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Effects of Agricultural Chemicals on Native Plants of the Northern Great Plains

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EFFECTS OF AGRICULTURAL CHEMICALS ON NATIVE PLANTS OF THE NORTHERN GREAT PLAINS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

EFFECTS OF AGRICULTURAL CHEMICALS ON NATIVE PLANTS OF THE NORTHERN GREAT PLAINS

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Agricultural chemicals are ubiquitous on the Northern Great Plains landscape and have negative impacts on non-target plant communities, even at small doses. Northern Great Plains grassland plant communities may experience herbicide drift from agricultural fields or be subject to livestock pharmaceuticals in grazing lands. My research objective was to evaluate if and how native plants are affected by agricultural chemical presence at different concentrations. In Chapter 2, I studied the effect of different concentrations of three common agricultural herbicides (2,4-D, atrazine, and trifluralin) on the germination, emergence, and growth of native plant species of the Northern Great Plains. I performed growth chamber and greenhouse experiments in which seeds were treated with a range of herbicide concentrations found in herbicide drift. My results show that these herbicides can negatively affect certain native plant species' germination, emergence, and growth at a wide range of concentrations. In Chapter 3, I performed a growth chamber experiment to study the effect of two concentrations of ivermectin on the germination and growth of Northern Great Plains native plant species. My results show that four of 14 study species were negatively affected by high and low residual levels of ivermectin. This information can be used to recommend which species may perform well in ecological restorations adjacent to agricultural fields and how livestock management decisions may affect native plant communities.

CHAPTER 1: INTRODUCTION

Grasslands are an important ecosystem worldwide, providing a variety of provisioning, supporting, and cultural ecosystem services such as water quality, erosion control, carbon storage, livestock production, recreation, and pollination (Bengtsson et al. 2019). In North America, one important grassland system is the Northern Great Plains (NGP). The NGP consists of areas in the United States (Nebraska, South Dakota, North Dakota, Wyoming, and Montana) as well as Canada (Saskatchewan and Alberta), and is home to 220 butterfly species, 95 mammal species, and over 1,600 species of native plants (Perkins et al. 2019).

Native plants are a crucial component of a healthy ecosystem and provide many ecosystem services that benefit wildlife, pollinators, and people (Plant Conservation Alliance 2021). Native plant communities are considerably more diverse than monocultures created when non-native plants invade an area (BLM 2015). The structural and functional complexity of diverse communities of native plants enhances water and nutrient uptake, reduces runoff (Hernandez-Santana et al. 2013) and decreases soil loss through wind and water erosion (Porensky et al. 2014). Additionally, native plant communities are a valuable source of food, habitat, and nesting areas for wildlife (BLM 2015), and are important for recreation activities including hunting, birding, hiking (Espenshade et al. 2018).

Unfortunately, many factors threaten the presence of native plant species in the landscape. Overgrazing can decrease plant species richness in grazing lands, therefore creating a less diverse native plant landscape (Liu et al. 2022). Invasive species may also decrease native plant presence by dominating the landscape through resource competition

or ecological engineering (Vilà and Weiner 2004). Another threat to native plants is habitat loss through the conversion of native grasslands to crop production in the NGP (Wright and Wimberly 2013). Furthermore, the remaining native plants adjacent to agricultural fields may be negatively impacted by commonly used agricultural chemicals (Wagner and Nelson 2014).

Anthropogenic chemicals that may affect non-target native plant species include fertilizers, herbicides, and veterinary medicines. Fertilizer exposure, even at low concentrations, can affect the timing of flowering, probability of flowering, and plant height (Russo et al. 2020). Exposure to lethal and sublethal concentrations of herbicides can impact native plants that exist in an agricultural matrix (Florencia et al. 2017) through community-level effects like decreased richness and cover (Peterson et al. 2020), or through effects to individual plants such as changes in phenology (Boutin et al. 2014) or germination and establishment (Helander et al. 2019).

Although many factors, such as agricultural chemicals, lead to the decline of native plant communities, there are also opportunities to support and conserve native plants. To assist in the recovery of ecosystems that have been degraded or destroyed, land managers can practice ecological restoration (Society for Ecological Restoration International Science & Policy Working Group 2004) in areas such as retired agricultural lands or degraded remnant grasslands. Ecological restoration may increase the presence of native species on the landscape compared to the prior land use in that area. Land managers can also sow desirable native species into already existent grassland to increase the diversity and abundance of native plants on the land (Johnson et al. 2018). Both

ecosystem restoration and responsible livestock management can allow for the reintroduction and maintenance of native plants on the NGP landscape.

The goal of my research was to study the effect of agricultural chemicals (herbicides and ivermectin) on native plant communities of the NGP. This information will be used to inform ecological restoration practitioners and livestock managers of best management practices for their operations to promote best germination, emergence, and growth of native plant species. In Chapter 2, I examined the effect of 2,4-D, atrazine, and trifluralin at different concentrations on the germination, emergence, and growth of NGP native plants. Based on the information gained, I provided recommendations for planting these species in an ecological restoration. In Chapter 3, I examined the effect of ivermectin at two concentrations on the germination of NGP native plants. Using the knowledge gained from my research, I discussed livestock management implications on native plant species.

CHAPTER 2: HERBICIDE EFFECTS ON NATIVE PLANTS OF THE NORTHERN GREAT PLAINS

Introduction

Herbicides can have negative impacts on non-target plants, even at concentrations below the recommended herbicide application rate such as those found in herbicide drift (Olszyk et al. 2013, Olszyk et al. 2017, Schmitz et al. 2014). Herbicide drift is the movement of herbicide particles through the air, at or soon after application, to an unintended area (US Environmental Protection Agency 2023a). For example, a common herbicide (glyphosate) applied at 10-20% of the recommended field application rate decreased reproduction in six out of the nine study species, consisting of native forbs and grasses. (Olszyk et al. 2017). Further, herbicide (sulfonylurea) application at 30% of the recommended field application rate caused sublethal effects to grassland plants, such as leaf damage and flower suppression, and caused significantly lower species diversity than in control plots (Schmitz et al. 2014). Although these examples demonstrate herbicide effects to mature non-target plants, there is a knowledge gap on the effect of many herbicides on non-target plants at the germination and emergence stage.

Germination and emergence are crucial, yet sensitive, stages in a plants' life history. In ecological studies, germination is the life stage in which the radicle protrudes from the seed (Baskin et al. 2002). Germination is a complex process (Nonogaki et al. 2010), and as the first step in establishing a new plant (Bewley 1997), determines whether the process of plant growth will begin. Emergence is when a seedling emerges from the soil (Heydecker 1956) and is weaned from its dependence on seed reserves (Forcella et al. 2000). Seedling emergence is another important plant life history event

(Forcella et al. 2000) that facilitates the transition from a germinated seed to a new plant. These critical life history stages are sensitive and may be affected by herbicide exposure (McManamen et al. 2018; Rokich et al. 2009).

Effective ecological restoration relies on the success of germination and emergence to begin the creation of a new plant. Ecological restoration assists in the recovery of ecosystems that have been degraded or destroyed (Society for Ecological Restoration International Science & Policy Working Group 2004), such as retired agricultural lands or degraded remnant grasslands, which are present throughout the Northern Great Plains (NGP) region of North America. Knowledge on what affects seed germination can enhance restoration of damaged ecosystems (Broadhurst et al. 2016; Baskin and Baskin 2014). However, because germination and emergence are relatively separate processes (Fenner and Thompson 2005; Zhang et al. 2021), both are important to consider in species selection for ecological restoration.

Successful germination, emergence, and growth of plants during seed-based ecological restoration may be threatened by the proximity to agricultural lands using herbicides, as seeds/seedlings are sensitive to external factors such as herbicides. Habitat fragmentation is common in the NGP (Wimberly et al. 2018) and therefore restoration sites are likely adjacent to agricultural lands where herbicides are used. The NGP provides habitat for 95 mammal species, 220 butterfly species, and over 1,600 species of native plants (Perkins et al. 2019). However, less than 63% of the NGP remains native grassland (Samson et al. 2004) due to development and conversion (Wimberly et al. 2018). The fragmentation that exists in the NGP today (Wimberly et al. 2018) coupled with the current extent of herbicide use (US Environmental Protection Agency 2023b)

means that herbicides may readily drift to adjacent nontarget lands (Kleijn and Snoeijing 1997; Vieira et al. 2020), exposing grasslands to herbicides (Wimberly et al. 2018). This phenomenon may affect the germination, emergence, and growth of native plants in ecological restoration sites, as non-target native plants can be affected by herbicides (Olszyk et al. 2013, Olszyk et al. 2017, Schmitz et al. 2014).

To examine the response of native plants used in NGP seed-based ecological restorations to below-recommended application rates of herbicide consistent with those present in drift, I chose to study three of the most commonly used herbicides in the United States: 2,4-D, atrazine, and trifluralin (US Environmental Protection Agency 2023b). Each of these herbicides have a different mode of action, and each can affect nontarget seeds or seedlings (Jacobs et al. 2007, Lair and Redente 2004, Erusha et al. 1991). 2,4-D is a synthetic auxin that targets post-emergent broadleaves (dicots) (Song 2014). Synthetic auxins are synthesized hormones made to mimic natural auxins and can have phytotoxic effects (Flasiński and Hąc-Wydro 2014) by causing rapid and uncontrolled growth (Grossmann 2010). Atrazine targets pre- and post-emergent grasses (monocots) and dicots by inhibiting photosystem II (Helena Chemical Company 2010; Steinback et al. 1981), a crucial part of the photosynthetic process (Nickelsen and Rengstl 2013). Trifluralin is a microtubule inhibitor which targets pre-emergent monocots and dicots (Vaughn and Lehnen 1991; Agri Star 2008). Microtubule inhibitors affect tubulin, an important microtubule protein, which leads to the disruption of mitosis (Callahan et al. 1996).

The objective of my study was to evaluate if native seed germination, emergence, and growth are affected by 2,4-D, atrazine, or trifluralin at different concentrations

representative of herbicide drift. To achieve this, I performed growth chamber and greenhouse experiments in which I subjected NGP native plant species to different concentrations of the study herbicides. I hypothesized that trifluralin would have the greatest effect on germination, as it inhibits root growth; that atrazine would have the greatest effect on plant growth, as it inhibits photosynthesis; and that 2,4-D would affect post-emergent dicots, as it is intended to target. The information learned will be used to inform land managers and restoration practitioners of recommendations and best practices when performing ecological restoration adjacent to agricultural land.

Methods

Germination Experiment

I conducted germination experiments to determine the effect of three agricultural herbicides, 2,4-D, atrazine, and trifluralin on the germination of 14 plant species, consisting of monocots and dicots, native to the Northern Great Plains (Table 1). Study species were chosen based on their widespread use in seed-based ecological restoration and species selection guides in the NGP (Lair and Redente 2004; Piper et al. 2007; Campbell and Hooymans 2016; Foster et. al 2007; USDA Natural Resource Conservation Service 2016; Xerces Society for Invertebrate Conservation 2018). Seeds were commercially sourced from Prairie Moon Nursery (Winona, MN) and Millborn Seeds (Brookings, SD), except for *P. albidus* which was harvested from the South Dakota State University Native Plant Initiative seed production plots (Table 1). Seeds were stored in a refrigerated environment at 5°C upon acquisition and were used within <5 months of storage.

I exposed eight replicates of 25 seeds from the study species to six different concentrations of each herbicide. Each herbicide was made into a stock solution of 100% of the recommended application rate for commercial corn production (2,4-D: 1 pint/acre, atrazine: 4 pints/acre, trifluralin: 1.5 pints/acre). I diluted that solution to 50, 10, 1, and 0.1% of the recommended application rate. These herbicide concentrations were intended to simulate the range of concentrations a seed may be exposed to from herbicide drift from adjacent agricultural land. Deionized water was used as a control. Germination paper (3.5" x 4") was saturated with a given herbicide solution and placed in a 6 mil reclosable plastic bag. Then, I placed twenty-five seeds of the given species in a five-by-five grid on the germination paper in each bag. The bags were put in a germination chamber for observations to begin the following day (day 1). The germination chamber was set to a 12-hour day/night cycle with 18°C during light periods and 6°C during dark. These temperatures were gathered from NOAA National Centers for Environmental Information in 2021 and reflect the 30-year average day and night temperatures of the NGP during May.

I counted the number of germinants once a day for the first seven days of the experiment, and then every other day for 45 days, as 45 days is a common germination experiment run-time (Sabina and Dorin 2015; Calles et al. 2020; Sorgato et al. 2020). If germination was still observed at day 45, observations continued every other day until there were two observation days without germination, or until day 91 when the experiment was ended. I considered a seed to be germinated when I observed a radicle protruding from the seed coat.

Data were analyzed with R (v4.1.2; R Core Team, 2021) using ANOVA and Tukey's HSD as a multiple comparison test among concentrations of each herbicide. Response variables include final germination percent (total germination / total seeds *100) and mean germination rate (total germination / length of experiment) of each study species and the explanatory variable was herbicide concentration.

Emergence and Growth Experiment

I conducted a greenhouse study to determine the effect of three herbicides (2,4-D, atrazine, and trifluralin) on the emergence and subsequent growth of 14 native plant species of the Northern Great Plains (Table 1). Research was conducted at South Dakota State University in Brookings, SD, USA. Seeds were commercially sourced from Prairie Moon Nursery (Winona, MN) and Millborn Seeds (Brookings, SD) and were stored in a refrigerated environment at 5°C upon acquisition and were used within <10 months of storage.

I exposed ten replicates of one seedling from the study species to five different concentrations of each herbicide. Each herbicide was made into a stock solution of 100% of the recommended application rate for commercial corn production (2,4-D: 1 pint/acre, atrazine: 4 pints/acre, trifluralin: 1.5 pints/acre), and then made into dilutions of 10, 1, and 0.1%. Deionized water was used as the control (0%). These concentrations simulate the potential range of herbicide concentration a seed may be exposed to in ecological restoration occurring near agricultural lands. I scaled the recommended amount of herbicide per acre to amount of herbicide per area of soil surface in a conetainer (Stuewe & Sons, Inc., 1in. x 4.75in.)

