seeding and herbicide applications significantly increased downy brome cover, as has been reported in other studies (Bradford and Lauenroth 2006). The difficulty of reestablishing native species within communities highly infested with downy brome, combined with the ability of downy brome to recover after disturbance, suggests that exotics need to be treated soon after their establishment, before they spread above an unmanageable threshold (Sieg et al. 2003). If imazapic was repeatedly applied to smaller downy brome patches before the invasion became too extensive, there might be a better chance of controlling it without adverse effects on nontarget species (Vollmer and Vollmer 2008). Reducing high thatch cover before imazapic application could increase the effectiveness of imazapic (Kyser et al. 2007). Thatch-removal methods, such as burning or grazing, however, can be lethal to the native shrubs that we studied (Lambert 2005). Imazapic ties up in thatch,

Table 1. (A) The percentage of downy brome mortality and average height and the percentage of plants that produced flowers after treatment with either imazapic or deionized (DI) water (controls) at three growth stages. (B) Mean percentage (± 1 SE) of seeds that germinated. (C) Final mean (± 1 SE) number of leaves and average plant height with MANOVA statistics and mean plant biomass with ANOVA statistics of three shrub species following treatment with either imazapic or DI water (controls).[a]

(A) Downy brome mortality, height, and flowers

Treatments	Mortality %	Average height cm	Plants that produced flowers %
Pregermination			
Herbicide	100	–	–
Control	0	20.8	80
2–4 leaves			
Herbicide	100	–	–
Control	1	22.5	95
5+ leaves			
Herbicide	90	5.6	0
Control	0	23.0	92

(B) Seed germination

Treatments	Percent Germination % (± SE)	ANOVA Results *F*; P
Wyoming big sagebrush		
Control	76 (5.1)	23.1; 0.001
Herbicide	28 (8.6)	
Mexican cliffrose		
Control	82 (3.7)	195.6; 0.001
Herbicide	10 (4.8)	
Four-wing saltbush		
Control	56 (5.1)	19.7; 0.002
Herbicide	24 (5.0)	

(C) Plant leaves, height, and biomass

Treatments	Leaves No. (± SE)	Height cm (± SE)	MANOVA treatment results *F*; P	Biomass g (± SE)	Biomass ANOVA results *F*; P
Wyoming big sagebrush					
Control	291.1 (41.6)	17.1 (1.6)	0.55; 0.46	7.5 (0.8)	0.76; 0.39
Herbicide	263.2 (37.5)	16.0 (1.8)		8.4 (0.7)	
Mexican cliffrose					
Control	70.1 (10.5)	10.4 (1.2)	2.08; 0.09	6.2 (0.9)	0.84; 0.27
Herbicide	64.6 (9.6)	8.6 (1.3)		5.1 (0.7)	
Fourwing saltbush					
Control	155.8 (25.5)	23.5 (2.7)	1.63; 0.42	12.56 (1.6)	0.83; 0.48
Herbicide	146.8 (24.6)	18.5 (2.6)		11.98 (1.4)	

[a] Abbreviation: –, no surviving plants

unlike herbicides such as clopyralid and aminopyralid that contact thatch and are released into the soil after the first rainfall to become available for plant uptake (DiTomaso et al. 2006). Reducing downy brome seed banks is vital to management efforts because even a small number of surviving plants can quickly reverse the effects of herbicide treatments if growing conditions are favorable. Seeding native plant barriers or using herbicides targeted at decreasing seed production could reduce the spread of annual invasive grasses (Davies et al. 2010, Rinella et al. 2010).

Although the spread of exotic species is the second greatest threat to biodiversity after habitat loss (Simberloff et al. 2005), management to control exotic species should also consider potential harm to established native species. Because imazapic can affect nontarget species, more greenhouse research is needed before treating large landscapes to quantify the effectiveness and unintended consequences of imazapic. Because the 4-mo-old shrub seedlings in the greenhouse were more tolerant of imazapic than were the germinating seeds and we observed that established shrubs in the field were not affected by the imazapic, managers should consider seeding shrubs first and applying herbicide after shrubs are established. Other strategies for establishing shrubs include planting containerized seedlings grown with local, native field inoculum (Lysne 2005) in some key locations. Managers could also consider seeding with faster-growing native competitors, such as squirreltail (Leger 2008).

Sources of Materials

[1] Teejet Technologies, 1801 Business Park Drive, Springfield, IL 62703.

[2] Imazapic, Plateau, BASF Corp., Research Triangle Park, NC 27709.

[3] Methylated seed oil surfactant, Loveland Products, Inc., P.O. Box 1286, Greeley, CO 80632.

[4] SAS software 2008, Version 8.0, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

[5] PC-ORD 1999, Version 5.1, MjM Software Design, P.O. Box 129, Gleneden Beach, OR 97388.

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