Effect of herbicide and herbicide concentration on *Elymus wawawaiensis* establishment in the shrub-steppe

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Abstract

Drill-seeding native bunchgrasses after prescribed fire and herbicide application is a technique used to restore shrub-steppe ecosystems infested with alien species. We conducted an experiment to determine the herbicide type (imazapic, glyphosate), minimum application rate, and application frequency that will result in high establishment of *Elymus wawawaiensis* in a shrub-steppe ecosystem infested with *Bromus tectorum.* The minimum concentration of imazapic needed for high establishment (0.55 plants m^{-2}) was 0.28 kg ha^{-1}. At this concentration and higher, soil cover was greater than 25% and alien species cover less than 38%, levels that likely reduced competition below a critical level needed for high establishment. Glyphosate resulted in lower establishment whether applied one time or repeated in a second year. Percent of seedlings in flower and height was about 47% and 51 cm at 0.56 kg ha^{-1} of imazapic, compared with about 8% and 32 cm for glyphosate at the same concentration applied one time. Under the conditions of the test, imazapic is the better herbicide.

Introduction

It has been estimated that there are at least 40 million hectares affected by the invasive alien annual grass, *Bromus tectorum* and associated fires (D'Antonio and Vitousek 1992; Whisenant 1990) in western North America. These fires lead to reductions in diversity and cover of native species (Billings 1990; Monsen 1994) which allows *B. tectorum* to increase its presence (Knick and Rotenberry 1997). This pattern can be reversed by restoring shrub-steppe ecosystems using large native bunchgrasses (Link and Hill 2005b). A shrub-steppe ecosystem dominated by native plant species can result in lowered fire risk (Link et al. 2005) and a return to near-historical fire frequencies (Whisenant 1990).

Ecosystems dominated by *B. tectorum* are usually very resistant to natural reinvasion of native species (Daubenmire 1970; Daubenmire 1975; Rickard and Vaughan 1988). The restoration of these ecosystems requires measures to control invasive species before seeding is attempted. A common practice is to reduce the cover of *B. tectorum* and other competitive exotic annuals using herbicides (Whitson and Koch 1998) before seeding. Seeding is often done by drill-seeding large bunchgrasses (Munshower 1994). The objective of this study was to determine the minimum amount of herbicide needed to establish a drill-seeded bunchgrass (*Elymus wawawaiensis*) in a shrub-steppe ecosystem infested with *B. tectorum*.

In this study, we examined the effect of two herbicides on establishment after a prescribed burn. Prescribed burns are often used to clear surfaces for efficient application of imazapic (Plateau®) to the soil as a pre-emergent herbicide. Imazapic remains active in the soil for a period where it can control germinating plants. Imazapic can control weeds without damaging other species (Vollmer 2005), but the application rate for control is sensitive to species and environmental conditions (Link and Hill 2005a). Pre-emergent application of imazapic improved native forb and grass establishment in Illinois (Masters et al. 1996) and Nebraska (Beran et al. 1999b; Beran et al. 2000). Glyphosate (Roundup®) is a general herbicide that can kill most plants at high concentrations (Zimdahl 1999). Application rates up to 0.84 kg ha^{-1} have been effective for *B. tectorum* control (Beck et al. 1995; Carpenter and Murray 2000; Whitson and Koch 1998). It is often applied to *B. tectorum* at the two to three leaf-stage to reduce its cover and competitive abilities. Glyphosate is applied to foliage and is quickly deactivated in the soil (Carpenter and Murray 2000). We also investigated repeated application of glyphosate because yearly application significantly reduced *B. tectorum* cover (Whitson and Koch 1998). We tested the hypothesis that these herbicides will have no effect on establishment of *E. wawawaiensis*. The hypothesis was tested after a prescribed fire at five application levels and with repeated application of glyphosate.

The purpose of this work was to determine the herbicide type, minimum application rate, and application frequency that will result in high establishment of *E. wawawaiensis* in a shrub-steppe ecosystem infested with *B. tectorum.* The work was done in a set of replicated field plots where we determined establishment characteristics of *E. wawawaiensis* and percent cover of soil and plant species in response to treatments.