Conetainers were filled with Pro-Mix BX potting medium and misted with deionized water. I chose to use potting medium as field soils generally do not have the necessary aeration, drainage, and water holding capacity for greenhouse production, and would also need to be pasteurized or fumigated to prevent issues from diseases and weeds (UMass Extension n.d.). I planted three seeds in each conetainer, then covered them with a fine layer of potting medium and misted them again. Using a pipette, I then applied 1 mL of herbicide solution to each conetainer in a circular motion to ensure even distribution. Conetainers were randomly arranged within trays, and tray positions were randomized weekly to eliminate the potential impacts of environmental variation. Trays were irrigated with water and fertilizer (3.38 oz/gal Jack's Professional 20-10-20 Peat-Lite) every two days for three minutes via overhead mist. I used this watering schedule to prevent over-saturation of the soil, which could cause microbial growth, and to prevent the soil from drying completely, which could cause plant stress. The greenhouse was set to 21°C and used only natural lighting. The greenhouse received approximately 10 hours of light each day for the duration of the experiment.

Conetainers were observed daily for emergence and seedling death. I defined emergence as the protrusion of any seedling part out of the soil. Seedlings were considered dead if over 75% of the seedling had senesced. If multiple seedlings emerged, they were thinned to one seedling per conetainer. Seedling height and leaf count were recorded on day 7, 14, 21, and 28 after emergence. After the measurements were taken on day 28, the seedlings were clipped at the soil surface and placed in a paper envelope. These dry biomass samples were oven dried at 60°C for a minimum of 48 hours, then weighed to the nearest tenth of a milligram.

Response variables were time to emergence (days), probability of death, final height (cm), final leaf count (number of leaves), and dry biomass (mg). The explanatory variable was herbicide concentration. Data were analyzed with R (v4.1.2; R Core Team, 2021). Time to emergence, final height, and dry biomass were analyzed using ANOVA and Tukey's HSD, with a log transformation on all time to emergence data. I performed log transformations on final height and dry biomass data on a species-basis as necessary for normality (Tables 3-5). Probability of death was analyzed with a binomial family generalized linear model and likelihood ratio test. No multiple comparison procedure was used for probability of death due to model constraints. Final leaf count was analyzed with a Poisson family generalized linear model and likelihood ratio test. No multiple comparison procedure was used for final leaf count due to model constraints.

Results

Germination Experiment

Germination of many study species decreased with the presence of herbicides at different concentrations. Final germination percent and germination rate of monocots and dicots were highly affected by exposure to increasing concentrations of 2,4-D (Table 2, Figure 1 and 4). No discernible trends were evident in germination response to atrazine and trifluralin concentrations representative of herbicide drift (Tables 3 and 4; Figures 2, 3, 5, and 6). The germination of *D. purpurea* was unaffected by any herbicide at any concentration.

The varying concentrations of 2,4-D elicited a broad range of effects on the final germination percent of monocot and dicot study species (Figure 1). Final germination percent decreased for eleven species when compared to the control treatment. At 100%

recommended application rate, final germination percent of *H. helianthoides* decreased by 10%. At 50% recommended application rate, final germination percent of *A. tuberosa*, *E. trachycaulus*, *M. fistulosa*, and *R. pinnata* decreased by 25-56%. At 10% recommended application rate, final germination percent of *A. gerardii*, *L. pycnostachya*, and *N. viridula* decreased by 26-79%. At 1% recommended application rate, *L. aspera*, *S. laeve*, and *S. nutans* decreased by 20-71%. Final germination percent of *D. purpurea*, *P. smithii*, and *P. virgatum* did not decrease when compared to the control (Table 2).

The differing concentrations of 2,4-D also affected germination rates of monocot and dicot study species at different levels (Figure 4). Germination rate decreased for eight species when compared to the control treatment. At 50% recommended application rate, germination rate of *L. aspera*, *L. pycnostachya*, and *P. virgatum* decreased by 21-43%. At 10% recommended application rate, germination rate of *M. fistulosa* decreased by 48%. At 1% recommended application rate, germination rate of *A. gerardii* and *S. nutans* decreased by 50-62%. At 0.1% recommended application rate, germination rate of *R. pinnata* and *S. leave* decreased by 20-33%. Germination rate of *A. tuberosa*, *D. purpurea*, *E. trachycaulus*, *H. helianthoides*, *N. viridula*, *P. smithii* did not decrease when compared to the control (Table 2).

Emergence and Growth Experiment

Herbicide exposure, even at concentrations below those recommended for use in agricultural settings, decreased emergence and growth of multiple study species. 2,4-D and trifluralin only affected emergence and growth at 100% recommended application rate. Atrazine affected emergence and growth for a greater number of species at

concentrations as low as 10%. All emergence and growth metrics for *E. trachycaulus* and *N. viridula* were affected by exposure to increasing concentrations of atrazine. No other herbicide affected all emergence and growth metrics for any species.

Application of 2,4-D at 100% recommended application rate affected emergence and growth of monocot and dicot species compared to the control (Table 5). Time to emergence increased by 34-156% for *D. purpurea*, *E. trachycaulus*, *H. helianthoide*s, *M. fistulosa*, *P. virgatum*, and *R. pinnata* (Figure 7). Probability of death was notably greater for *A. tuberosa* and *R. pinnata*, with no death occurring at the lower concentrations (Figure 8). Final height decreased by 22-47% for *E. trachycaulus*, *H. helianthoides*, and *R. pinnata* (Figure 9). Final leaf count decreased by 52% for *M. fistulosa* (Figure 10). Dry biomass decreased by 45-71% for *E. trachycaulus*, *H. helianthoides*, *M. fistulosa*, and *R. pinnata* (Figure 11). Tukey's HSD was unable to differentiate the effect by concentration on dry biomass for *L. aspera*, despite significance shown in the ANOVA.

Trifluralin application at 100% recommended application rate affected emergence and growth of monocot and dicot species compared to the control (Table 6). Time to emergence increased by 33% for *E. trachycaulus* (Figure 12). Probability of death increased for *D. purpurea*, *N. viridula*, and *P. virgatum* (Figure 13). Final height decreased by 20-96% for *E. trachycaulus*, *P. smithii*, and *P. virgatum* (Figure 14). Final leaf count decreased by 42-52% for *A. tuberosa*, *D. purpurea*, *M. fistulosa*, and *P. smithii* (Figure 15). Dry biomass decreased by 54-94% for *E. trachycaulus*, *M. fistulosa*, *P. smithii*, *P. virgatum*, *S. laeve*, and *S. nutans* (Figure 16). Tukey's HSD was unable to differentiate the effect by concentration on final leaf count for *P. virgatum*, despite significance shown in the ANOVA.

Atrazine application at different concentrations negatively affected emergence and growth of monocot and dicot species compared to the control (Table 7). Time to emergence, at 100% recommended application rate, decreased by 13-36% for *A. tuberosa*, *E. trachycaulus*, *H. helianthoides*, *L. aspera*, *M. fistulosa*, and *N. viridula;* and at 50% recommended application rate, decreased by 5-23% for *D. purpurea*, *P. smithii*, and *R. pinnata* (Figure 17). Probability of death, at 100% recommended application rate, increased for *A. tuberosa*, *E. trachycaulus* and *N. viridula*; and at 10% recommended application rate, increased for *D. purpurea*, *H. helianthoides*, *M. fistulosa*, *P. smithii*, *R. pinnata*, and *S. laeve* (Figure 18). Final height, at 100% recommended application rate, decreased by 37% for *P. virgatum*; and at 10% recommended application rate, decreased by 29-43% for *E. trachycaulus*, *H. helianthoides*, *N. viridula*, and *P. smithii* (Figure 19). Final leaf count, at 10% recommended application rate, decreased by 53-62% for *E. trachycaulus* and *M. fistulosa* (Figure 20). Dry biomass, at 100% recommended application rate, decreased by 53-79% for *P. virgatum* and *S. nutans*; and at 10% recommended application rate, decreased by 69-84% for *E. trachycaulus*, *H. helianthoides*, *M. fistulosa*, *N. viridula*, and *P. smithii* (Figure 21). Tukey's HSD was unable to differentiate the effect by concentration on final leaf count for *N. viridula*, despite significance shown in the ANOVA.

Discussion

The objective of my study was to evaluate if native seed germination, emergence, and growth was affected by herbicide at different concentrations representative of herbicide drift. Many NGP native plant species are vulnerable to 2,4-D, atrazine, and trifluralin below the recommended application rate, as demonstrated in the growth

chamber and greenhouse experiments. Germination decreased in monocot and dicot study species with exposure to 2,4-D at a variety of concentrations, contrary to my hypothesis that only emerged dicots would be affected. Other studies found similar results, demonstrating that monocots and dicots at various life stages can be negatively affected by a range of 2,4-D concentrations (Raza and Saiprakash 1978, Triques et al. 2021; Ogura et al. 2022). Atrazine had the greatest effect on plant growth of the study species, supporting my hypothesis. The observed effects of atrazine on seedling emergence and growth in my experiments align with another study in which no seedlings of their five study species survived treatment with atrazine (Jacobs et al. 2007). Trifluralin did not greatly affect germination of the study species, contrary to my hypothesis. Similarly, trifluralin caused the least seedling mortality of all the study herbicides in an experiment on wildflower establishment for native seed production (Jacobs et al. 2007). None of the study species were unaffected by all study herbicides in the experiments; each study species responded negatively to at least one herbicide. The germination, emergence, and growth response to herbicides were plant species-specific.

2,4-D negatively impacted the final germination percent of more species than atrazine and trifluralin. This result was unexpected, as 2,4-D is a synthetic auxin that is intended to target post-emergence dicots (Song 2014). Conversely, I observed impacts on both monocots and dicots that have yet to emerge from the soil, similar to other studies (Raza and Saiprakash 1978, Triques et al. 2021; Ogura et al. 2022). Dicot-selective herbicides such as 2,4-D may affect monocot seeds because the seeds do not have the morphology to protect them from herbicide effects as a mature monocot plant does, such as leaf sheaths that enclose the meristem (Wagner and Nelson 2014). Another synthetic

auxin, quinclorac, targets monocots in addition to dicots by mediating the ethylene biosynthesis pathway (Grossmann 2003). In the ethylene biosynthesis pathway, cyanide and ethylene are produced in stoichiometrically equivalent amounts from the process of 1-Aminocyclopropane-1-carboxylate synthase (ACC) oxidation (Seo et al. 2011), which can be induced by quinclorac application (Grossmann 2003). Cyanide is toxic to living organisms and can inhibit certain intracellular enzymes important to plant metabolic pathways (Siegień and Bogatek 2006). Depending on the concentration present, cyanide can either inhibit or stimulate seed germination pathways (Siegień and Bogatek 2006). If 2,4-D were to work in a similar way to quinclorac, the cyanide concentration present in the seeds may have been great enough to inhibit germination.

Overall, seedlings were more vulnerable to herbicides than germinating seeds. Atrazine and trifluralin affected more species in the emergence and growth experiment than in the germination experiment. Similarly, James et al. (2011) found that seedling death after germination but before emergence was the most limiting transition for native plant recruitment. Further, the seedling stage was the most sensitive period for species sprayed with 1 and 10% recommended application rate of metsulfuron methyl (Boutin et al. 2000). To reduce harm inflicted on seedlings from herbicide exposure, ecological restoration practitioners should take care to mitigate the effects of herbicides early in the restoration process, and herbicide applicators should attempt to reduce herbicide drift from their applications.

Herbicides may affect non-target plants differently at various points of development (Boutin et. al 2014), so I recommend further study of these herbicides and species at different life stages. Although my study shows that seed-based restoration may

be affected by herbicide drift, young and established native plant communities can also be negatively impacted. Some of the negative effects seen in young and established plant communities from herbicide drift include impaired reproduction (Olszyk et al. 2017), decreased plant biomass and species richness in experimental plots (Kleijn and Snoeijing 1997), and decreased plant cover and shifts in community composition (Nelemans et al. 2017).

Management Considerations

An important factor that can influence the effect of herbicide drift on restoration seeds and seedlings is the timing of seeding and herbicide application. Restoration seedings in the NGP typically occur in spring, around April and May (Wilson et al. 2004; Alstad et al. 2018; Carter and Blair 2012). Herbicide applications for conventional agriculture also typically occur in spring, in May and June (DeWerff et al. 2015; Loux et al. 2011). Due to this overlap in time, there is potential for restoration seeding to occur concurrently to herbicide application, increasing the potential for adverse effects to germination, emergence, and growth from herbicide drift. Even if the seeding and application do not occur at the same time, ecological restoration success may still be affected by seeds encountering herbicide residue in the soil (Schroeder et al. 2023), or by plants in early stages of growth encountering herbicide drift (Boutin et al. 2014). The amount of herbicide residue in soil varies based on herbicide type and environmental factors (Faroon et al. 2020). 2,4-D has a half-life of 6-68 days (Faroon et al. 2020); atrazine has a half-life of 60-150 days (Ribaudo and Bouzaher 1994); trifluralin has a half-life of 81-356 days (European Commission 2007).

The knowledge gained from my research can inform land management decisions for seed-based ecological restoration in a fragmented landscape. In a field setting, one can presume that the closer a seed is to the point of herbicide application, the more likely it is that the seed may experience herbicide exposure from drift (Kleijn and Snoeijing 1997; Vieira et al. 2020). Because of this, seeds planted closer to field borders are more likely to experience the effects of herbicide than seeds planted away from field borders. Ecological restoration sites that share a border with agricultural lands, which is likely in the NGP due to fragmentation, are therefore more subject to potential damage from herbicide drift. However, significant effects on non-target plants caused by herbicide drift may be confined to plants within approximately 6-8 meters from field borders (Brain et al. 2017). Ecological restoration practitioners can use this information to plan which species should be planted in certain areas of a field to ensure the best possible establishment of their restoration (Table 8).

To minimize the non-target effects of herbicides, producers should follow herbicide application laws and regulations, pay attention to weather conditions, and use drift-reducing application methods/additives when possible. To reduce herbicide drift, applicators can reduce spray pressure, increase nozzle orifice size, use additives to increase spray viscosity, reduce the boom height, and use spray shields on ground sprayers (Dexter 1993). These methods reduce drift by changing the herbicide droplet size, volatility, and physical ability for herbicide to drift (Dexter 1993). By using drift reducing methods, herbicide drift can be reduced up to ~85-90% compared to other methods (Foster et al. 2018; Wang et al. 2023) Additionally, herbicides are least likely to drift when the wind velocity is low, temperatures are low, and relative humidity is high

(Dexter 1993). Ecological restoration practitioners may also minimize the non-target effects of herbicide drift by using seed enhancement technologies to reduce the potential effects of herbicide drift on germinating seeds. Activated carbon can be used as a seed coating or as an inclusion in extruded seed pellets to mitigate the effects of herbicides on sowed seeds, as activated carbon can adsorb and immobilize herbicides (Clenet et al 2020; Svejcar et al. 2022).

