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SHORT COMMUNICATION

# Herbicides Can Negatively Affect Seed Performance in Native Plants

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## Abstract

**Herbicides are widely used to control invasive non-native plants in wildlands, yet there is little information on their non-target effects, including on native plants that are intended to benefit from the treatment. Effects at the seed stage have been particularly understudied, despite the fact that managers commonly seed native plants immediately after herbicide application. We conducted a greenhouse experiment to explore the effects of two broadleaf-specific herbicides (aminopyralid and picloram) on seedling emergence and biomass for 14 species that grow in dry grasslands of NW North America. For each species, we placed 50 seeds in soil-filled pots that were sprayed with a water control or one of the herbicides at one of two rates (1× and 0.01× of the recommended rate). After 5 weeks, we assessed seedling emergence and dry aboveground biomass per pot. At the recommended rate (1×), both herbicides significantly suppressed seedling emergence and lowered biomass. At the diluted rate (0.01×), the effect of picloram was comparable to the effect at the recommended rate, whereas aminopyralid had no effect. There was no difference in effects of herbicides on native versus non-native species. Although both herbicides are considered to be broadleaf-specific, monocots were just as vulnerable as dicots at the recommended rate. Our results show that herbicides can harm non-native and native plants at the seed stage, alike. Land managers should avoid spraying if recruitment of native species from the seedbank is a goal and should not seed directly after spraying.**

**Key words: aminopyralid, exotic plants, germination, invasive plants, invasive species management, land management, non-target effects, pesticides, picloram, restoration, seeding, seeds, toxicity.**

## Introduction

In many countries, land managers use herbicides to combat invasive non-native plants (e.g. in Australia: Rokich et al. 2009, in Canada and the United States: Appleby 2005). Non-selective herbicides target all plant species, whereas selective herbicides target specific groups, such as broadleaf plants (Davy 2002). Seeds are often sown immediately after herbicide application to enrich the depauperate native seed bank (Biggerstaff & Beck 2007). Because of their toxicity, herbicides can pose a risk to non-target plants (Follak & Hurle 2002). Whereas their effects on the cover of native species have been studied (Tyser et al. 1998; Sheley & Denny 2006), little is known about their effects on seed performance of native plants, despite the importance of viable seeds for restocking the native seed bank. We carried out a greenhouse experiment to test the effects of two herbicides on seed performance of selected dry grassland plants from western North America. We asked: (1) Do herbicides affect seedling emergence and biomass of non-target native and target invasive plants? (2) Is there a difference between the recommended and a diluted application rate? (3) Do effects differ between monocot and dicot plants, and native and non-native plants?

## Methods

### Study Herbicides

We used aminopyralid (Milestone®, Dow AgroSciences, Indianapolis, IN, U.S.A.) and picloram (Tordon K®, Dow AgroSciences), two active ingredients commonly used in invasive species management. Both herbicides are auxin-mimicking and dicot-selective (Fedtke & Duke 2005). They can harm seeds by inhibiting oxygen utilization or can stimulate germination when present at very low dosages (Hsueh & Lou 1947).

### Experimental Design

We used 14 species for our greenhouse experiment (Table S1, Supporting Information). Pots (9.6 cm in diameter, 9 cm high) were filled with a 1:2 mixture of 2 mm-sieved washed sand and loamy topsoil that had been sterilized at 180°C for 1 hour to kill the soil seed bank. We applied herbicide at a recommended rate (1×) based on the manufacturer's instructions, to simulate direct exposure, and a diluted rate (0.01× of the recommended rate, mixed with water), to simulate exposure to herbicide drift. We also hypothesized that low dosages might trigger a positive

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effect as has been shown for other auxin mimicking herbicides (e.g. Hsueh & Lou 1947). Spraying was carried out using a hand-held sprayer, with a rate of 4.78 L/ha of Tordon (1.17 L/ha of active ingredient) and 0.52 L/ha of Milestone (0.21 L/ha) for the 1× rate. We used water as a control treatment.

The study design included 280 pots (five treatments [aminopyralid 1×, aminopyralid 0.01×, picloram 1×, picloram 0.01×, control] × four replicates × 14 species). Owing to an insufficient number of seeds in some species, the realized number was 275 pots. On 3 April 2012, we sprayed pots outdoors (0 km/hour wind speed, 16°C) and, after 2 hours, transferred them to the greenhouse. We immediately added 50 seeds per pot, with 20 pots per species, and incorporated the seeds into the upper 1 cm soil layer using a clean toothpick.