Methods

Study Area

The study area is on the Columbia National Wildlife Refuge in Grant County, Washington. The refuge is semi-arid with most precipitation (annual average 203 mm) in the fall and winter. Snowfall is variable with winter high temperatures usually near freezing. Lightning during the summer is a frequent cause of wildfire. Slope of the study area is about 10° and aspect is about 270°.

Plant communities have been dominated by *Artemisia tridentata*, *Pseudoroegneria spicata*, and *P. secunda* and are classified as an *A. tridentata – P. spicata* association (Daubenmire 1970). Cattle and sheep were introduced in the 1800's. The area was severely overgrazed and soon dominated by *B. tectorum*. Grazing was halted more than 20 years ago and fire is the most prominent disturbance to upland areas. The study area burned in 1993. *Bromus tectorum* remains the dominant cover, with variable amounts of annual and perennial forbs (Link and Hill 2006).

Experimental Design and Treatment Application

Forty-five plots (8.2 m x 33 m) were established in spring 2002. The study area was burned in the early afternoon on October 1, 2002. The prescribed burn was conducted as a flanking fire, with some backing, and brief periods of head fire. Treatments were then randomly applied to the plots.

Imazapic was applied on Nov. 14, 2002 as a pre-emergent. Glyphosate was applied on March 4, 2003 and again on March 10, 2004 to half the glyphosate plots. In the second glyphosate application, concentration levels matched those of the first application. Glyphosate and imazapic were applied at five concentrations (0, 1, 2, 4, 8, oz acre^{-1} or 0, 0.07, 0.14, 0.28, and 0.56 kg ha^{-1}) with a boom sprayer (Spider Sprayer, West Texas Lee Company). Glyphosate was applied with a surfactant and ammonium sulfate at a rate of 5.71 kg ha^{-1}. Herbicides were applied using water at a rate of 281 kg ha^{-1}. There are three replicates for each level of herbicide.

Elymus wawawaiensis (Snake River wheatgrass, Secar cultivar) was drill seeded at a rate of about 7.9 kg ha $^{-1}$ (about 215 PLS m^{-2}) on Feb. 19, 2003. Drill rows were 0.3 m apart. The old name for the Secar cultivar was *Pseudoroegneria spicata* (Carlson and Barkworth 1997).

Measurements

Measurements were taken before the fire in 2002, 2003, and 2004. The response variables were cover, drill-seeded grass density, flowering status, and height.

Percent cover of each vascular plant species, bare soil, soil cryptogams, and litter was determined in May and June in each year of the study. Cover was determined using a tape (Bonham 1989; Elmore et al. 2003; Link et al. 2005) and identifying the first observed (tallest) cover type at each 0.25 m hash mark on the tape. The tape was stretched tightly between two pieces of rebar metal stakes in the ground at the ends of any transect. Two tape transects were observed between plot corners in each plot. Observations were initiated and ended 5 m from the ends of the tape to minimize edge effects. Where transects crossed, data were thrown out from one transect to minimize over-sampling, resulting in 205 observations in a plot.

All drill-seeded grasses were counted, flowering status (in flower or not) recorded, and measured for height in each plot in 2004. A meter stick was used to measure height. The age of each established drill-seeded grass was determined by noting if the individual was in the second year of growth or a current year seedling. Only 1.7% of the drill-seeded grasses were current year seedlings.

Analysis

Given that nearly all drill-seeded grasses germinated in 2003, measurements taken on drill-seeded grasses in 2004 were related to cover conditions in 2003.

The relation between *E. wawawaiensis* density (ρ) and [imazapic] or % soil cover (x) was described using:

$$\rho = \frac{\rho_{\max} x^n}{\kappa^n + x^n}, \tag{1}$$

where $\rho_{\max}$ is an estimate of maximal ρ when "x" is large, κ is the value of "x" when ρ is half-maximal, and *n* defines the sensitivity of ρ to increasing values of "x".