Ecological restoration is an important tool for combating the loss of grasslands due to conversion to agriculture. Implementing seed-based ecological restoration in a fragmented landscape may subject the seeds to herbicide drift, which can impact the germination of some native plant species. When selecting which species to include in an ecological restoration setting near agricultural fields, land managers should choose species which may be tolerant to common herbicides (Table 8). However, environmental stress can affect plant tolerance of herbicides (Jacobs et al. 2007), so even a generally tolerant species in poor environmental conditions may be affected by herbicides. My research showed that the most tolerant of the study species were *L. pycnostachya, S. laeve, and D. purpurea*, as they were not greatly affected by presence of atrazine, trifluralin, or 2,4-D at recommended herbicide application rates or herbicide drift concentrations. These species should be prioritized in seed-based restoration located near agricultural fields, and they have the best chance of survival and growth in an herbicide-affected environment.

Table 1. Experimental families, species, and seed source used in the germination, emergence and growth, and ivermectin experiments. PM = Prairie Moon Nursery (Winona, MN); MB = Millborn Seeds (Brookings, SD). D = dicot; M = monocot.

Family	Scientific name	Common name	Source	Cotyledon type
Apocynaceae	*Asclepias tuberosa*	Butterfly weed	PM	D
Asteraceae	*Heliopsis helianthoides*	Smooth oxeye	PM	D
	Liatris aspera	Tall blazing star	PM	D
	Liatris pycnostachya	Prairie blazing star	PM	D
	Ratibida pinnata	Pinnate prairie coneflower	PM	D
	Symphyotrichum laeve	Smooth blue aster	PM	D
Fabaceae	*Dalea purpurea*	Purple prairie clover	PM	D
Lamiaceae	*Monarda fistulosa*	Wild bergamot	PM	D
Poaceae	*Andropogon gerardii*	Big bluestem	PM	M
	Elymus trachycaulus	Slender wheatgrass	MB	M
	Nassella viridula	Green needlegrass	MB	M
	Panicum virgatum	Switchgrass	PM	M
	Pascopyrum smithii	Western wheatgrass	MB	M
	Sorghastrum nutans	Indiangrass	PM	M

Table 2. Effect of 2,4-D on final germination percent and germination rate of 14 NGP native species. Bold p-values indicate significance (alpha = 0.05). df = 5, 42.

	Germination %		Germination rate (seeds/day)	
Species	F	p	F	p
A. gerardii	9.12	< **0.001**	10.7	< **0.001**
A. tuberosa	9.83	< **0.001**	3.05	**0.020**
D. purpurea	0.857	0.518	0.623	0.683
E. trachycaulus	15.7	< **0.001**	2.84	**0.027**
H. helianthoides	4.33	**0.003**	1.28	0.292
L. aspera	43.5	< **0.001**	11.8	< **0.001**
L. pycnostachya	33.0	< **0.001**	10.5	< **0.001**
M. fistulosa	18.2	< **0.001**	13.0	< **0.001**
N. viridula	12.1	< **0.001**	0.667	0.651
P. smithii	4.80	**0.002**	3.71	**0.007**
P. virgatum	1.52	0.203	1.64	0.171
R. pinnata	37.5	< **0.001**	29.2	< **0.001**
S. laeve	110	< **0.001**	122	< **0.001**
S. nutans	37.9	< **0.001**	31.79	< **0.001**

Table 3. Effect of atrazine on final germination percent and germination rate of 14 NGP native species. Bold p-values indicate significance (alpha = 0.05). df = 5, 42.

	Germination %		Germination rate (seeds/day)	
Species	F	p	F	p
A. gerardii	0.695	0.630	0.909	0.484
A. tuberosa	2.24	0.068	5.20	< **0.001**
D. purpurea	0.935	0.468	0.297	0.912
E. trachycaulus	1.53	0.201	0.231	0.947
H. helianthoides	0.910	0.484	0.413	0.837
L. aspera	3.98	**0.005**	5.25	< **0.001**
L. pycnostachya	0.944	0.463	1.24	0.309
M. fistulosa	0.572	0.721	2.15	0.078
N. viridula	0.153	0.978	0.725	0.609
P. smithii	2.11	0.083	2.25	0.067
P. virgatum	0.911	0.483	0.808	0.551
R. pinnata	0.606	0.696	0.545	0.741
S. laeve	1.56	0.193	0.919	0.478
S. nutans	1.68	0.162	1.82	0.130

Table 4. Effect of trifluralin on final germination percent and germination rate of 14 NGP native species. Bold p-values indicate significance (alpha = 0.05). df = 5, 42.

	Germination %		Germination rate (seeds/day)	
Species	F	p	F	p
A. gerardii	1.15	0.350	0.0239	1.00
A. tuberosa	3.24	**0.015**	0.617	0.688
D. purpurea	0.355	0.876	0.643	0.669
E. trachycaulus	0.942	0.464	1.24	0.306
H. helianthoides	1.76	0.143	0.183	0.967
L. aspera	0.688	0.635	0.770	0.577
L. pycnostachya	0.551	0.737	0.577	0.717
M. fistulosa	4.71	**0.002**	1.88	0.119
N. viridula	3.46	**0.010**	0.754	0.588
P. smithii	3.36	**0.012**	0.329	0.893
P. virgatum	0.221	0.952	1.10	0.373
R. pinnata	3.51	**0.010**	0.798	0.558
S. laeve	1.03	0.413	1.06	0.394
S. nutans	3.27	**0.014**	5.49	< **0.001**

Table 5. Emergence and growth metrics for 14 NGP native species when exposed to varying concentrations of 2,4-D. Underline indicates log transformed data. Bold p-values indicate significance (alpha = 0.05).

	Time to emergence (days)		Probability of death		Final height (cm)		Final leaf count (#)		Dry biomass (mg)	
Species	F_{df}	p	χ^2_{df}	p	F_{df}	p	χ^2_{df}	p	F_{df}	p
A. gerardii	$3.61_{4, 37}$	**0.014**	$0_{4, 45}$	0.999	$1.95_{4, 37}$	0.122	$0.54_{4, 37}$	0.970	$1.33_{4, 36}$	0.278
A. tuberosa	$1.96_{4, 34}$	0.123	$10.5_{4, 45}$	**0.033**	$0.50_{4, 31}$	0.738	$2.01_{4, 31}$	0.735	$1.31_{4, 31}$	0.290
D. purpurea	$13.5_{4, 44}$	**<0.001**	$2.27_{4, 45}$	0.686	$1.85_{4, 37}$	0.140	$3.33_{4, 37}$	0.504	$1.52_{4, 37}$	0.217
E. trachycaulus	$10.3_{4, 45}$	**<0.001**	$0_{4, 45}$	0.999	$5.48_{4, 45}$	**0.001**	$8.46_{4, 45}$	0.076	$4.87_{4, 45}$	**0.002**
H. helianthoides	$9.81_{4, 41}$	**<0.001**	$0_{4, 45}$	0.999	$3.48_{4, 41}$	**0.015**	$4.38_{4, 41}$	0.357	$3.98_{4, 41}$	**0.008**
L. aspera	$5.15_{4, 37}$	**0.002**	$3.19_{4, 45}$	0.526	$3.40_{4, 34}$	**0.019**	$1.36_{4, 34}$	0.851	$2.84_{4, 34}$	**0.039**
L. pycnostachya	$0.42_{4, 33}$	0.796	$0.68_{4, 45}$	0.954	$1.25_{4, 28}$	0.312	$0.79_{4, 28}$	0.940	$0.44_{4, 28}$	0.780
M. fistulosa	$5.48_{4, 38}$	**0.001**	$3.00_{4, 45}$	0.559	$3.61_{4, 33}$	**0.015**	$15.7_{4, 33}$	**0.003**	$4.93_{4, 33}$	**0.003**
N. viridula	$1.55_{4, 45}$	0.205	$0_{4, 45}$	0.999	$2.20_{4, 45}$	0.084	$1.28_{4, 45}$	0.864	$1.26_{4, 45}$	0.302
P. smithii	$0.84_{4, 40}$	0.508	$0_{4, 45}$	0.999	$0.55_{4, 40}$	0.702	$1.29_{4, 40}$	0.864	$0.25_{4, 39}$	0.906
P. virgatum	$6.99_{4, 45}$	**<0.001**	$3.30_{4, 45}$	0.509	$0.48_{4, 44}$	0.753	$0.03_{4, 44}$	0.999	$0.69_{4, 44}$	0.601
R. pinnata	$14.9_{4, 42}$	**<0.001**	$10.5_{4, 45}$	**0.033**	$5.13_{4, 39}$	**0.002**	$4.01_{4, 39}$	0.405	$13.9_{4, 39}$	**<0.001**
S. laeve	$0.69_{4, 8}$	0.689	$0_{4, 45}$	0.999	$1.92_{4, 8}$	0.200	$0.97_{4, 8}$	0.914	$1.15_{4, 8}$	0.401
S. nutans	$0.58_{4, 13}$	0.593	$0_{4, 45}$	0.999	$0.99_{4, 13}$	0.446	$0.35_{4, 13}$	0.986	$1.63_{4, 13}$	0.226

Table 6. Emergence and growth metrics for 14 NGP native species when exposed to varying concentrations of trifluralin. Underline indicates log transformed data. Bold p-values indicate significance (alpha = 0.05).

	Time to emergence (days)		Probability of death		Final height (cm)		Final leaf count (#)		Dry biomass (mg)	
Species	F_{df}	p	$\chi2_{df}$	p	F_{df}	p	$\chi2_{df}$	p	F_{df}	p
A. gerardii	$5.66_{4, 29}$	**0.002**	$7.29_{4, 45}$	0.121	$6.27_{4, 25}$	**0.001**	$1.12_{4, 25}$	0.891	$4.40_{4, 25}$	**0.008**
A. tuberosa	$2.55_{4, 32}$	0.058	$6.79_{4, 45}$	0.148	$2.26_{4, 31}$	0.085	$20.1_{4, 31}$	**<0.001**	$4.85_{4, 31}$	**0.004**
D. purpurea	$2.18_{4, 45}$	0.087	$12.9_{4, 45}$	**0.012**	$1.43_{4, 32}$	0.248	$13.3_{4, 32}$	**0.010**	$2.20_{4, 32}$	0.091
E. trachycaulus	$3.59_{4, 44}$	**0.013**	$6.79_{4, 45}$	0.148	$4.78_{4, 42}$	**0.003**	$6.65_{4, 42}$	0.155	$9.49_{4, 42}$	**<0.001**
H. helianthoides	$1.35_{4, 41}$	0.269	$4.87_{4, 45}$	0.301	$1.63_{4, 37}$	0.188	$0.623_{4, 37}$	0.961	$1.27_{4, 37}$	0.298
L. aspera	$0.932_{4, 16}$	0.470	$6.19_{4, 45}$	0.186	$3.25_{4, 23}$	**0.030**	$0.578_{4, 23}$	0.965	$3.46_{4, 23}$	**0.024**
L. pycnostachya	$2.78_{4, 7}$	0.113	$3.19_{4, 45}$	0.526	$1.40_{4, 20}$	0.272	$0.627_{4, 20}$	0.960	$1.33_{4, 20}$	0.292
M. fistulosa	$0.573_{4, 29}$	0.685	$14.4_{4, 45}$	**0.006**	$1.89_{4, 17}$	0.158	$12.8_{4, 17}$	**0.013**	$5.83_{4, 17}$	**0.004**
N. viridula	$2.70_{4, 37}$	**0.046**	$14.4_{4, 45}$	**0.006**	$6.13_{4, 33}$	**0.001**	$6.16_{4, 33}$	0.188	$2.12_{4, 33}$	0.100
P. smithii	$0.294_{4, 42}$	0.880	$0_{4, 45}$	1.000	$11.3_{4, 42}$	**<0.001**	$12.1_{4, 42}$	**0.017**	$10.2_{4, 42}$	**<0.001**
P. virgatum	$1.20_{4, 42}$	0.325	$16.3_{4, 45}$	**0.003**	$9.71_{4, 36}$	**<0.001**	$9.80_{4, 36}$	**0.044**	$6.90_{4, 36}$	**<0.001**
R. pinnata	$1.34_{4, 41}$	0.272	$0_{4, 45}$	1.000	$1.71_{4, 40}$	0.166	$0.90_{4, 40}$	0.925	$1.28_{4, 40}$	0.295
S. laeve	$0.918_{3, 10}$	0.467	$6.19_{4, 45}$	0.186	$2.20_{3, 8}$	0.166	$0.346_{3, 8}$	0.951	$6.00_{3, 8}$	**0.019**
S. nutans	$0.253_{4, 13}$	0.903	$7.86_{4, 45}$	0.097	$27.1_{4, 10}$	**<0.001**	$5.00_{4, 10}$	0.287	$9.66_{4, 10}$	**0.002**

Table 7. Emergence and growth metrics for 14 NGP native species when exposed to varying concentrations of atrazine. Underline indicates log transformed data. Bold p-values indicate significance (alpha = 0.05).

