Research

Growth Regulator Herbicides Prevent Invasive Annual Grass Seed Production

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Auxinic herbicides, such as 2,4-D and dicamba, that act as plant growth regulators are commonly used for broadleaf weed control in cereal crops (e.g., wheat, barley), grasslands, and noncroplands. If applied at late growth stages, while cereals are developing reproductive parts, the herbicides can reduce seed production. We tested whether growth regulators have this same effect on the invasive annual grass Japanese brome. The herbicides 2,4-D, dicamba, and picloram were applied at typical field use rates to Japanese brome at various growth stages in a greenhouse. Picloram reduced seed production nearly 100% when applied at the internode elongation, boot, or heading stages of growth, whereas dicamba appeared to be slightly less effective and 2,4-D was much less effective. Our results indicate it may be possible to control Japanese brome by using growth regulator herbicides to reduce its seed production, thereby depleting its short-lived seed bank.

Nomenclature: 2,4-D; dicamba; picloram; Japanese brome, *Bromus japonicus* Thunb.; barley, *Hordeum vulgare* L.; wheat, *Triticum aestivum* L.

Key words: Seed bank, seed development, rangeland.

Exotic annual grasses such as cheatgrass (*Bromus tectorum* L.), Japanese brome (*Bromus japonicus* Thunb.), and medusahead (*Taeniatherum caput-medusae* L.) are negatively impacting millions of hectares of grassland in the western United States (Davies and Svejcar 2008; DiTomaso 2000; Sheley and Petroff 1999; Sperry et al. 2006). Efforts to control these plants typically focus on grazing (Harmoney 2007), seeding competitive species (Whitson and Koch 1998), fire (DiTomaso et al. 2006a), and herbicides or an integration of these practices (Masters and Sheley 2001). The most widely used classes of herbicides for invasive annual grass control are amino acid synthesis inhibitors and photosynthetic inhibitors, and within these classes, glyphosate, imazapic, and tebuthiuron are currently used. Shinn and Thill (2002) reported that imazapic provided up to 76% control of annual grasses and Whitson and Koch (1998) found that glyphosate combined with grazing provided greater than 90% control of cheatgrass. However, in addition to damaging target invasive grasses, amino acid synthesis inhibitors and photosynthetic inhibitors often damage desirable grasses growing with the weed. For example, Shinn and Thill (2004) found that the rates of imazapic required to control cheatgrass caused up to a 63% decrease in biomass of perennial grasses and Lym and Kirby (1991) reported that glyphosate reduced western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Löve] yield up to 78%.

Plant growth regulator herbicides such as 2,4-D, aminopyralid, clopyralid, dicamba, and picloram may control annual grasses by preventing seed production, thereby providing an alternative to amino acid synthesis inhibitors and photosynthetic inhibitor herbicides. If effective, growth regulators could offer the advantage of being less injurious to desirable cool- and warm-season perennial grasses that grow in association with invasive annual grasses.

Growth regulators are regularly used for broadleaf weed control in cereal crops, rangeland, pastures, and noncropland. Growth regulators sometimes reduce viable seed production in cereals, such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), and oats

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Interpretive Summary

This research tested a novel approach for controlling invasive annual grasses with growth regulator herbicides such as dicamba, picloram, and 2,4-D. It has been known for decades that growth regulators can dramatically reduce cereal seed production if applied for broadleaf weed control late in the growing season while cereals are developing reproductive parts. We tested whether or not growth regulators have this same effect on seed production of an invasive annual grass (Japanese brome). Our results show that growth regulators can dramatically reduce invasive annual grass seed production. Therefore, it may be possible to use growth regulators to control invasive annual grasses by depleting their short-lived seed banks. Growth regulator herbicides are less damaging to desirable perennial grasses than currently used herbicides.

(*Avena sativa* L.), when applied at late phenological stages; i.e. after initiation of grass jointing but before seeds begin to form (Friesen et al. 1968; Rinella et al. 2001; Sikkema et al. 2007). For example, Rinella et al. (2001) applied dicamba late in the development of winter wheat and found that harvestable grain yield was reduced by as much as 100%. The authors concluded that dicamba caused development of minute, nongerminable seeds in winter wheat.