The relation between *E. wawawaiensis* density (ρ) and the [glyphosate] when applied once or twice was described using:

$$\rho = \alpha e^{\beta[glyphosate]}, \tag{2}$$

where α is an estimate of ρ when [glyphosate] = 0 and β defines the sensitivity of ρ to increasing [glyphosate]

The relation between the percent of *E. wawawaiensis* in flower (*f*) and [imazapic] was described using:

$$f = \frac{f_{\max}[imazapic]}{\kappa + [imazapic]}, \tag{3}$$

where $f_{\max}$ is an estimate of maximal *f* when [imazapic] is large and κ is [imazapic] when *f* is half-maximal.

The relation between the percent of *E. wawawaiensis* in flower, height, % soil cover, or % alien species cover (y) and [herbicide] was described using:

$$y = b_0 + b_1[\text{herbicide}], \tag{4}$$

where b_0 is the estimated response without herbicide and b_1 is slope of the relationship.

The relation between *E. wawawaiensis* density (ρ) and % alien species cover (x) was described using:

$$\rho = \frac{\rho_{\max}\kappa^n}{\kappa^n + x^n}, \tag{5}$$

where $\rho_{\max}$ is an estimate of maximal ρ when “x” is large, κ is the value of “x” when ρ is half-maximal, and *n* defines the sensitivity of ρ to increasing values of “x”.

Data Analysis

Analyses were done using JMP version 5, software (SAS Institute 2002). Linear and non-linear regression was used to assess herbicide concentration effects. Error terms are one standard error of the mean. Statistical significance is set at the $\alpha = 0.05$.

Results

In 2002, before treatment application, cover of *B. tectorum* was 47.2 ± 1.5% (n = 45). Cover of all alien species was 54.4 ± 1.5% (n = 45).

In 2004, of nine control plots without herbicide, three had established *E. wawawaiensis* plants for a mean of 50 ± 38 plants ha^{-1}. This was not significantly different from zero.

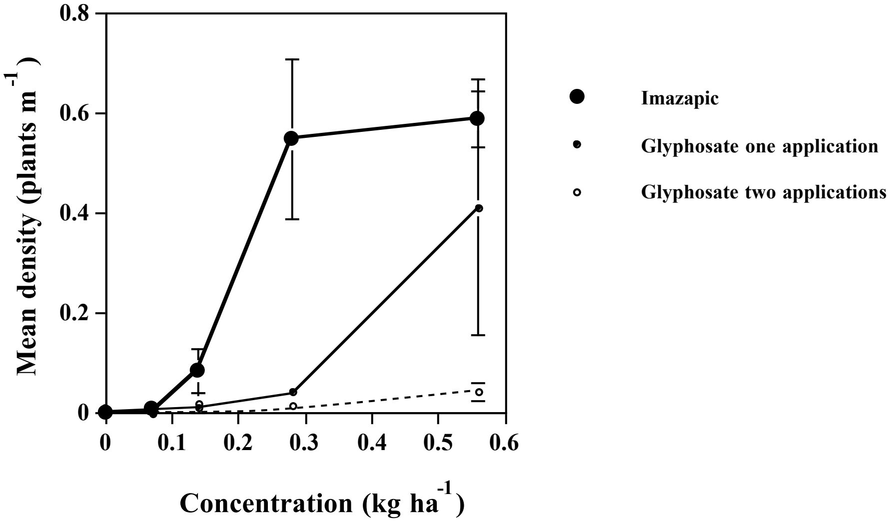

Figure 1. Relationships between herbicide concentration and density of *E. wawawaiensis*. Predicted lines are from equations 1 and 2. Error bars are one standard error of the mean (n = 3).

There was a significant (p = 0.0035) nonlinear regression (Eq. 1, $\rho_{max} = 0.590 \pm 0.073$, $\kappa = 0.185 \pm 0.037$, $n = 6.29 \pm 3.86$, $r^2 = 0.49$) between *E. wawawaiensis* density and concentration of imazapic. Imazapic at 0.28 kg ha^{-1} resulted in a density of 0.55 plants m^{-2}. There was a significant (p = 0.0035) nonlinear regression (Eq. 2, $\alpha = 0.00459 \pm 0.00356$, $\beta = 8.034 \pm 1.132$, $r^2 = 0.49$) between *E. wawawaiensis* density and concentration of Roundup when applied once. At 0.56 kg ha^{-1}, density (0.41 ± 0.26 plants m^{-2}) was highly variable and not significantly different from zero. There was a significant (p = 0.0443) nonlinear regression (Eq. 2, $\alpha = 0.000957 \pm 0.000280$, $\beta = 6.934 \pm 0.073$, $r^2 = 0.28$) between *E. wawawaiensis* density and concentration of Roundup when applied twice.