	Time to emergence (days)		Probability of death		Final height (cm)		Final leaf count (#)		Dry biomass (mg)	
Species	F_{df}	p	$\chi2_{df}$	p	F_{df}	p	$\chi2_{df}$	p	F_{df}	p
A. gerardii	$0.559_{4, 31}$	0.699	$2.99_{4, 45}$	0.559	$1.94_{4, 26}$	0.133	$1.65_{4, 26}$	0.799	$2.97_{4, 26}$	**0.038**
A. tuberosa	$8.22_{4, 38}$	**<0.001**	$35.9_{4, 45}$	**<0.001**	$0.702_{3, 24}$	0.560	$2.71_{3, 24}$	0.439	$0.582_{3, 24}$	0.633
D. purpurea	$97.6_{4, 44}$	**<0.001**	$61.5_{4, 45}$	**<0.001**	$0.910_{2, 25}$	0.415	$0.040_{2, 25}$	0.982	$0.0417_{2, 25}$	0.959
E. trachycaulus	$7.67_{4, 44}$	**<0.001**	$33.2_{4, 45}$	**<0.001**	$8.33_{4, 33}$	**<0.001**	$41.1_{4, 33}$	**<0.001**	$4.46_{4, 33}$	**<0.001**
H. helianthoides	$18.6_{4, 45}$	**<0.001**	$47.2_{4, 45}$	**<0.001**	$4.73_{3, 31}$	**0.008**	$1.08_{3, 31}$	0.781	$4.46_{3, 31}$	**0.010**
L. aspera	$4.22_{4, 19}$	**0.013**	$2.68_{4, 45}$	0.612	$1.69_{2, 7}$	0.252	$1.03_{2, 7}$	0.597	$1.27_{2, 7}$	0.339
L. pycnostachya	$3.23_{4, 13}$	**0.048**	$4.72_{4, 45}$	0.317	$0.985_{3, 3}$	0.505	$<0.001_{3, 3}$	1.000	$1.97_{3, 3}$	0.296
M. fistulosa	$9.29_{4, 34}$	**<0.001**	$18.5_{4, 45}$	**0.001**	$2.51_{3, 20}$	0.088	$16.3_{3, 20}$	**0.001**	$3.49_{3, 20}$	**0.035**
N. viridula	$13.9_{4, 39}$	**<0.001**	$46.2_{4, 45}$	**<0.001**	$7.96_{3, 29}$	**0.001**	$7.81_{3, 29}$	**0.050**	$13.7_{3, 29}$	**<0.001**
P. smithii	$57.8_{4, 45}$	**<0.001**	$45.1_{4, 45}$	**<0.001**	$13.1_{3, 33}$	**<0.001**	$7.35_{3, 33}$	0.062	$13.7_{3, 33}$	**<0.001**
P. virgatum	$0.823_{4, 45}$	0.518	$3.30_{4, 45}$	0.509	$5.87_{4, 44}$	**<0.001**	$0.870_{4, 44}$	0.929	$4.14_{4, 44}$	**0.006**
R. pinnata	$38.6_{4, 43}$	**<0.001**	$59.9_{4, 45}$	**<0.001**	$0.217_{2, 26}$	0.806	$0.0938_{2, 26}$	0.954	$0.392_{2, 26}$	0.680
S. laeve	$2.73_{4, 17}$	0.064	$14.8_{4, 45}$	**0.005**	$0.676_{3, 11}$	0.585	$1.25_{3, 11}$	0.741	$2.40_{3, 11}$	0.123
S. nutans	$0.735_{4, 36}$	0.577	$3.79_{4, 45}$	0.435	$2.62_{4, 24}$	0.060	$1.67_{4, 24}$	0.797	$4.56_{4, 24}$	**0.007**

Table 8. Planting recommendations for 14 NGP native species based on their tolerance to varying concentrations of 2,4-D, atrazine, and trifluralin. A green rectangle with an "L" indicates that no germination, emergence, or growth metrics were affected by that chemical, and are therefore most tolerant to drift of that given herbicide. A yellow rectangle with an "M" indicates that germination, emergence, and growth metrics were affected by that herbicide at 100 or 50% recommended application rate, and these species may be negatively affected close to field borders where herbicide drift potential is higher. A red rectangle with an "H" indicates that germination, emergence, and growth metrics were affected by that herbicide at 10 to 0.1% recommended application rate, and these species may be negatively affected by herbicide drift at a greater distance than "M" species.

Scientific name	Common name	2,4-D	Atrazine	Trifluralin
Andropogon gerardii	Big bluestem	L	L	L
Asclepias tuberosa	Butterfly weed	L	L	L
Dalea purpurea	Purple prairie clover	L	M	L
Elymus trachycaulus	Slender wheatgrass	M	H	M
Heliopsis helianthoides	Smooth oxeye	M	H	L
Liatris aspera	Tall blazing star	L	L	L
Liatris pycnostachya	Prairie blazing star	L	M	L
Monarda fistulosa	Wild bergamot	M	H	M
Nassella viridula	Green needlegrass	L	M	L
Panicum virgatum	Switchgrass	L	L	M
Pascopyrum smithii	Western wheatgrass	L	H	M
Ratibida pinnata	Pinnate prairie coneflower	M	M	L
Sorghastrum nutans	Indiangrass	L	L	M
Symphyotrichum laeve	Smooth blue aster	L	L	L

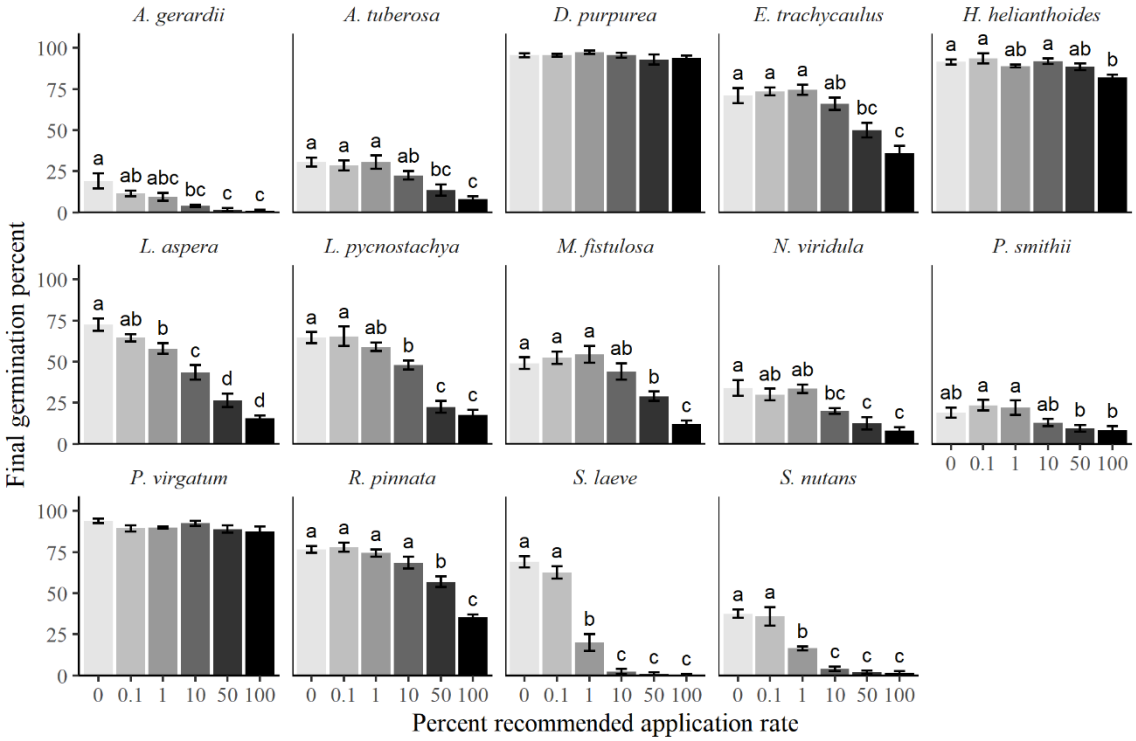

Figure 1. Effect of different 2,4-D application rates on final germination percent. p=Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

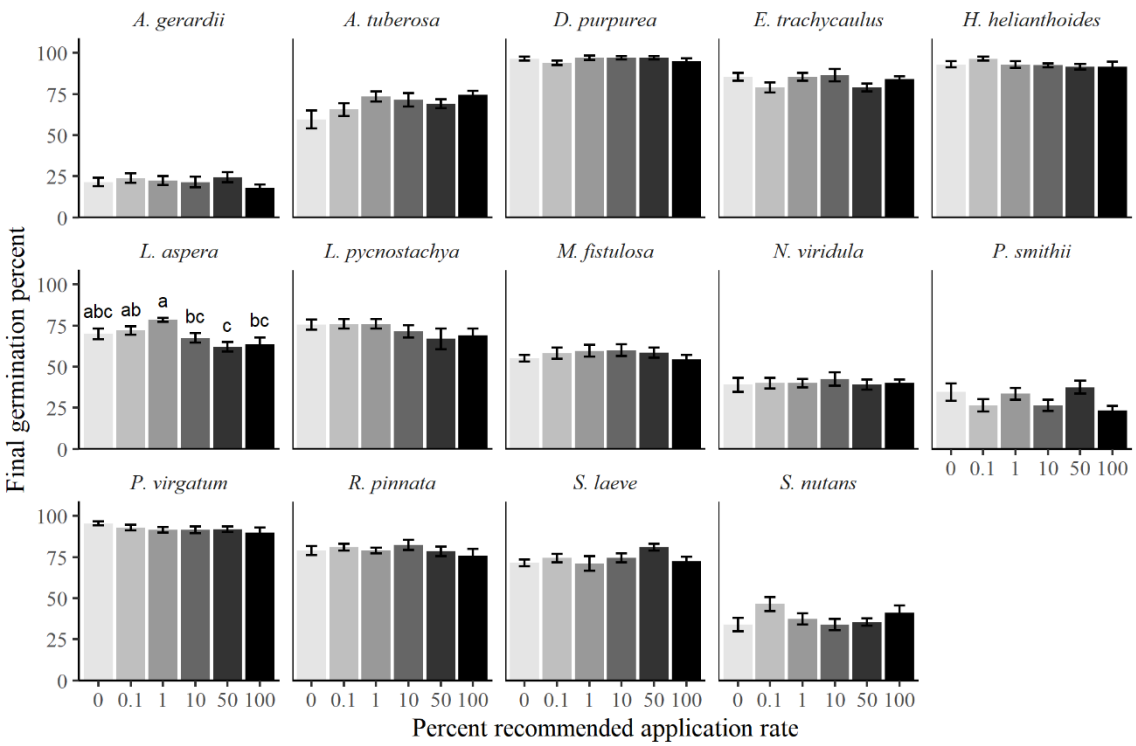

Figure 2. Effect of different atrazine application rates on final germination percent. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

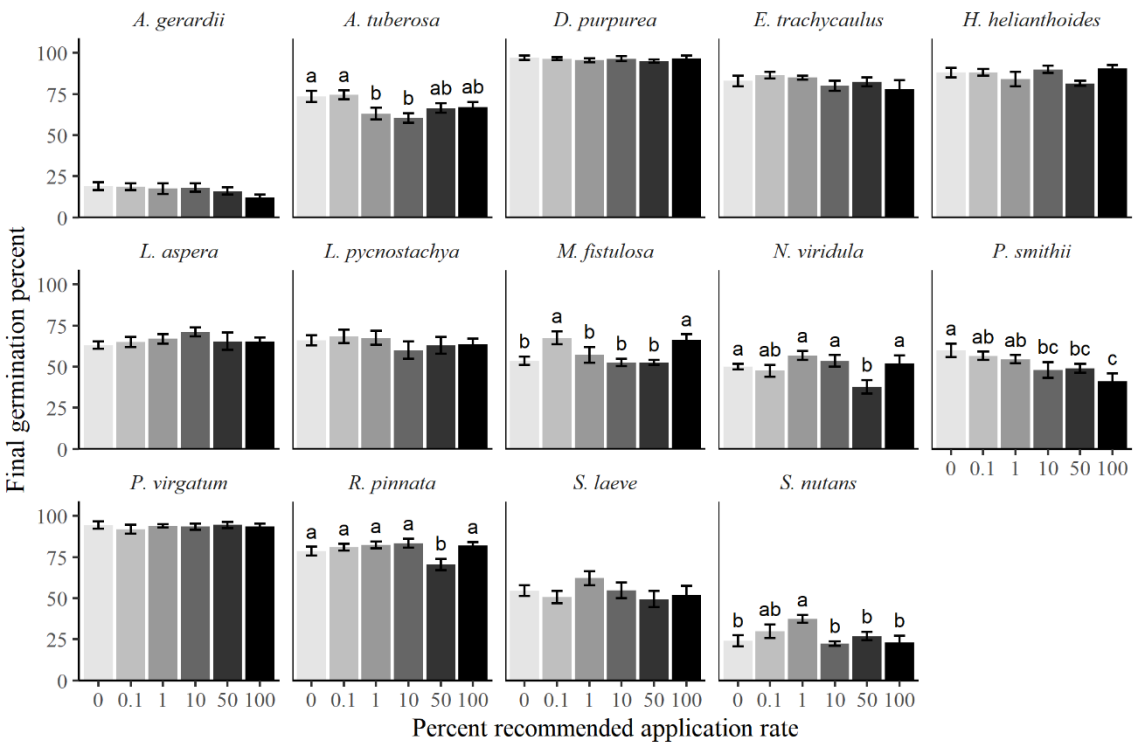

Figure 3. Effect of different trifluralin application rates on final germination percent. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

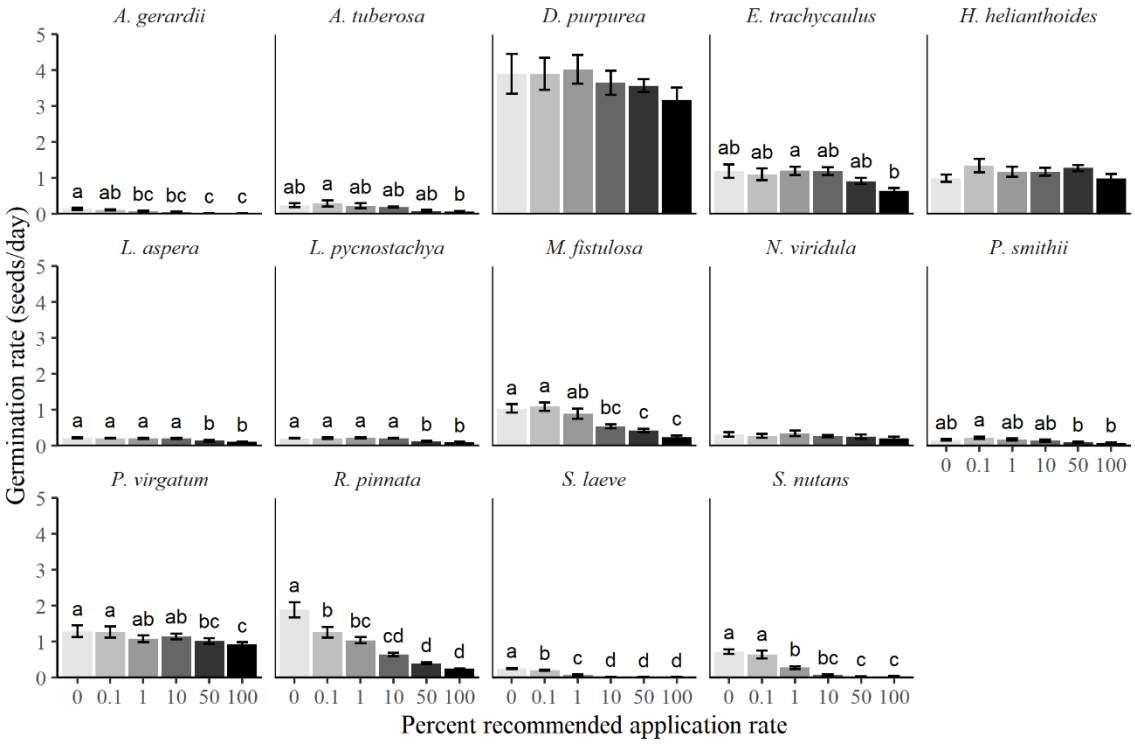

Figure 4. Effect of different 2,4-D application rates on germination rate. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