If growth regulator herbicides adversely affect invasive annual grass seed production as they do cereal seed production, it may be possible to use these chemicals to deplete invasive annual grass seed banks, because invasive annual grass seeds tend to be short-lived in soil (i.e., 0 to 3 yr) (e.g., Smith et al. 2008). Alternatively, desirable perennial grasses rely more on vegetative means of propagation, as opposed to seeds, so these species are unlikely to be appreciably damaged by growth regulator herbicides. The purpose of our research was to evaluate effects of growth regulator herbicides on invasive annual grass seed production. Specifically, we evaluated effects of 2,4-D, dicamba, and picloram on Japanese brome seed production in the greenhouse. Japanese brome is very similar to its more well-known congeneric cheatgrass in terms of biology, ecology, and impacts. Detailed information on the current and projected distribution of Japanese brome is unavailable, but it has invaded expansive areas in the central and northern Great Plains (Haferkamp et al. 2001b; Harmoney 2007), where it often co-occurs with cheatgrass (Ogle et al. 2004). Japanese brome is known to compete with native vegetation (Haferkamp and Heitschmidt 1999; Perry et al. 2009), and reduce livestock performance below that obtained on native range (Haferkamp et al. 2001a).

Materials and Methods

Two greenhouse experiments were conducted November through June 2004 to 2005 (experiment 1) and 2005 to 2006 (experiment 2) to evaluate sensitivity of Japanese brome to 2,4-D, picloram, and dicamba applied at four growth stages. Japanese brome seeds were collected from a grassland site on the Fort Keogh Livestock and Range Research Station (46°22′N 105°5′W) near Miles City, MT. Seeds for experiments 1 and 2 were collected in July 1999 and July 2005, respectively. Seeds were germinated in flats (51 by 26 by 6 cm [20 by 10 by 2 in]) containing commercial potting mix.[1] When the seedlings were at the two- to three-leaf stage they were transplanted to pots (21 cm diam by 21 cm deep, 7.6 L [2.0 gal]) containing commercial potting mix. Two seedlings were planted per pot, and pots were placed in the greenhouse in a randomized complete block design (6 blocks × 3 herbicides × 4 plant growth stages + 6 controls = 78 pots per experiment).

Greenhouse temperatures were initially set at 13 C (55 F) during the day and 2 C (36 F) at night. Temperatures were gradually increased over the course of the experiments to mimic spring conditions, until the end of March when they were fixed at 24 C during the day and 10 C at night. To attain desired day and nighttime temperatures, greenhouse controls were set so that temperatures began gradually increasing 2 h before sunrise and began decreasing 2 h after sunset. Throughout the experiment, plants were not exposed to supplemental artificial light and were watered as needed.

Our herbicide rates were equal to those commonly used for broadleaf weed control. Herbicide treatments were (1) untreated, (2) 2,4-D (1.12 kg ae/ha [1.0 lb ae/ac]), (3) picloram (0.42 kg ae/ha [0.4 lb ae/ac]), and (4) dicamba (0.56 kg ae/ha [0.5 lb ae/ac]). Plants (both treated and untreated) were transferred outside for herbicide application. Herbicides were applied using a CO_2-pressurized backpack sprayer with 4-XR TeeJet 8200VS nozzles[2] calibrated to deliver 131 L/ha. Plants receiving the same herbicide treatment were aligned in a row and herbicide was applied in a single pass with the applicator walking parallel to the row of plants.

Herbicides were applied at seedling, initiation of internode elongation, boot, or heading stages of development. At the seedling stage, plants were approximately 10 cm tall. At the internode elongation stage, tillering was complete and a node could be felt through the sheath at the base of the stems. At the boot stage, the developing inflorescence began to grow into the flag leaf causing it to swell. At the heading stage, the inflorescence began to emerge from the sheath and the first spikelets became visible.