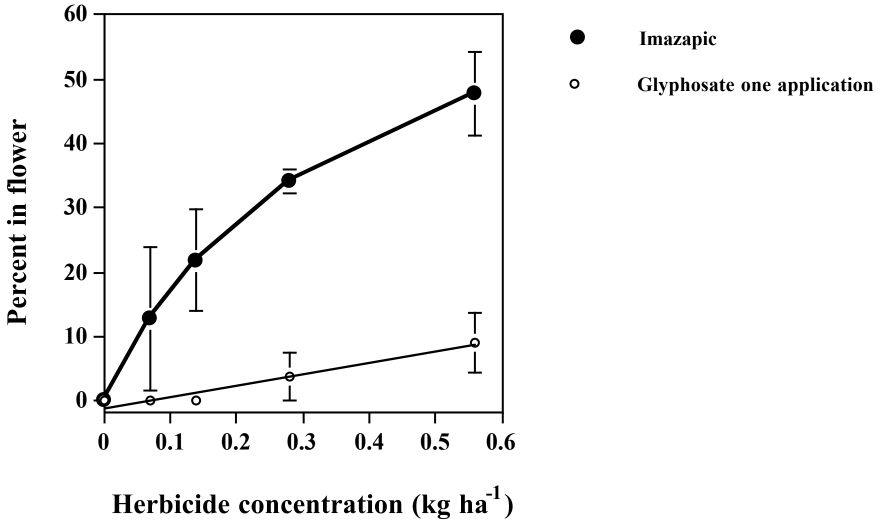

Figure 2. Relationships between herbicide concentration and percent of *E. wawawaiensis* in flower. Predicted lines are from equations 3 and 4. Error bars are one standard error of the mean (n = 3).

There was a significant ($p < 0.0001$) nonlinear regression (Eq. 3, $f_{\max} = 79.25 \pm 31.0$, $\kappa = 0.369 \pm 0.279$, $r^2 = 0.75$) between the percent of *E. wawawaiensis* in flower (*f*) and the concentration of imazapic (Fig. 2). There was a significant ($p = 0.0062$) linear regression ($b_0 = -1.10 \pm 1.53$, $b_1 = 17.3 \pm 5.31$, $r^2 = 0.45$) between glyphosate concentration applied one time and the percent of *E. wawawaiensis* in flower (Fig. 2). No plants flowered when glyphosate was applied two times.

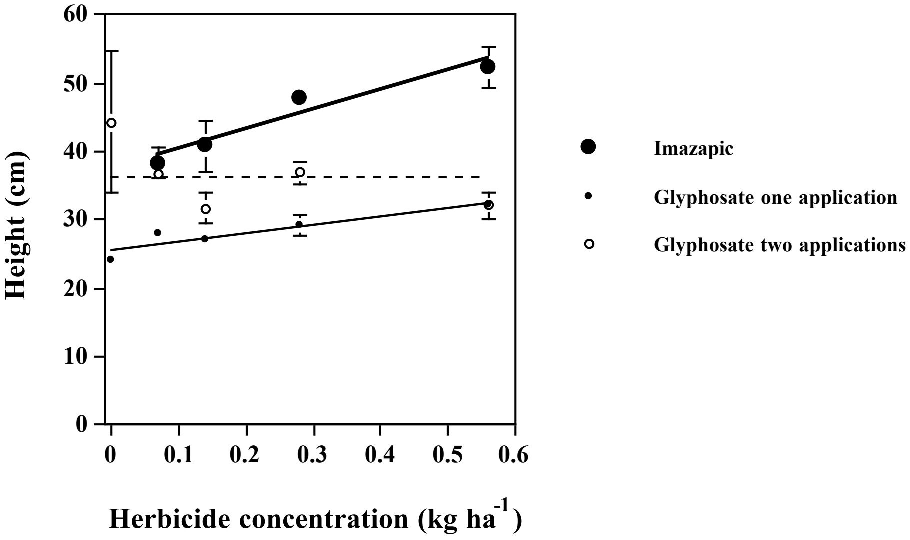

Figure 3. Relationships between herbicide concentration and height of *E. wawawaiensis*. Predicted lines are from equation 4. Error bars are one standard error of the mean (n <= 3).