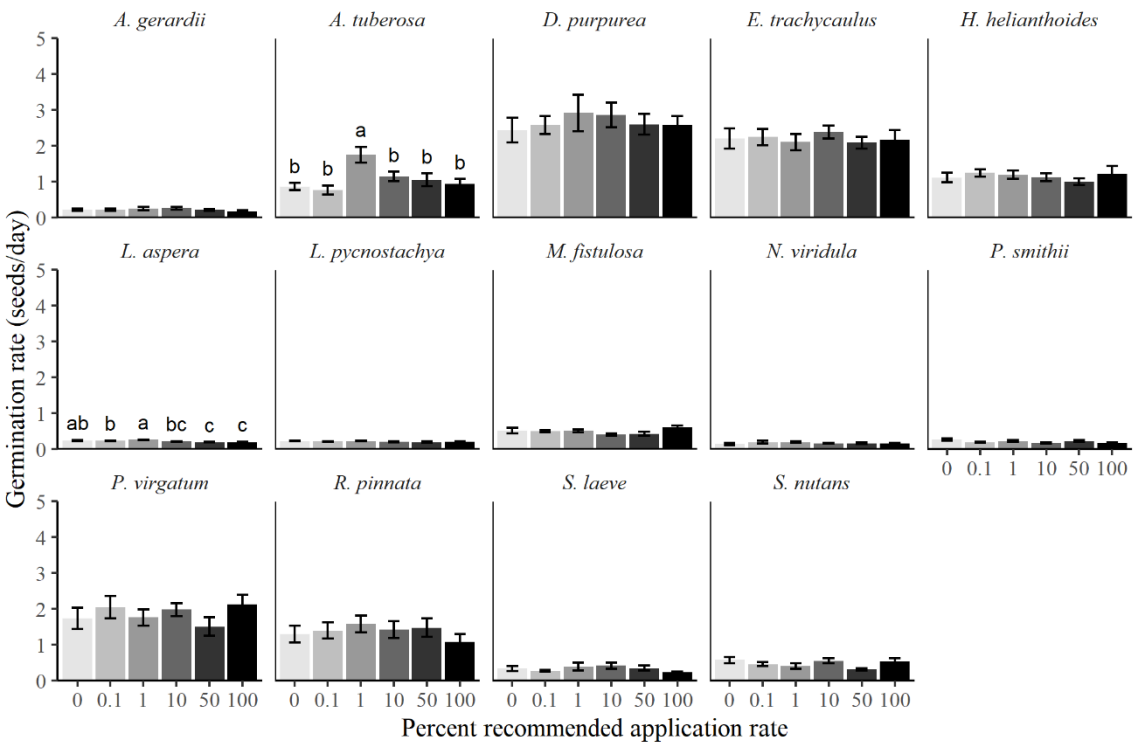

Figure 5. Effect of different atrazine application rates on germination rate. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

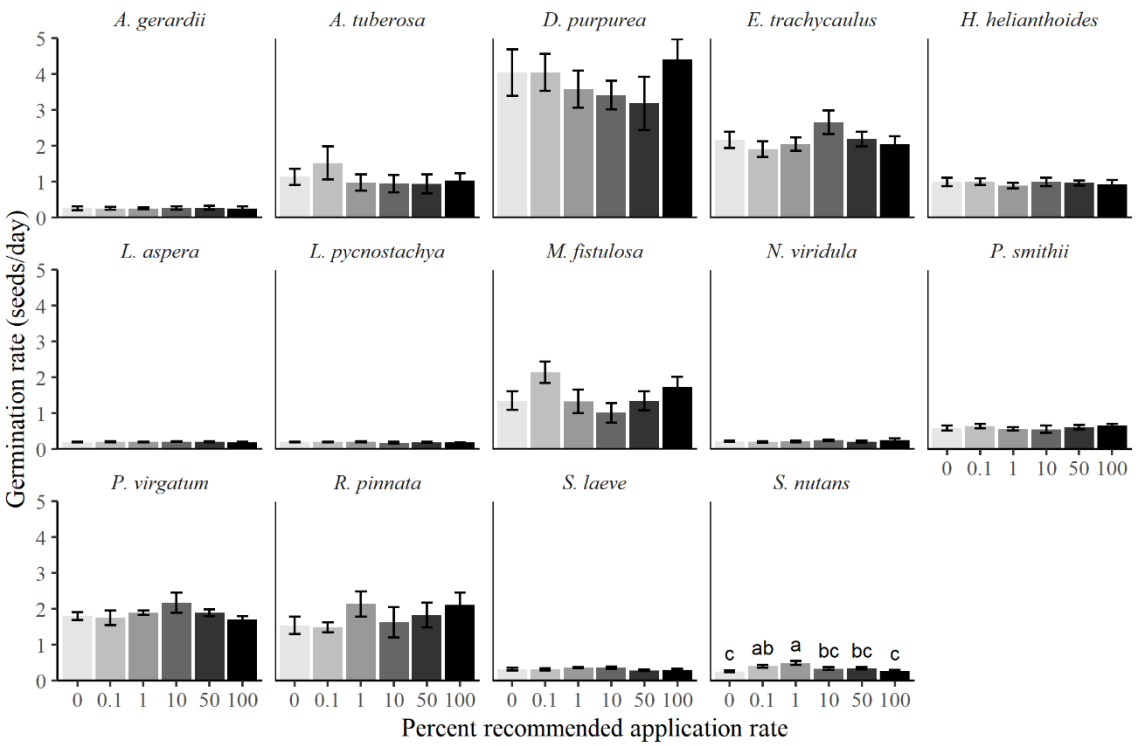

Figure 6. Effect of different trifluralin application rates on germination rate. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

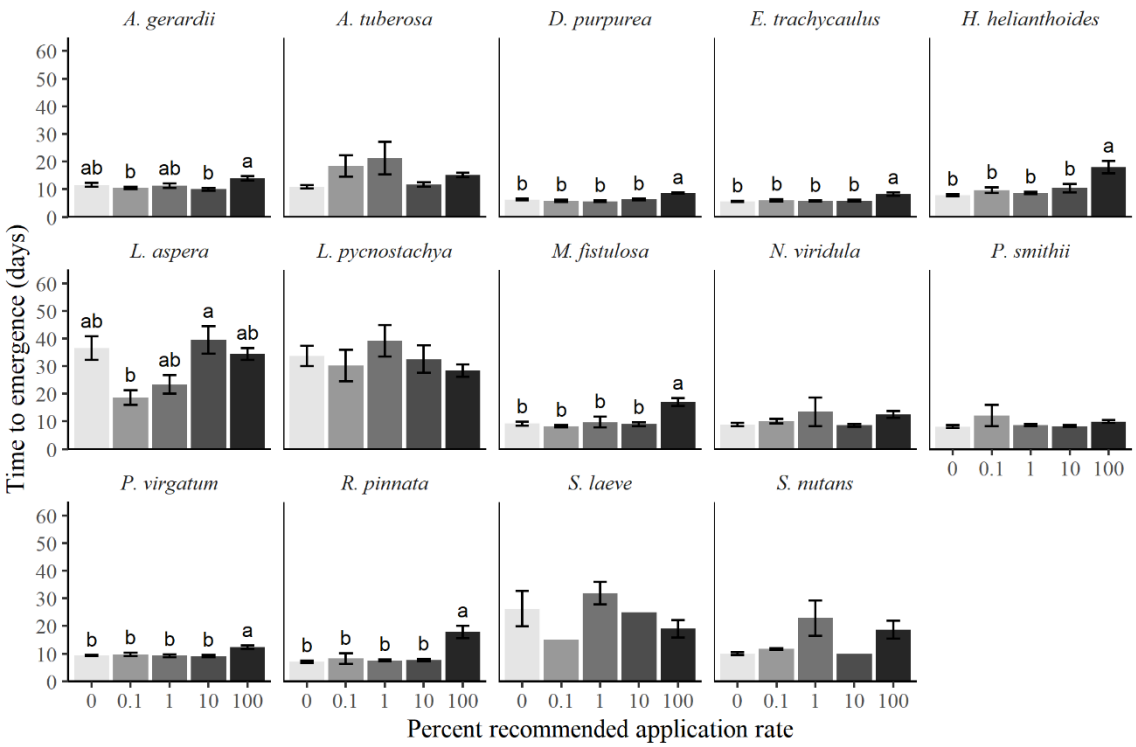

Figure 7. Effect of different 2,4-D application rates on time to emergence. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

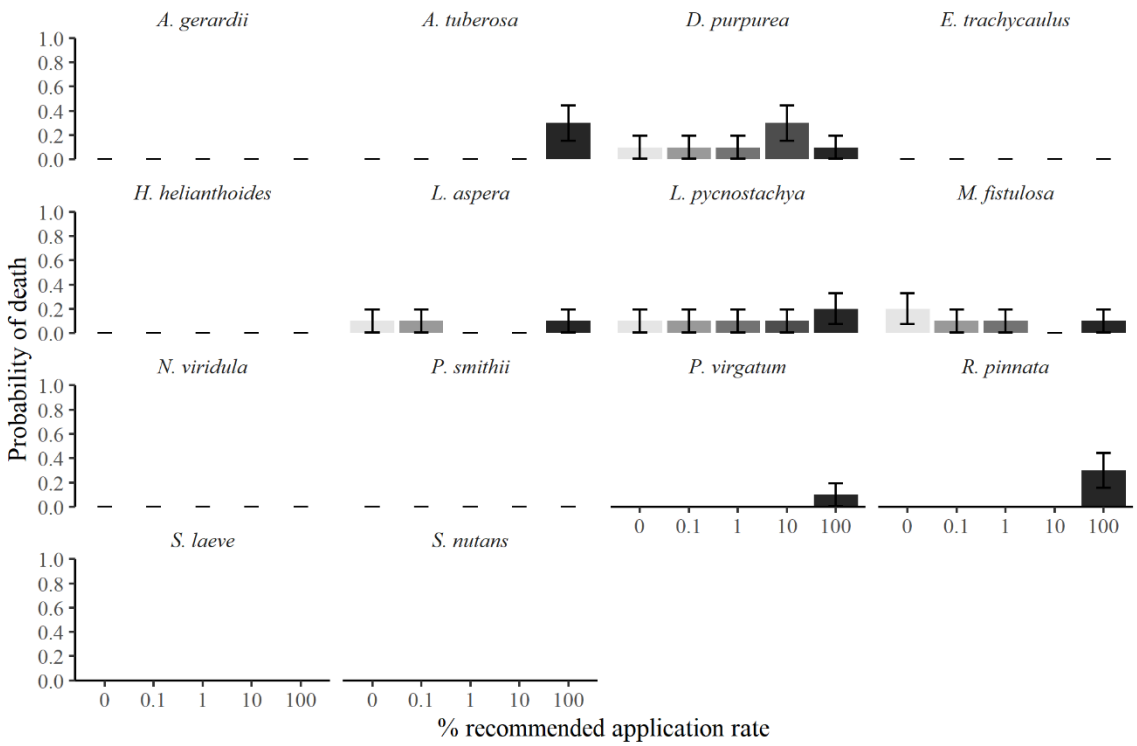

Figure 8. Effect of different 2,4-D application rates on probability of death. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

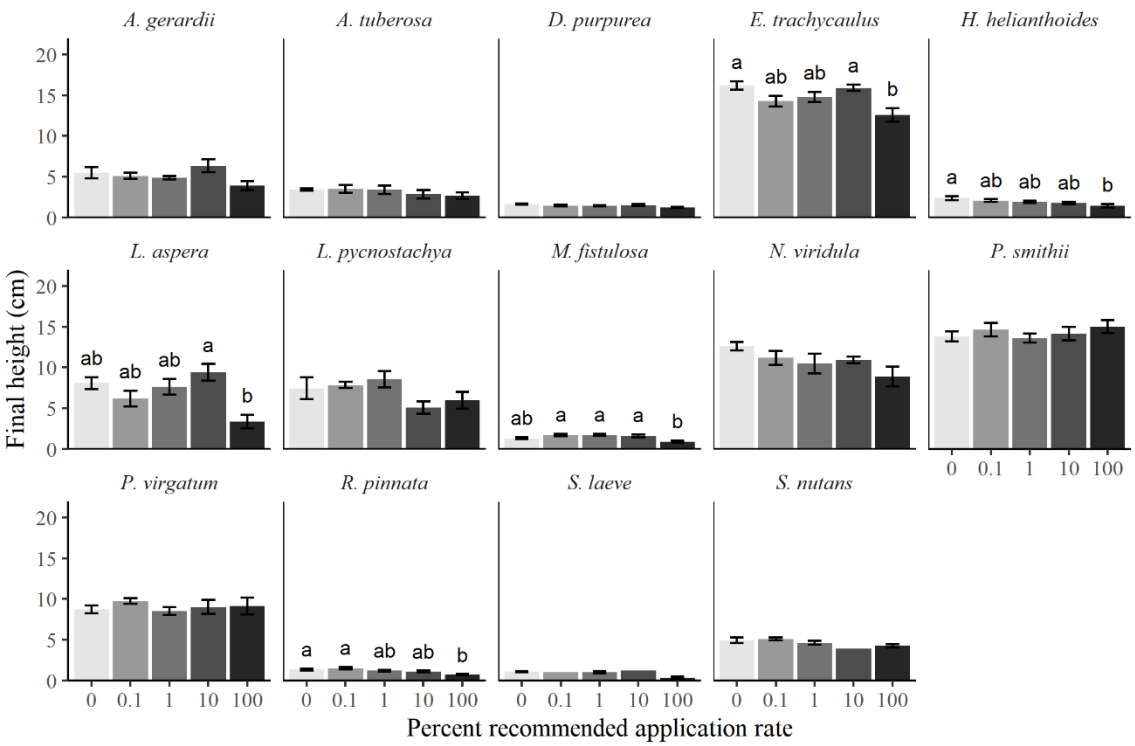

Figure 9. Effect of different 2,4-D application rates on final height. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