Seeds were clipped from plants when mature but before dropping from the plant. Immediately following seed harvest, plants were clipped at soil level, dried at 60 C for 48 h, and weighed. Seeds were counted, weighed, and placed into one of five categories according to appearance: (1) normal mature, (2) normal immature, (3) damaged

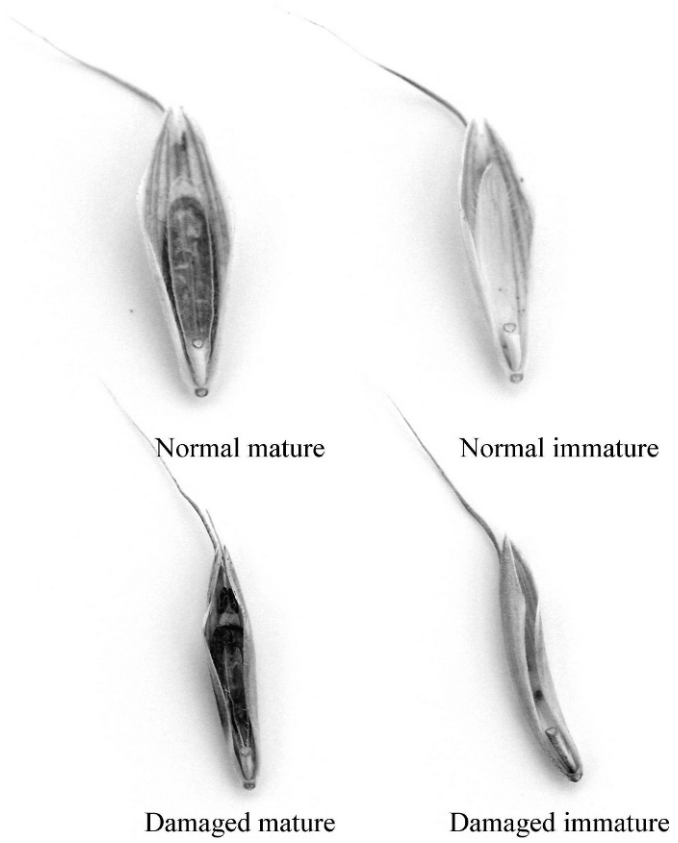

Figure 1. Seed types observed in a study that applied growth regulator herbicides to Japanese brome.

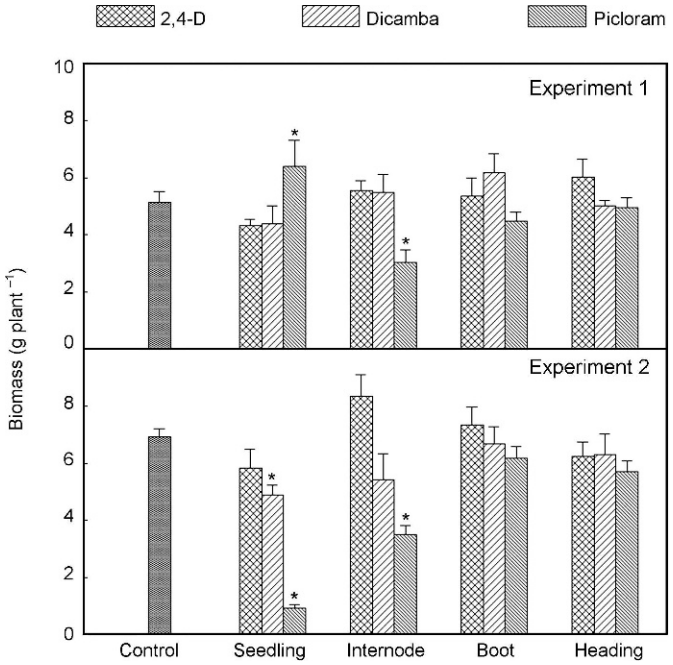

Figure 2. Effects of growth regulator herbicides on Japanese brome biomass. Bars denote standard error and asterisks denote significant differences from control at the 5% level.

mature, (4) damaged immature, or (5) smutty, as some seeds developed smut (Figure 1). Normal immature seeds had lemma shapes and sizes similar to normal mature seeds, but the caryopses were absent or only partially filled. Damaged mature seeds were small and elongated compared to nondamaged seeds with the lemma curled around the caryopsis and twisted laterally. Damaged immature seeds were shaped like damaged mature seeds, but the caryopsis was absent or only partially filled.