There was a significant ($p = 0.0023$) linear regression ($b_0 = 37.3 \pm 2.30$, $b_1 = 28.8 \pm 7.11$, $r^2 = 0.62$) between imazapic concentration and the height of *E. wawawaiensis* (Fig. 3). There was a significant ($p = 0.0119$) linear regression ($b_0 = 25.6 \pm 1.29$, $b_1 = 11.9 \pm 3.68$, $r^2 = 0.57$) between glyphosate concentration applied once and the height of *E. wawawaiensis* (Fig. 3). There was no relationship (grand mean = 36.1 ± 2.06 cm) between glyphosate concentration applied twice and height of *E. wawawaiensis* (Fig. 3).

There was a significant ($p < 0.0001$) linear regression ($b_0 = 12.4 \pm 1.30$, $b_1 = 40.4 \pm 4.53$, $r^2 = 0.86$) between imazapic concentration and % soil cover (Fig. 4). There was no ($p = 0.69$) relationship between glyphosate herbicide applied one time and % soil cover, thus the grand mean of % soil cover was 14.0 ± 1.2 % (n = 15).

There was a significant ($p < 0.0001$) linear regression ($b_0 = 60.7 \pm 3.23$, $b_1 = -79.1 \pm 11.2$, $r^2 = 0.79$) between imazapic concentration and % alien species cover (Fig. 4). There was no ($p = 0.61$) relationship between glyphosate herbicide applied one time and % alien species cover, thus the grand mean of % alien species cover was 60.4 ± 2.9 % (n = 15).

There were no significant relationships between herbicide concentration and % native species cover, thus the grand mean for imazapic was 21.4 ± 2.0% (n = 15), and glyphosate applied one time was 14.8 ± 1.9% (n = 15).

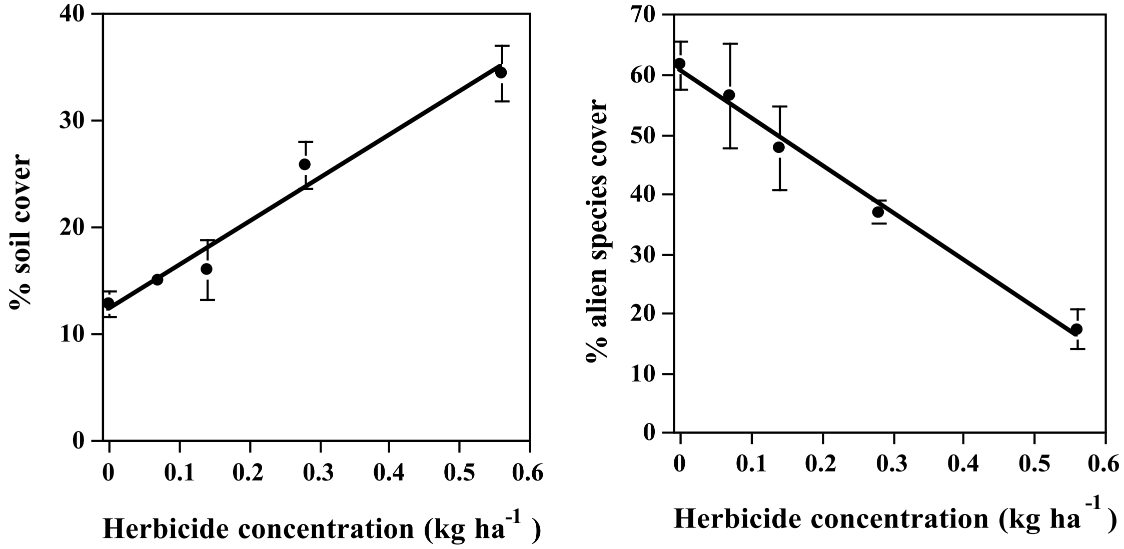

Figure 4. Relationships between imazapic concentration and % soil plus % alien species cover in 2003. Predicted lines are from equation 4. Error bars are one standard error of the mean (n = 3).