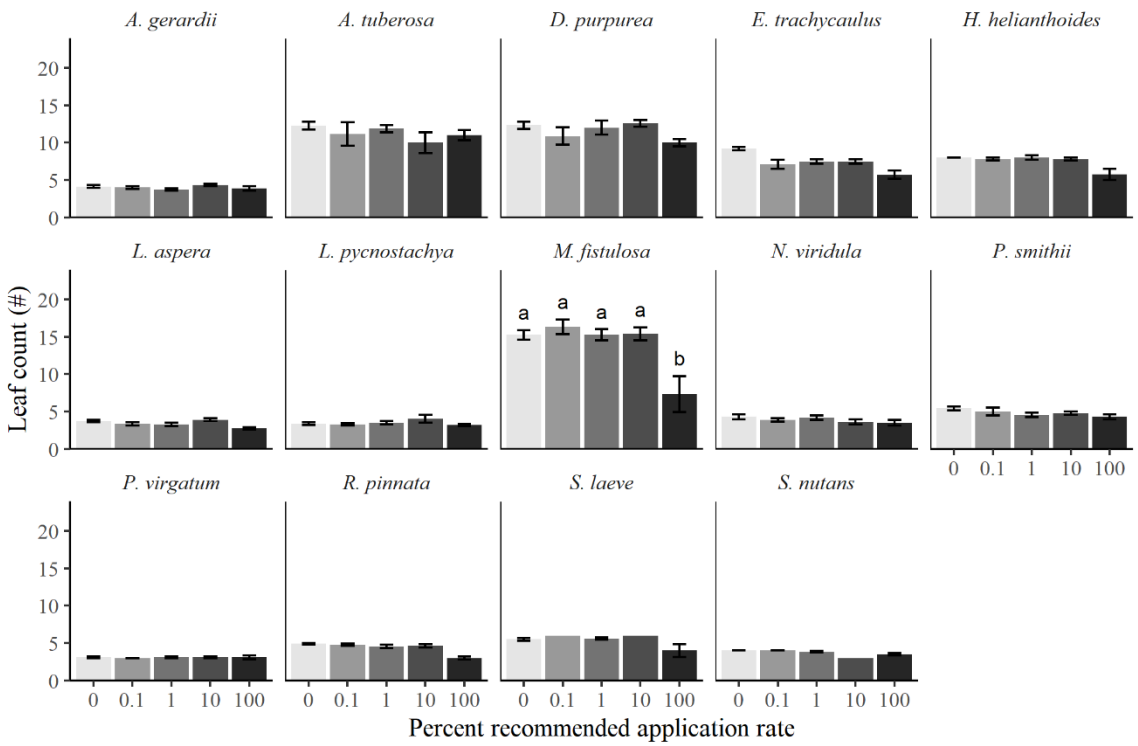

Figure 10. Effect of different 2,4-D application rates on final leaf count. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

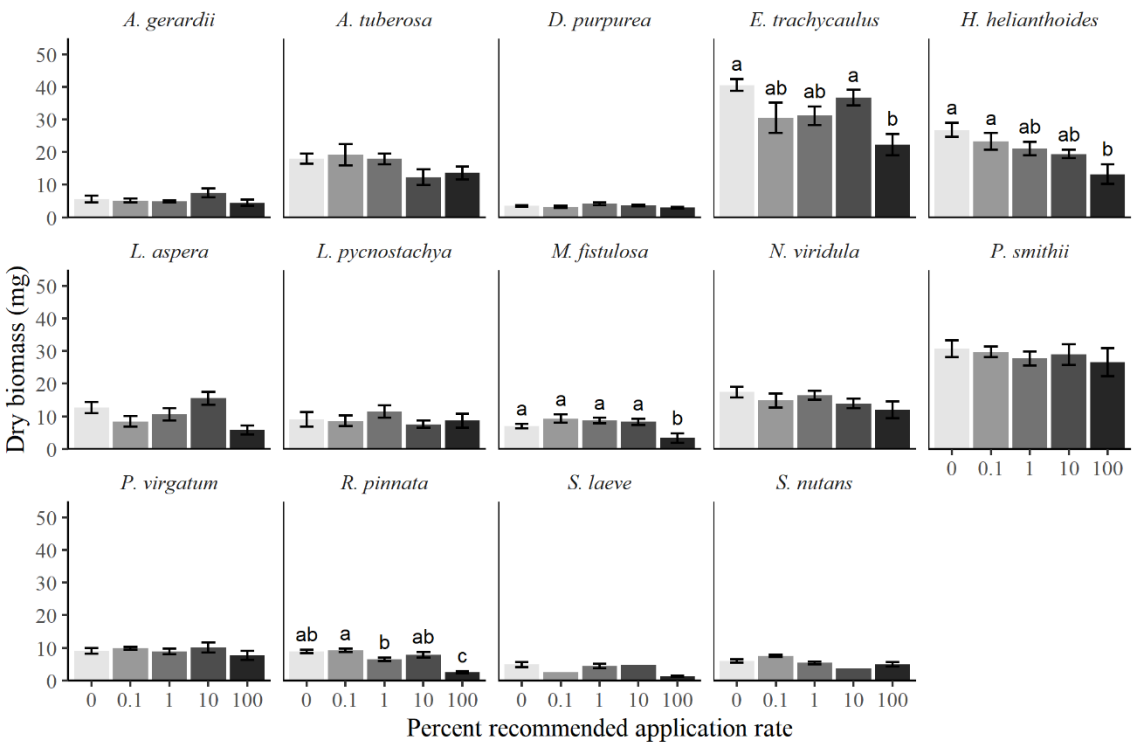

Figure 11. Effect of different 2,4-D application rates on dry biomass. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

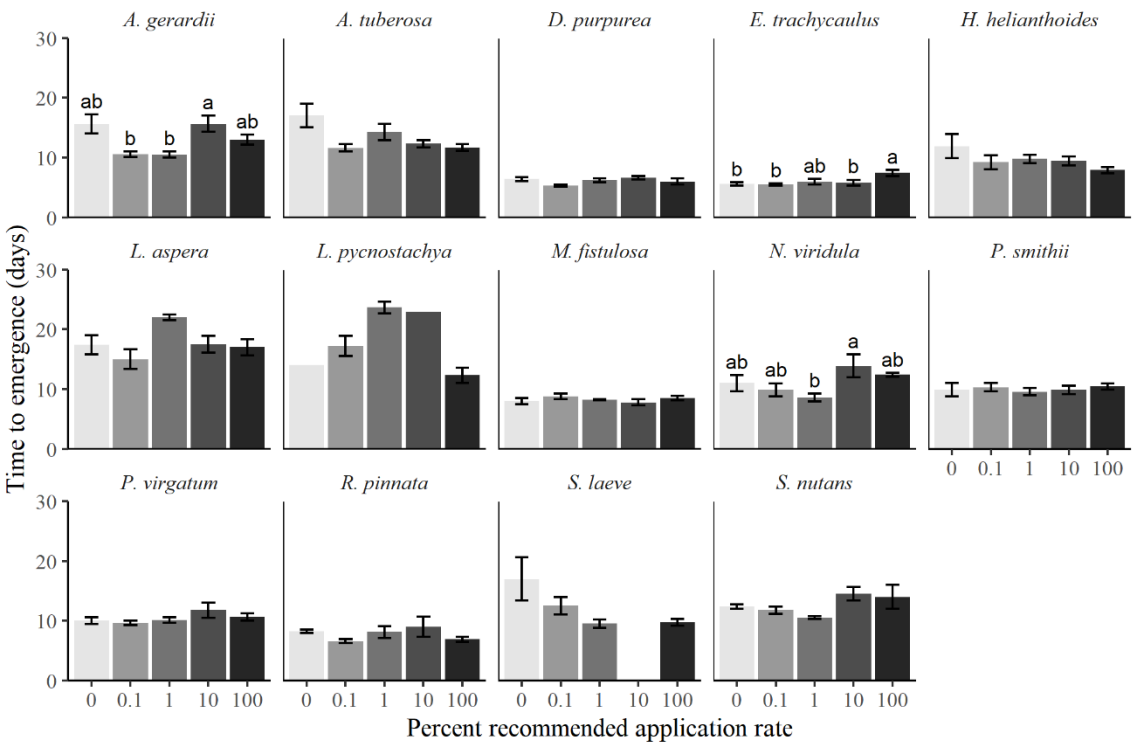

Figure 12. Effect of different trifluralin application rates on time to emergence. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

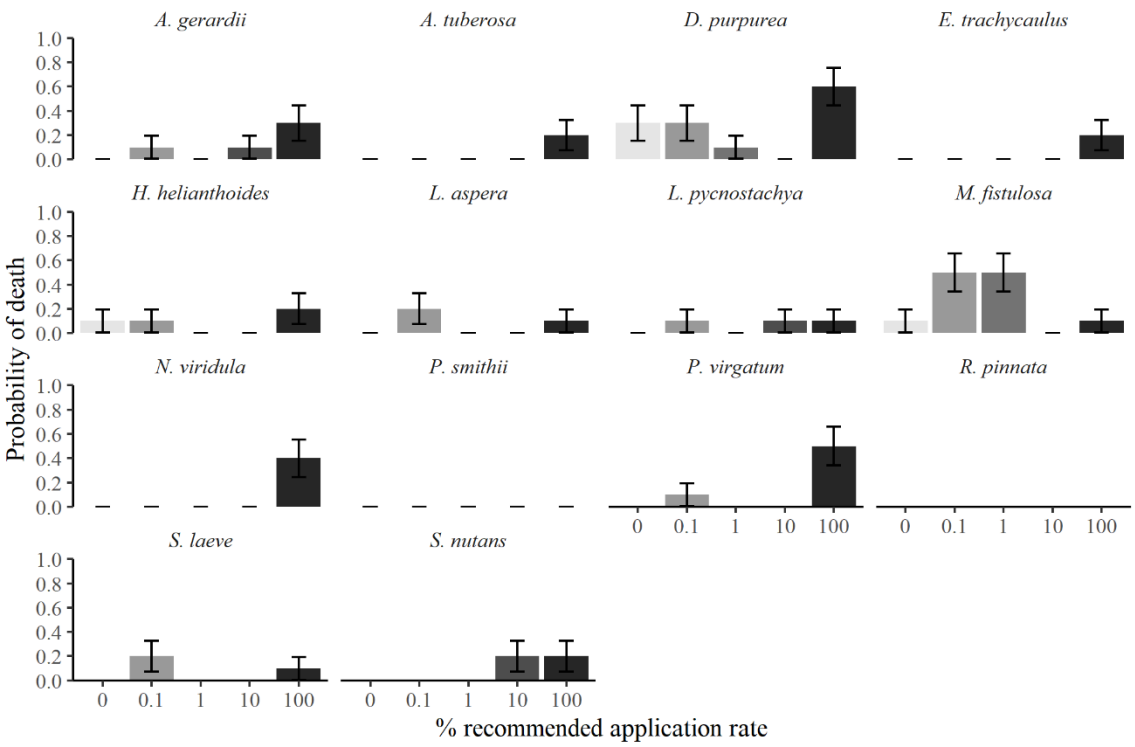

Figure 13. Effect of different trifluralin application rates on probability of death. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

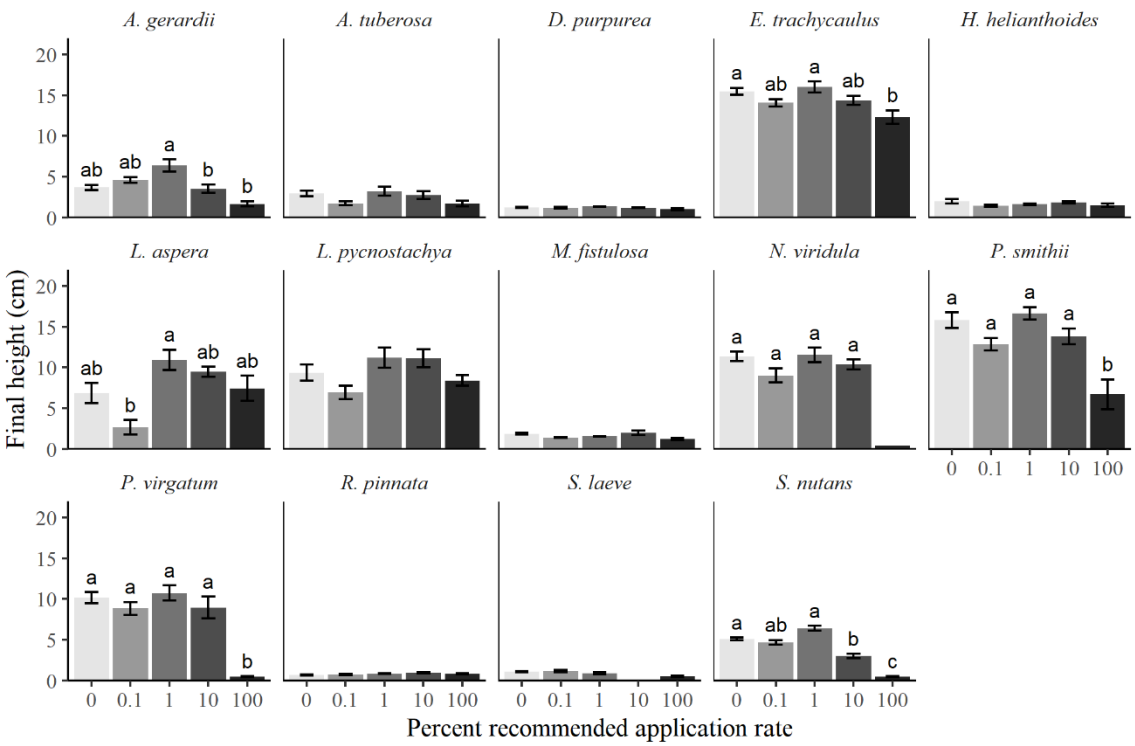

Figure 14. Effect of different trifluralin application rates on final height. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