Seedling emergence was quantified after 5 weeks as the number of living seedlings in the pot, including those that showed only a radicle. In addition, at the end of the 5 weeks we harvested all emerged plants (referred to as seedlings) at the soil surface and dried them at 70°C for 24 hour prior to weighing to determine final biomass.

### Data Analysis

Seedling emergence data was highly zero inflated, causing strong overdispersion in Poisson regression and producing biased estimates, even with quasipoisson or negative binomial error families. Consequently, we analyzed seedling number using linear models with a Gaussian error family. Biomass for all pots was analyzed using the same model type. We tested for differences in response between native and non-native plants (origin group), and between monocots and dicots (functional group) using analysis of variance (ANOVA). All analyses were performed in R (R Development Core Team 2012).

## Results

All herbicide treatments suppressed seedling emergence when species were pooled for analysis (Table 1). However, when species was incorporated as an additional factor, the fit of the model improved significantly (AIC 2111.2 vs 1913.6), indicating strong species-specific responses to herbicide treatments. Picloram at both concentrations and aminopyralid at the recommended rate significantly suppressed seedling emergence in all species (except in non-native *Linaria dalmatica*; Fig. 1).

**Table 1.** Test of herbicide treatments on the number of emerged seedlings per pot.

| | *Estimate* | *Standard Error* | t | p |
|---|---|---|---|---|
| Intercept (control) | 25.6 | 1.5 | 17.1 | **<0.05** |
| Aminopyralid 0.01× | −6.6 | 2.1 | −3.1 | **<0.05** |
| Aminopyralid 1× | −25.4 | 2.1 | −12.0 | **<0.05** |
| Picloram 0.01× | −20.8 | 2.1 | −9.8 | **<0.05** |
| Picloram 1× | −25.5 | 2.1 | −12.0 | **<0.05** |

Results are based on analysis of variance and shown as treatment contrasts for estimated parameters. Significant $p$ levels (<0.05) are in bold.

Native and non-native plants did not differ in their response (ANOVA, treatment × origin group, $F_{[4, 265]} = 0.36$, $p = 0.8$). Functional groups (monocots vs dicots) responded differently to herbicide treatments (ANOVA, treatment × functional group, $F_{[4, 265]} = 18.59$, $p < 0.05$) but only for the control (Tukey Honest Significant Differences test, 32.2 vs 13.2, $p < 0.05$) and aminopyralid 0.01× treatment (27.3 vs 3.3, $p < 0.05$).

Biomass per pot was significantly correlated with seedling emergence (Pearson's $r = 0.807$, $p < 0.05$) and herbicides affected biomass, in a similar way (Table S2; Fig. S1).

## Discussion

We found that two common herbicides can negatively affect seedling emergence and biomass of both non-native and native plants. Seeds might be harmed at the recommended field application rates and even at low-rate exposure. Our findings corroborate the results of Biggerstaff and Beck (2007) and Rokich et al. (2009), who also detected negative effect of herbicides on seed performance in native plants in North America and Australia.

Although our study herbicides are marketed as dicot-specific, they had a negative effect on monocots at the seed stage. This could be explained by the fact that monocot seeds lack the morphological features that protect mature monocot plants from detrimental effects, such as leaf sheaths that enclose the meristem (Cobb & Reade 2010). Furthermore, they have a lower biomass and could have a higher relative exposure than mature monocots.

The negative effect of herbicides on seed germination can be beneficial for land management when the seed bank is mostly made up of non-native species. By contrast, when the soil seed bank harbors significant amounts of desired species, such as native ones, spraying herbicides could undermine restoration efforts. Applying herbicides immediately before or after sowing seeds of native species might be similarly counterproductive. The ultimate effects of herbicides under field conditions will depend on a variety of factors that need to be considered in future studies, including the depth of the soil seed bank, soil and climate conditions, and the type of herbicide. As effects at the seed stage are difficult to assess visually in the field, land managers will have to monitor responses at the seed stage through experiments.

### Implications for Practice

- Herbicides are routinely used in land management to control non-native invasive plants in favor of native species. However, when applied at the recommended rate, they can harm desired native plants at the seed stage.
- Although the studied herbicides are marketed as broadleaf selective herbicides, they can harm seeds of monocot and dicot species, alike.
- The herbicide's toxic properties might be beneficial when the seed bank is almost entirely made up of undesired non-native species.