There was a significant ($p < 0.0001$) nonlinear regression (Eq. 1, $\rho_{max} = 0.64 \pm 0.047$, $\kappa = 0.224 \pm 0.008$, $n = 18.9 \pm 11.6$, $r^2 = 0.92$) between *E. wawawaiensis* density and % soil cover (Fig. 5). There was a significant ($p < 0.0001$) nonlinear regression (Eq. 5, $\rho_{max} = 0.629 \pm 0.054$, $\kappa = 0.388 \pm 0.016$, $n = 193.2 \pm 553.9$, $r^2 = 0.88$) between *E. wawawaiensis* density and % alien species cover (Fig. 5).

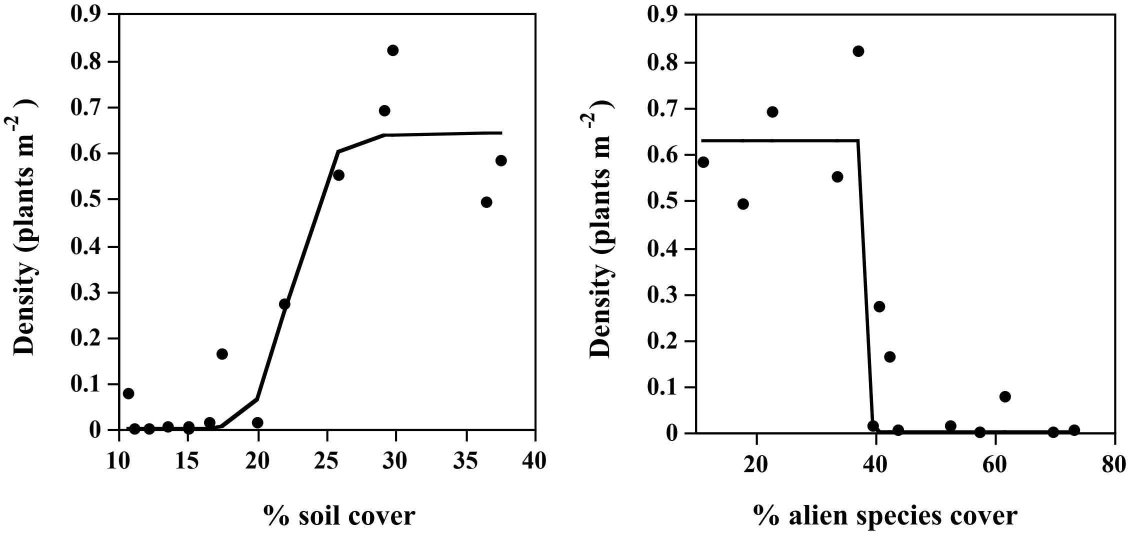

Figure 5. Relationships between *E. wawawaiensis* density and % soil cover plus % alien species cover. Predicted lines are from equations 3 and 4.

Discussion

The entire area was burned and then herbicides applied, thus conclusions are most relevant to herbicide use after a prescribed fire. Results may also be applied to wildfires in the fall where herbicides are used in similar ecosystems. The main finding was that imazapic concentration at or above 0.28 kg ha $^{-1}$ resulted in the greatest density of drill-seeded *E. wawawaiensis.* The hypothesis that these herbicides will have no effect on establishment of *E. wawawaiensis* was false.

Drill-seeded *E. wawawaiensis* established in control plots, but in very low numbers. It is likely that chance establishment without herbicides is because some seeding occurred in open spots where competition was low. We observed soil cover of about 13% without imazapic, some, of which, is likely associated with open spaces caused by burrowing mammals.

Significant establishment occurred at imazapic concentrations of 0.28 kg ha^{-1} and above. At these levels of imazapic, competition with alien species was reduced sufficiently to allow a large number of plants to establish. Under the conditions of the test, when % soil cover was greater than 25% and alien species cover less than 38% (Fig. 5), competition was below a critical level allowing high establishment. This is in contrast to establishment with glyphosate, where, even though there was an increasing relation between density and concentration, the effect was small at 0.28 kg ha^{-1} and highly variable at 0.56 kg ha^{-1}. One plot had 0.91 plants m^{-2} and another only 0.04 plants m^{-2}. Alien species cover was about 60% across all concentrations in the single application treatment, which is too much competition for high and consistent establishment. The treatment with repeated application of glyphosate resulted in low establishment at all concentrations, which may, in part, be caused by the direct effect of the herbicide on *E. wawawaiensis* seedlings.