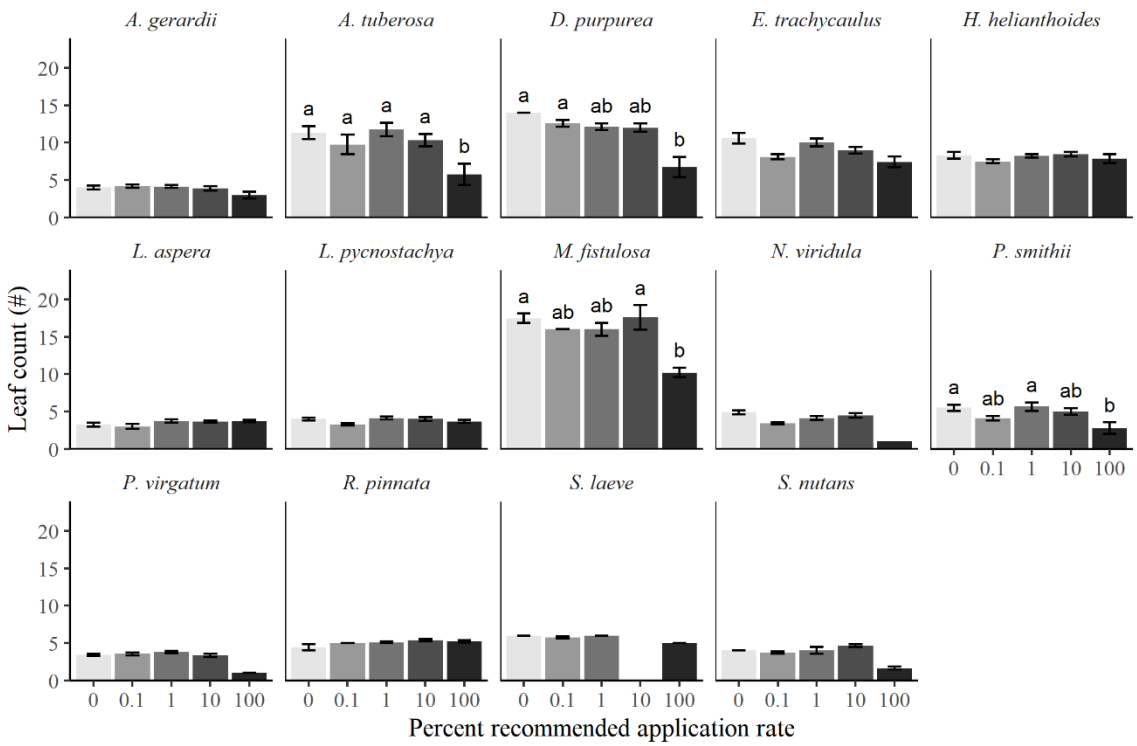

Figure 15**.** Effect of different trifluralin application rates on final leaf count. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

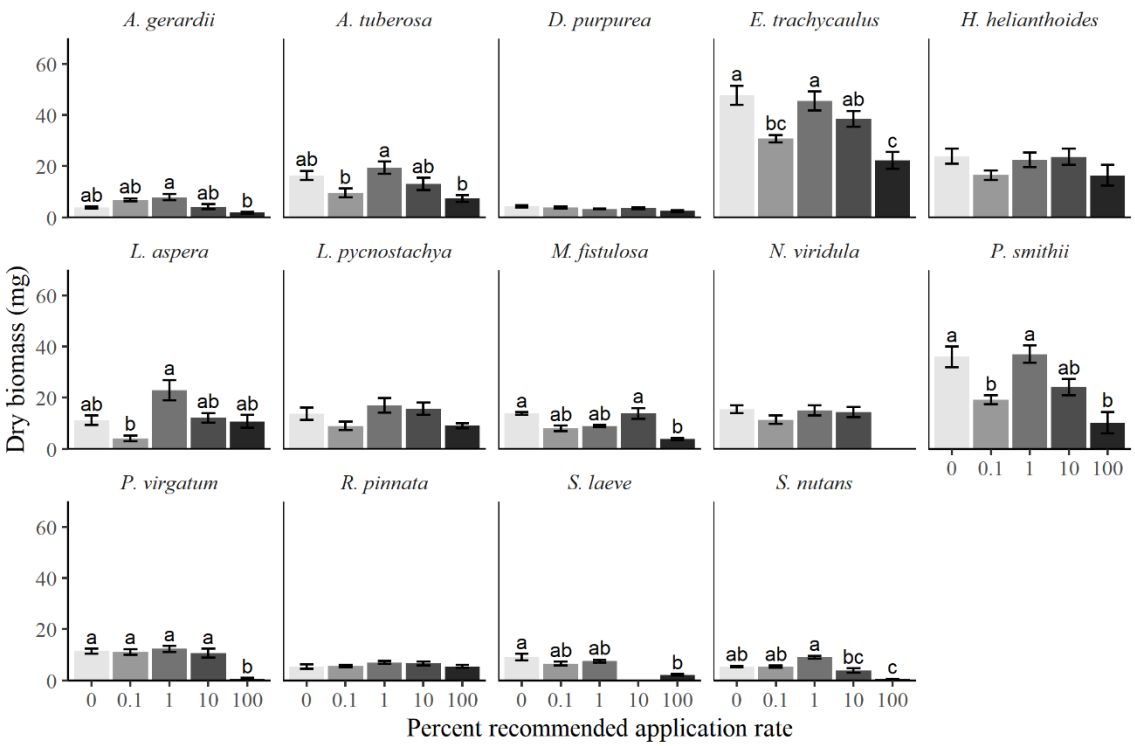

Figure 16**.** Effect of different trifluralin application rates on dry biomass. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

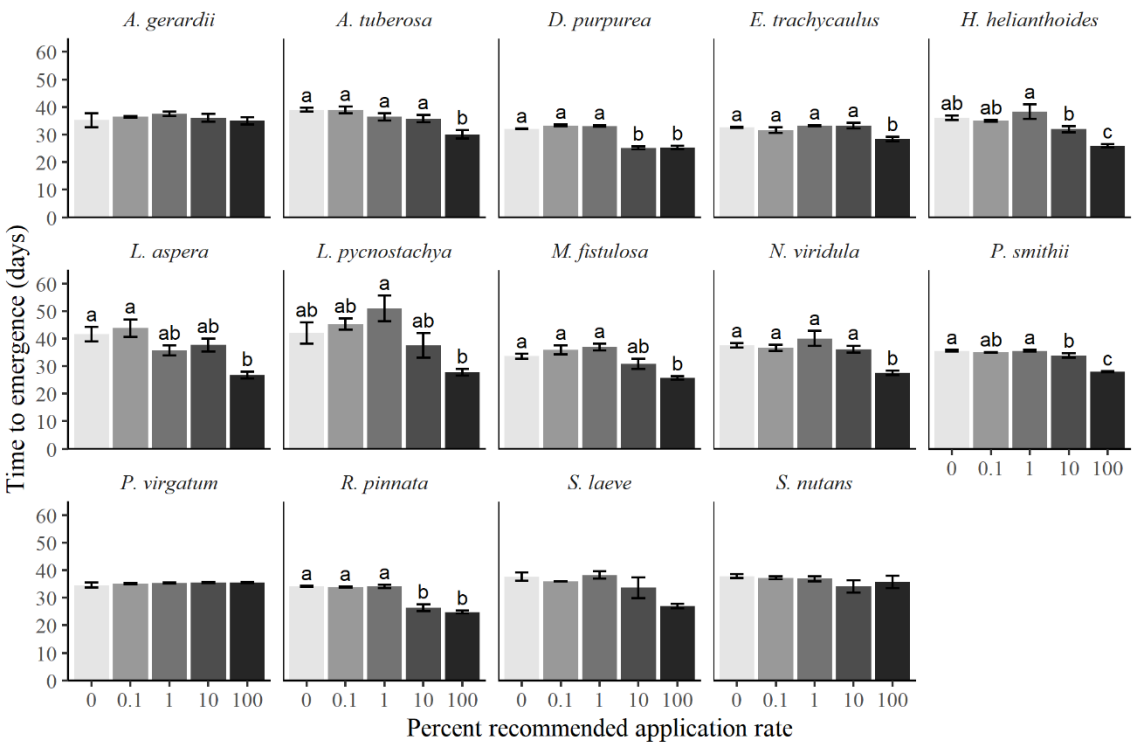

Figure 17. Effect of different atrazine application rates on time to emergence. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

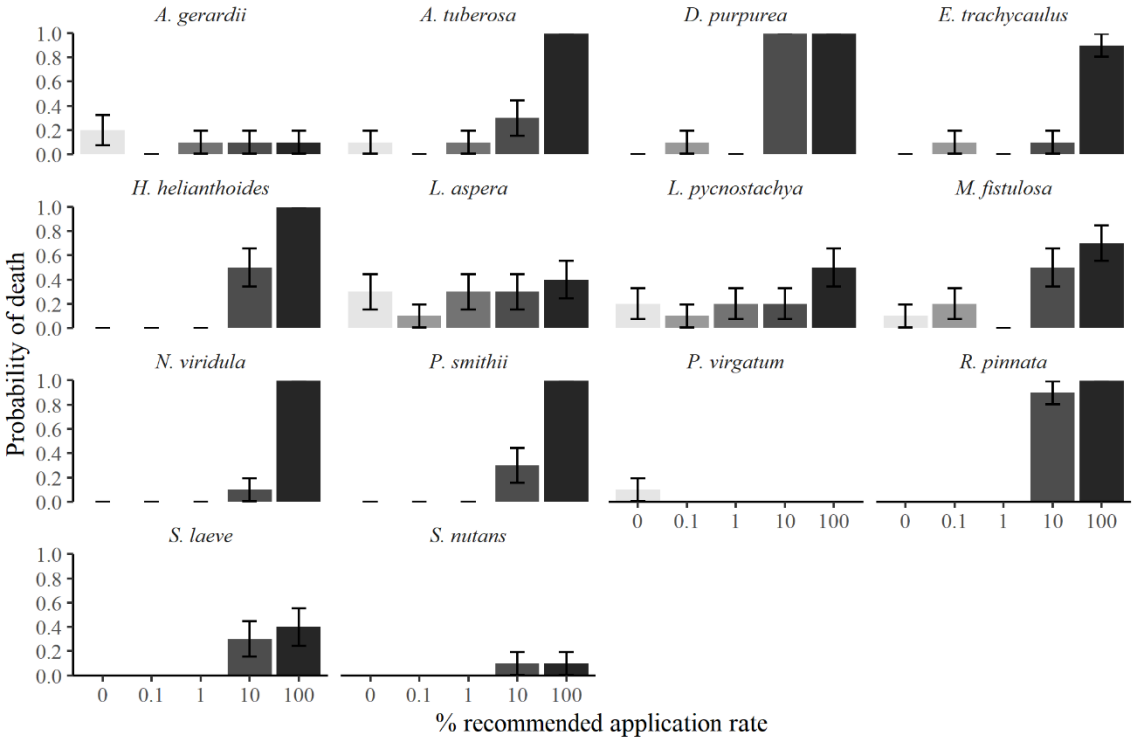

Figure 18. Effect of different atrazine application rates on probability of death. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

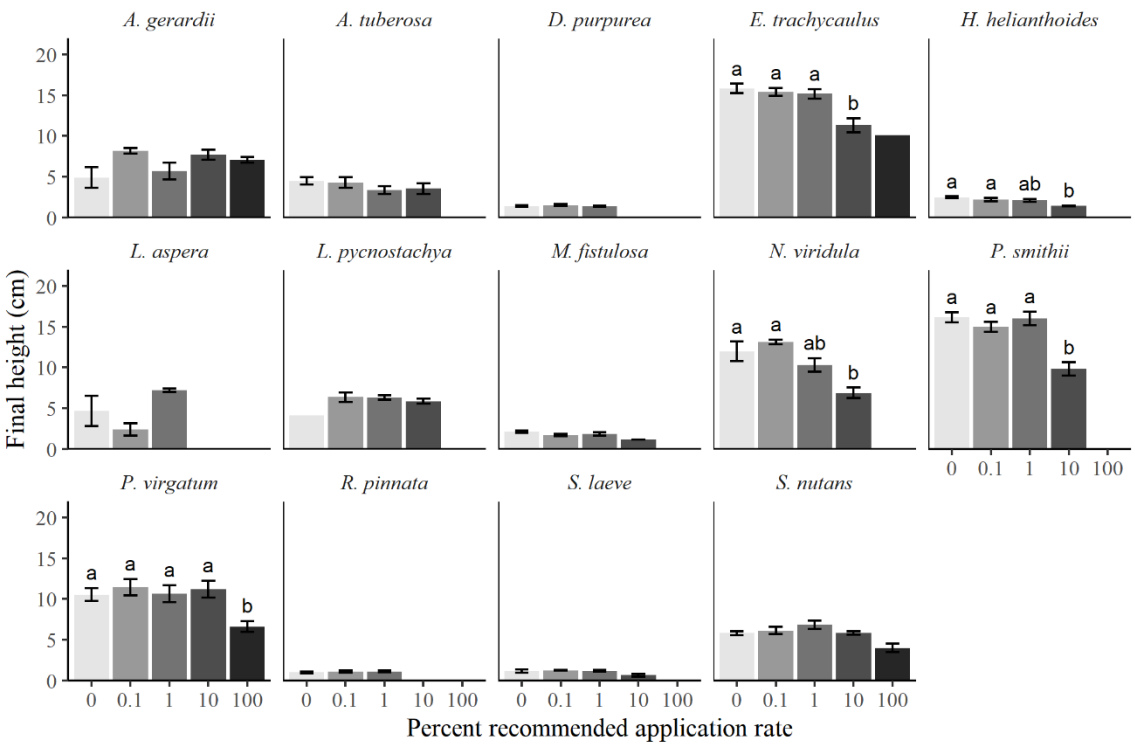

Figure 19. Effect of different atrazine application rates on final height. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

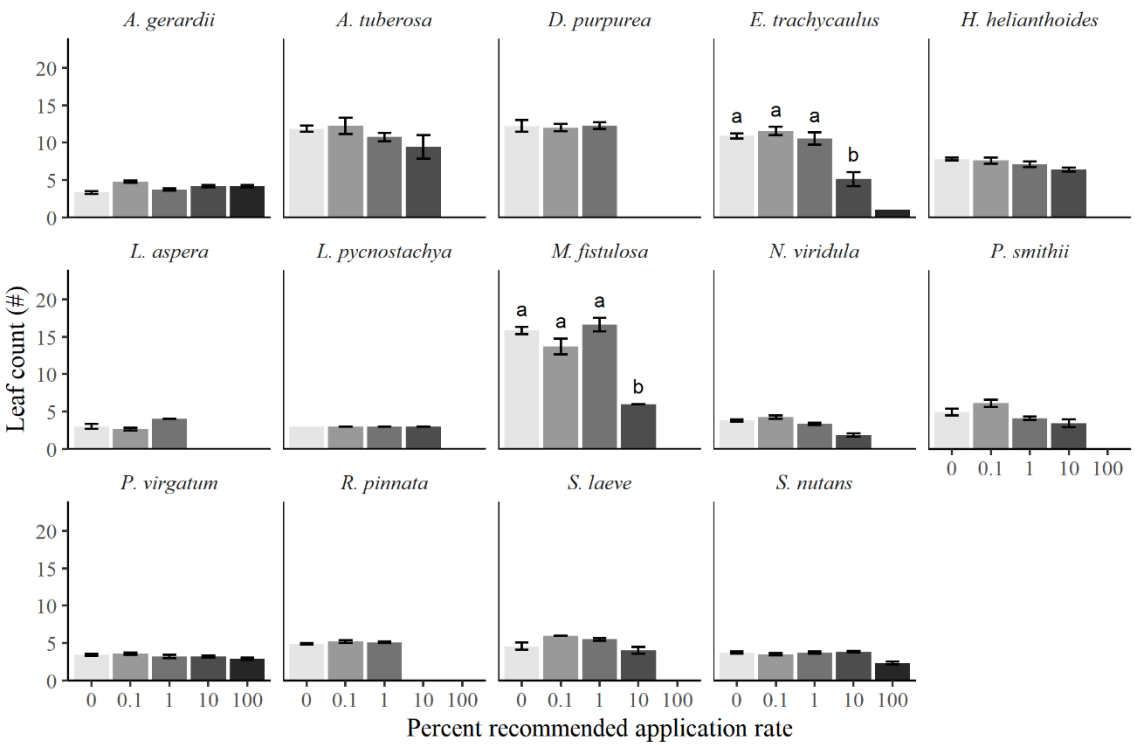

Figure 20. Effect of different atrazine application rates on final leaf count. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish between percent recommended application rate. Error bars represent one SE.