Damaged immature, normal immature, and smutty seeds were not viable. The viability of damaged mature and normal mature seeds was assessed via a germination test. All damaged mature and normal mature seeds from a pot were tested, unless the number of seeds exceeded 200, in which case only 200 seeds were tested. Seeds were incubated in 100 by 15–mm petri dishes (100 or less seeds per dish) for 30 d or until they germinated. Each dish contained a piece of filter paper supported by a polyurethane foam disc. Distilled water was supplied continuously via a cotton wick inserted in a hole in the center of the disc. Light was supplied for 12 h a day with cool-white fluorescent bulbs (PAR = 30 μmol/m^2/s [= 2.8 μmol/ft^2/s]) and temperature was held at 21 and 15 C during the light and dark periods, respectively. Seeds were recorded as germinable and removed from the petri dishes if they developed radicles and coleoptiles exceeding 5 mm in length. The number of germinable seeds per plant was estimated by multiplying the total number of damaged mature and normal mature seeds by the proportion of seeds that germinated.

Aboveground biomass data were analyzed with ANOVA for a randomized complete block design using the MIXED procedure of SAS[3] (Littell et al. 1996). The model included fixed effects for treatment, experiment (1 or 2), and their interaction and a random block effect. Significant differences were declared at $P < 0.05$, and means from significant main and interaction effects were separated using tests of simple effects.

Seed data (i.e., number of seeds per plant) were not well approximated by normal distributions because they contained a large number of zeros. Therefore, seed data were analyzed with a nonparametric procedure (i.e., bootstrap) (Efron and Tibshirani 1993; Hjorth 1994). For each treatment, we gathered 10,000 bootstrap samples by repeatedly calculating the mean of n (n = number of repetitions) data points sampled randomly with replacement. These bootstrap samples were used to make two-tailed tests of controls vs. treatments at the 5% level. All bootstrap calculations were conducted in Mathematica 6.0.[4]

Results and Discussion

Most herbicide treatments had only minor effects on biomass production relative to controls (Figure 2). Excep-

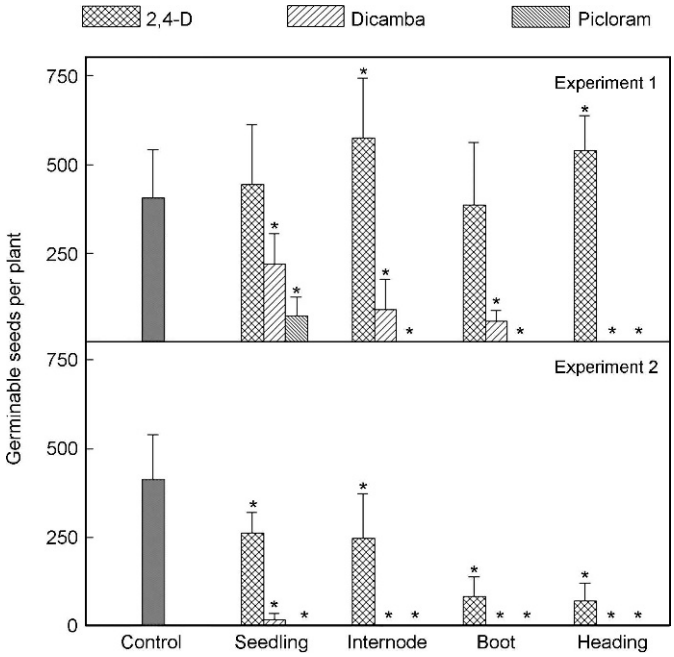

Figure 3. Effects of growth regulator herbicides on germinable seed production of Japanese brome. Bars denote standard error and asterisks denote significant differences from control at the 5% level.

tions included (1) picloram at the seedling stage, which increased and decreased biomass in experiments 1 and 2, respectively; (2) picloram at the internode stage, which decreased biomass in both experiments; and (3) dicamba at the seedling stage, which reduced biomass in experiment 2. These responses aside, our findings are consistent with other research suggesting growth regulators often do not appreciably reduce biomass production of established annual grass plants (e.g., DiTomaso et al. 2006b; Shinn and Thill 2002).

Compared to the plant biomass responses, seed production responses were more consistently and dramatically negative (Figure 3). Picloram was the most effective herbicide; when applied at the internode stage or later, it all but eliminated germinable seed production in both experiments. Somewhat similarly, dicamba reduced seed production at all timings in both experiments and nearly eliminated seed production when applied at heading. Compared to picloram and dicamba, 2,4-D had a less consistent effect, though it did reduce seed production at all application timings in experiment 2. The finding that 2,4-D was less effective than dicamba agrees with findings from one winter wheat study (M. J. Rinella, personal communication) but is inconsistent with another winter wheat study where both chemicals reduced seed production similarly (Robison and Fenster 1973).