It is likely that increased establishment success of *E. wawawaiensis* seedlings with high concentration of herbicides is caused by reductions in competition. This was strongest with imazapic and is reflected in effects on flowering status and growth of the seedlings. Percent of seedlings in flower was about 47% at the highest concentration of imazapic, which is much greater than about 8% for glyphosate applied one time. Height increased with increasing concentrations of the herbicides to about 51 cm with imazapic and only 32 cm with glyphosate applied one time. Similar effects have been observed with other perennial grasses. Growth and reproduction of *Nassella pulchra* was negatively affected by interspecific competition with annual grasses in California (Dyer and Rice 1999). *Pseudoroegneria spicata,* which is similar to *E. wawawaiensis,* grew less and produced fewer flowering culms when planted 6 cm from established *Achnatherum lemmonii* bunchgrasses than when planted at least 18 cm away (Huddleston and Young 2004). Increasing soil and decreasing alien species cover with increasing imazapic concentration are both indicators of reduced competitive interference for seeded *E. wawawaiensis* in this study.

Response of seeded *E. wawawaiensis* to increasing herbicide concentration was positive except for height after two applications of glyphosate. This is in contrast to a negative response of *E. wawawaiensis* to increasing concentration of imazapic applied pre-emergent to a tilled field where other competing plants were eliminated (Shinn and Thill 2004). Shinn and Thill (2004) removed competitors, applied imazapic in the spring, and seeded the next day. It is possible that application in the spring and seeding immediately after application subjects the seed to a stronger imazapic effect. We applied imazapic in the fall and seeded on February 19. This time lag may have reduced the activity of imazapic to levels where damage does not occur. Removal of competitive plants (Shinn and Thill 2004) may also account for some of the differences with our results where competitive plants were present, but affected by imazapic. Imazapic positively affected seeded dicots when weed interference was high and negatively affected the seeded dicots when weed interference was low (Beran et al. 1999a). Imazapic should be used with caution when applied in the spring and/or weed competition is low.

Management implications and Conclusions

The purpose of this work was to determine the herbicide type, minimum application rate, and application frequency that will result in high establishment of *E. wawawaiensis* in a shrub-steppe ecosystem infested with *B. tectorum.* Imazapic applied at 0.28 kg ha^{-1} resulted in high establishment. This is the minimum application rate under the conditions of the test. A lower rate may be sufficient, but would require further testing. Glyphosate resulted in lower establishment whether applied one time or repeated in a second year. Glyphosate damaged native species while imazapic positively affected native species in a companion study (Link and Hill 2005a). Under the conditions of the test, imazapic is the better herbicide.

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Literature cited

Beck, G. K., J. R. Sebastian, and P. L. Chapman. 1995. Jointed goatgrass (*Aegilops cylindrica)* and downy brome (*Bromus tectorum*) control in perennial grasses. Weed Technology 9:255-259.

Beran, D. D., R. E. Gaussoin, and R. A. Masters. 1999a. Native wildflower establishment with imidazolinone herbicides. Hortscience 34:283-286.

Beran, D. D., R. A. Masters, and R. E. Gaussoin. 1999b. Grassland Legume Establishment with Imazethapyr and Imazapic. Agronomy Journal 91:592-596.

Beran, D. D., R. A. Masters, R. E. Gaussoin, and F. Rivas-Pantoja. 2000. Establishment of big bluestem and Illinois bundleflower mixtures with imazapic and imazethapyr. Agronomy Journal 92:460-465.

Billings, W. D. 1990. *Bromus tectorum*, a biotic cause of ecosystem impoverishment in the Great Basin. Pp. 301-322 *in* G. M. Woodwell, ed. The earth in transition: patterns and processes of biotic impoverishment. Cambridge University Press, Cambridge.