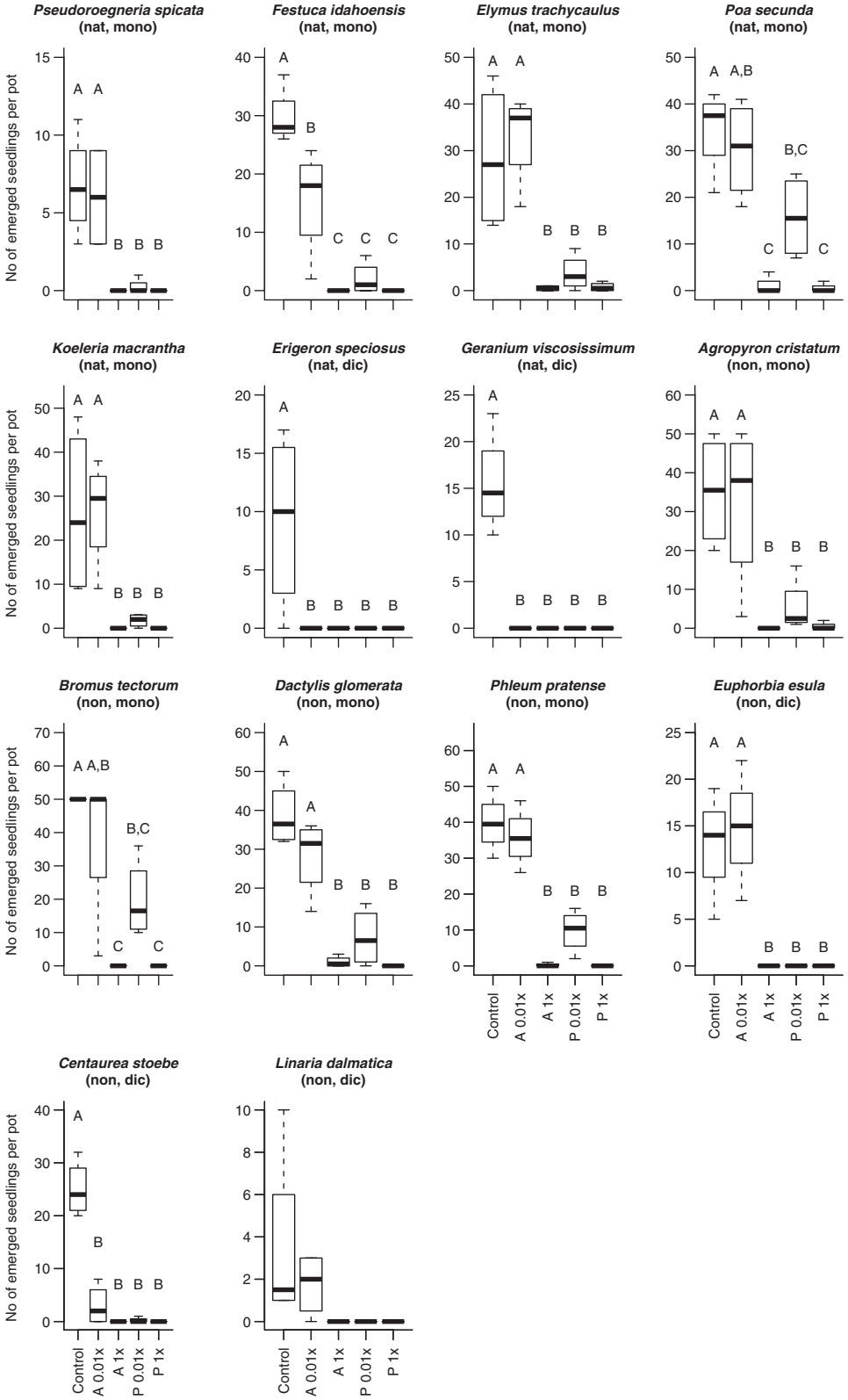


Figure 1. Overview of treatment effects on the number of emerged seedlings per pot. Same letters above boxplots indicate no significant differences among treatments ($p \geq 0.05$), based on a Tukey Honest Significant Differences test. Except for *Linaria dalmatica*, all species showed significant treatment effects according to an ANOVA. A 0.01×, aminopyralid (0.01× rate); A 1×, aminopyralid (1× rate); P 0.01×, picloram (0.01× rate); and P 1×, picloram (1× rate); nat, native; mono, monocot; non, non-native invasive; dic, dicot.

- When the soil contains substantial amounts of seeds of native species, it might be counterproductive to spray with herbicides.
- We suggest avoiding seeding native plants immediately after spraying.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of study species by functional group, including family and seed source.

Table S2. Test of herbicide treatments on biomass per pot.

Figure S1. Overview of herbicide effects on biomass per pot.