Growth regulator herbicides are most damaging to grass seed production when applied after initiation of jointing but before seeds begin to form. Applying growth regulators for effective control in this window could be difficult if invasive grass plants are not developmentally synchronized, i.e., if spatial variation exists in plant phenology across the landscape being treated. However, in observing Japanese brome in the field, we have noted that phenotypic development of individuals within populations tends to be highly uniform, which suggests timing herbicide applications to susceptible growth stages may be possible. Nonetheless, it will be important to test growth regulators under field conditions before promoting their use for invasive annual grass management.

The application timing window we identified for most effective control of invasive annual grasses with growth regulators partially overlaps the (much wider) window for controlling leafy spurge (*Euphorbia esula* L.), spotted knapweed (*Centaurea stoebe* L., = *Centaurea maculosa* Lam.), and other broadleaf weeds with these chemicals. So it may be possible to time growth regulator herbicide applications to simultaneously target both grass and broadleaf weeds on degraded grasslands. If effective, this approach could overcome the common problem of invasive annual grasses proliferating after herbicidal control of broadleaf weeds (e.g., DiTomaso et al. 2006b).

Growth regulators can cause long-term damage to native dicot (i.e., forb and shrub) populations (Crone et al. 2009; Rinella et al. 2009), so use of these herbicides for invasive annual grass control may be unadvisable where native dicots are present, or it may be necessary to reestablish dicots following annual grass control. Use may also be unadvisable in the presence of native annual grasses such as sixweeks fescue [*Vulpia octoflora* (Walter) Rydb. var. *glauca* (Nutt.) Fernald] or desert fescue [*Vulpia microstachys* (Nutt.) Munro var. *microstachys*], as growth regulators could reduce seed production of these species. However, it is important to note that currently used invasive annual grass herbicides pose similar risks to dicots and annual grasses. Furthermore, compared to growth regulators, currently used herbicides likely pose greater risks to perennial grasses, because they are capable of extensively damaging perennial grasses (Lym and Kirby 1991; Shinn and Thill 2004). Conversely, perennial grasses very often proliferate after growth regulators are used to control perennial weeds (Lym and Messersmith 1985; Sheley et al. 2000), suggesting growth regulator damage to perennial grasses is negligible.

Growth regulator herbicides drastically reduce seed production of Japanese brome and several cereal grasses (Friesen et al. 1968; Rinella et al. 2001; Sikkema et al. 2007). That growth regulators reduce seed production in so many grasses suggests that cheatgrass, medusahead, and other untested invasive cool-season annual grasses may also prove sensitive to growth regulator herbicides. Furthermore, although we did not test multiple herbicide rates,

Rinella et al. (2001) found that winter wheat seed production responded similarly to low and high rates of dicamba. This leaves open the possibility that rates much lower than those tested might be sufficient for preventing seed production in invasive annual grasses.

Invasive annual grass populations tend to decline rapidly when seed inputs are prevented. Smith et al. (2008) measured cheatgrass seedling emergence after clipping inflorescences to prevent seed rain. They found that many seedlings continued to emerge following 1 yr of clipping but that germination was reduced by more than 96% after seed bank entry was prevented for 2 yr. Hulbert (1955) conducted a similar study and found that preventing seed input for just 1 yr nearly eradicated cheatgrass from plots. Burnside et al. (1996) buried Japanese brome and cheatgrass seeds 20 cm deep in field conditions and found that cheatgrass germination dropped from 95 to 2% after 1 yr and to 0% after 2 yr. Corresponding numbers for Japanese brome were 95, 5, and 1%. The short seed lives of the grasses in these studies suggest that using growth regulator herbicides to reduce seed inputs for 1 to 3 yr could greatly reduce invasive annual grass populations.

Sources of Materials

[1] Sunshine Mix #1, Sun Gro Horticulture, Inc., 15831 NE 8th Street, no. 100, Bellevue, WA 98008.

[2] T-Jet Technologies, 4-XR TeeJet 8200VS nozzles, Wheaton, IL.

[3] SAS Institute, Cary, NC.

[4] Mathematica, Version 6.0, Wolfram Research, Inc., Champaign, IL.

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