Bonham, C. D. 1989. Measurements for Terrestrial Vegetation. John Wiley & Sons, New York.

Carlson, J. R., and M. E. Barkworth. 1997. *Elymus wawawaiensis*: A species hitherto confused with *Pseudoroegneria spicata* (Triticeae, Poaceae). Phytologia 83:312-330.

Carpenter, A. T., and T. A. Murray. 2000. Element stewardship abstract for *Bromus tectorum* L. (*Anisantha tectorum* (L.) Nevski) cheatgrass, downy brome. http://tncweeds.ucdavis.edu/esadocs/brometec.html. The Nature Conservancy, Arlington, VA.

D'Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annu. Rev. Ecol. Syst 23:63-87.

Daubenmire, R. 1970. Steppe vegetation of Washington, Pullman, Washington.

Daubenmire, R. 1975. Plant succession on abandoned fields, and fire influences, in a steppe area in southeastern Washington. Northwest Science 49:36-48.

Dyer, A. R., and K. J. Rice. 1999. Effects of competition on resource availability and growth of a California bunchgrass. Ecology 80:2697-2710.

Elmore, A. J., J. F. Mustard, and S. J. Manning. 2003. Regional patterns of plant community response to changes in water: Owens Valley, California. Ecological Applications 13:443-460.

Huddleston, R. T., and T. P. Young. 2004. Spacing and competition between planted grass plugs and preexisting perennial grasses in a restoration site in Oregon. Restoration Ecology 12:546-551.

Knick, S. T., and J. T. Rotenberry. 1997. Landscape characteristics of disturbed shrubsteppe habitats in southwestern Idaho (U.S.A.). Landscape Ecology 12:287-297.

Link, S. O., and R. W. Hill. 2005a. Effects of herbicides on a shrub-steppe plant community after a prescribed fire. submitted to Ecological Applications

Link, S. O., and R. W. Hill. 2005b. Fire risk of restored shrub-steppe plant communities. (submitted to the International Journal of Wildland Fire)

Link, S. O., and R. W. Hill. 2006. Effect of prescribed fire on a shrub-steppe plant community infested with *Bromus tectorum*. International Journal of Wildland Fire:(submitted).

Link, S. O., C. W. Keeler, R. W. Hill, and E. Hagen. 2005. *Bromus tectorum* cover mapping and fire risk. International Journal of Wildland Fire (accepted)

Masters, R. A., S. J. Nissen, R. E. Gaussoin, D. D. Beran, and R. N. Stougaard. 1996. Imidazolinone herbicides improve restoration of Great Plains grasslands. Weed Technology 10:392-403.

Monsen, S. B. 1994. The competitive influences of cheatgrass (*Bromus tectorum*) on site restoration. Proceedings - ecology and management of annual rangelands,

Munshower, F. E. 1994. Practical Handbook of Disturbed Land Revegetation. Lewis Publishers, Boca Raton.

Rickard, W. H., and B. E. Vaughan. 1988. Plant community characteristics and responses. Pp. 109-179 *in* W. H. Rickard, L. E. Rogers, B. E. Vaughan and S. F. Liebetrau, eds. Shrub-steppe: balance and change in a semi-arid terrestrial ecosystem. Elsevier, Amsterdam.

SAS Institute. 2002. JMP Statistics and Graphics Guide, Version 5. SAS Institute Inc, Cary.

Shinn, S. L., and D. C. Thill. 2004. Tolerance of several perennial grasses to imazapic. Weed Technology 18:60-65.

Vollmer, J. L. 2005. New technology for fuel breaks and green strips in urban interface and wildland areas. Eighth International Wildland Firefighter Safety Summit - Human Factors - 10 Years Later, Missoula, MT pp. 1-7.

Whisenant, S. G. 1990. Changing fire frequencies on Idaho's Snake River plains: ecological and management implication. USDA Forest Service Intermountain Research Station General Technical Report: INT-276:4-10

Whitson, T. D., and D. W. Koch. 1998. Control of downy brome (*Bromus tectorum*) with herbicides and perennial grass competition. Weed Technology 12:391-396.

Zimdahl, R. L. 1999. Fundamentals of Weed Science. Academic Press, San Diego.