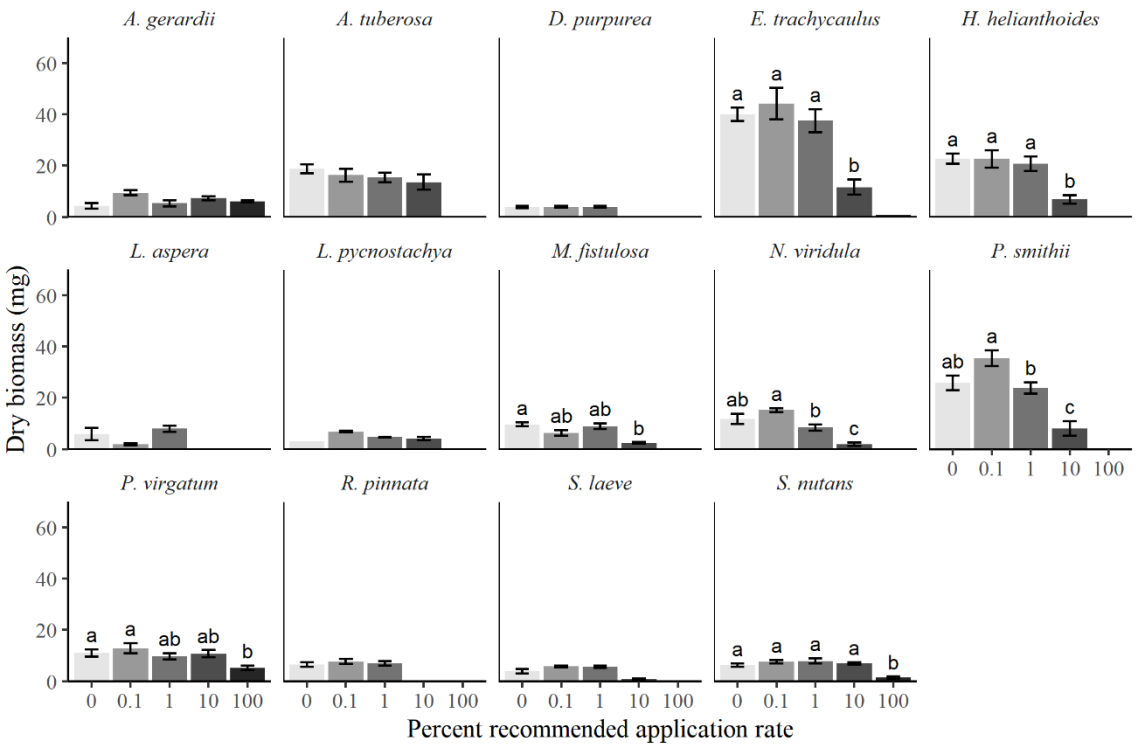

Figure 21. Effect of different atrazine application rates on dry biomass. Different letters indicate statistically different values ($\alpha = 0.05$). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among percent recommended application rate. Error bars represent one SE.

CHAPTER 3: IVERMECTIN EFFECTS ON NATIVE PLANTS OF THE NORTHERN GREAT PLAINS

Introduction

Ivermectin is a common anti-parasitic veterinary drug used to treat livestock (Laber et al. 2023) but can have unintended effects on non-target organisms. Ivermectin is used to control parasitic nematodes, one of the main health threats to livestock production (van Dijk et al. 2010; Martin et al. 2021), as they can reduce the productivity of the animals (Laber et al. 2023). These parasites can affect the economy and livelihoods of livestock producers, as producers rely on livestock as a source of income. In South Dakota alone, there are over 22 million acres in pastureland, supporting over 13,000 cattle and calf farms (USDA National Agricultural Statistics Service 2017). To rid livestock of these parasites, producers can use veterinary drugs like anthelmintics (Laber et al. 2023). One of the most commonly used anthelmintic drugs in South Dakota is ivermectin (Ulrich-Shad et al. 2021). Although ivermectin is a useful antiparasitic, it also negatively affects a variety of organisms such as aquatic invertebrates (Mesa et al. 2020), dung beetles (Liebig et al. 2010), and agricultural crops (Navrátilová et al. 2020). Although ivermectin can negatively affect agricultural crops, little to no research has been done on the effect of ivermectin on grassland vegetation (Vokřál et al. 2019).

Seeds may encounter ivermectin through several pathways: while passing through the digestive tract of livestock (Laber et al. 2023), when dung from a treated animal is deposited on grazing lands (Vokřál et al. 2019), or when ivermectin-treated animal dung is used as fertilizer on agricultural fields (Navrátilová et al. 2020). The ivermectin

administered to the animal undergoes little metabolism and some of the dose initially given to the animal is excreted in the dung (Lumaret et al. 2012). For example, dung deposited on soil can contain approximately 35% of the administered dose of ivermectin four days after application (Fernandez et al. 2009).

After contact with seeds, ivermectin may impact seeds through multiple mechanisms. Ivermectin may seep into the seed coat and contact the embryo tissue (Laber et al. 2023), which may have phytotoxic effects including inhibition of root growth, even at small doses such as 0.044 µg/mL (Vokřál et al. 2019). Ivermectin may also affect seeds indirectly through changes to the seed microbiome. Microbes in the soil around seeds can influence seed germination timing and percentages (Balshor et al. 2016; Ge and Wei 2023), and ivermectin can alter soil microbiomes (Torabian 2023). Therefore, by affecting the soil microbiome, ivermectin may indirectly affect seed germination.

The objective of my study was to evaluate the effects of ivermectin on native seed germination at concentrations consistent with those found in treated cattle dung. To achieve this, I performed a laboratory experiment in which I subjected seed of 14 NGP native plant species to two concentrations of ivermectin found in cattle dung. This information will be used to inform land managers of recommendations and best practices when grazing livestock on grasslands.

Methods

I conducted an experiment to determine the effect of different ivermectin concentrations on the germination of 14 plant species native to the NGP (Table 1). Study species were chosen based on their widespread use in ecological restoration and species

selection guides in the NGP (Lair and Redente 2004; Piper et al. 2007; Campbell and Hooymans 2016; Foster et. al 2007; USDA Natural Resource Conservation Service 2016; Xerces Society for Invertebrate Conservation 2018). Seeds were commercially sourced from Prairie Moon Nursery (Winona, MN) and Millborn Seeds (Brookings, SD) (Table 1) and were stored in a refrigerated environment at 5°C upon procurement.

I exposed eight replicates of 25 seeds to two ivermectin (Ivermax®, 5mg/mL) concentrations (low = 3 mg ivermectin/kg, and high = 10 mg ivermectin/kg) and a deionized water control. These concentrations represent a range of ivermectin found in ivermectin-treated cattle dung (Conforti et al. 2018; Pérez-Cogollo et al. 2015). The ivermectin was mixed with 0.2 mL of alcohol before adding it to water, as ivermectin is relatively insoluble in water on its own (Fernandez et al. 2009). Germination paper (3.5" x 4") was saturated with the ivermectin solution and placed in a 6 mil reclosable plastic bag. Then, 25 seeds of a single species were placed in a five by five grid on the germination paper in each bag. The bags were put in a germination chamber for observations to begin the following day (day 1). The germination chamber was set to a 12-hour day/night cycle with 18°C during light periods and 6°C during dark. These temperatures were gathered from NOAA National Centers for Environmental Information in 2021 and reflect the 20-year average day and night temperatures of the NGP during springtime.

The number of germinants were counted once a day for the first seven days of the experiment, and then every other day for 45 days. If germination was still observed at day 45, observations continued every other day until there were two observation days without germination, or until day 91 when the experiment was ended.

Data were analyzed with R (v4.1.2; R Core Team, 2021) using ANOVA and Tukey's HSD as a multiple comparison test among concentrations of ivermectin. Response variables include final germination percent (total germination / total seeds *100) and mean germination rate (total germination / length of experiment) of each study species and the explanatory variable was ivermectin concentration.

I also obtained seed weights for each species from Seed Information Database, except for *A. tuberosa* and *E. trachycaulus,* for which there were no seed weights available (SER, INSR, RBGK, Seed Information Database (SID), 2023). Species seed weights were matched with species final germination percent data for each concentration of ivermectin to examine the effect of seed weight on final germination percent. Data were analyzed with R (v4.1.2; R Core Team, 2021) using ANCOVA. The final germination percent was arcsine square root transformed, and seed weight was log transformed.

Results

Exposure to ivermectin concentrations representative of those found in ivermectin-treated cattle dung decreased germination of five species (Table 9). Ivermectin treatment at low and high concentration decreased final germination percent of *M. fistulosa* by 74% compared to the control (Figure 22). Final germination percent of *R. pinnata* decreased by 24% when treated with the high concentration of ivermectin compared to the control (Figure 22). Low and high concentrations of ivermectin decreased the germination rate of *M. fistulosa* by 84%, *R. pinnata* by 62%, and *S. laeve* by 30% compared to the control. The germination rates of *A. gerardii* and *A. tuberosa* were affected by ivermectin, but Tukey's HSD was unable to differentiate the effect of

concentration, despite significance shown in the ANOVA test. All other species germination metrics were unaffected by the presence of ivermectin. Seed weight did not affect final germination percent among ivermectin concentrations ($F_{2,330} = 1.10$, $p = 0.335$).

Discussion

The objective of my study was to evaluate the effects of ivermectin on native seed germination at concentrations consistent with those found in treated cattle dung. I found that exposure to ivermectin decreased the germination of five species (*A. gerardii, A. tuberosa, M. fistulosa, R. pinnata,* and *S. laeve*). Germination of all other study species was unaffected by ivermectin at low and high doses, consistent with other studies (Vokřál et al. 2019; Laber et al. 2023).

Ivermectin may influence seed germination and growth through two possible pathways. One possibility is that the chemical enters the seed via the seed coat and acts on the tissues to affect germination percentage, time, and synchrony before the radicle emerges (Laber et al. 2023). Another possibility is that the chemical may affect germination and growth when it encounters the radicle or the seed parts exposed when the radicle breaks the seed coat (Laber et al. 2023). To further examine the interaction between ivermectin and seeds, studies could focus on seed coat traits like thickness, chemical composition, and structural elements (Laber et al. 2023).

To potentially explain the varying effect of ivermectin on different species, I examined the effect of seed weight on final germination percent, as seed weight may be correlated with germination success (Bebawi et el. 1984, Hamza et al. 2019). Seed weight and seed coat thickness are correlated (Wada and Reed 2011), so I would expect that

ivermectin would have a larger effect on small seeds with thinner seed coats. When I regressed final germination percentages by seed weight for each species, I found that seed weight was not correlated with final germination percent when exposed to varying concentrations of ivermectin.

Ivermectin at differing concentrations can impact soil microbial communities (Torabian 2023), which may also impact seed germination. Seeds host a variety of microbes that play a role in the germination process (Glassner et al. 2018). Therefore, by affecting the soil microbial community, ivermectin may have an indirect impact on seed germination. For example, fungicides, which are not intended to impact seed growth processes, decreased germination percent and germination rate in wheat seeds (Gogna and Dorai 2015). This example reinforces the idea that seeds require a beneficial microbiome, which can be negatively affected by the presence of various chemicals. More research is needed to determine the effects of ivermectin on important microbes for seed germination in grassland species.

If managing for native species in grazing lands, I recommend being cautious about ivermectin use. The presence of phytotoxic concentrations of ivermectin (approximately 0.044 µg/mL) in livestock feces (Vokřál et al. 2019) can affect the final germination percent and germination rate of desirable native plant species, which may have consequences for species compositions in affected grazing lands (Laber et al. 2023). For example, *A. gerardii* is an excellent source of forage for livestock (Johnson and Larson 1999), so as ivermectin-treated livestock consume these plants, it may affect the germination of the seeds present in or under the excreted dung. *R. pinnata* is also palatable forage (Johnson and Larson 1999), but *A. tuberosa* (USDAS NRCS National

Plant Data Center 2006), *M. fistulosa* (Johnson and Larson 1999), and *S. laeve* (MSU Extension n.d.) are not readily consumed by livestock. Although most perennial species can reproduce through vegetative propagules, they also use seeds as a means of reproduction (Takada and Nakajima 1996). Ivermectin-sensitive perennial plants used for forage, such as *A. gerardii* and *R. pinnata*, may still be able to reproduce, but at a lower capacity than if their seeds were not exposed to ivermectin in treated livestock dung. If ivermectin will be used on livestock, be aware of the potential to see a decrease in certain native species that may be sensitive to ivermectin presence, such as those shown in my study.

Table 9. Effect of ivermectin on final germination percent and germination rate of 14 NGP native species. Bold p-values indicate significance (alpha = 0.05). df = 5, 42.

	Germination %		Germination rate (seeds/day)	
Species	F	p	F	p
A. gerardii	1.53	0.240	4.00	**0.034**
A. tuberosa	2.15	0.141	3.46	**0.050**
D. purpurea	2.66	0.093	1.21	0.318
E. trachycaulus	0.11	0.892	0.676	0.520
H. helianthoides	0.03	0.973	2.07	0.151
L. aspera	1.78	0.193	0.988	0.389
L. pycnostachya	0.52	0.600	0.751	0.484
M. fistulosa	38.1	**<0.001**	24.7	**<0.001**
N. viridula	2.86	0.080	2.22	0.134
P. smithii	0.04	0.965	0.012	0.988
P. virgatum	1.72	0.204	0.354	0.706
R. pinnata	6.50	**0.006**	46.4	**<0.001**
S. laeve	2.97	0.073	5.31	**0.014**
S. nutans	2.44	0.112	2.77	0.085

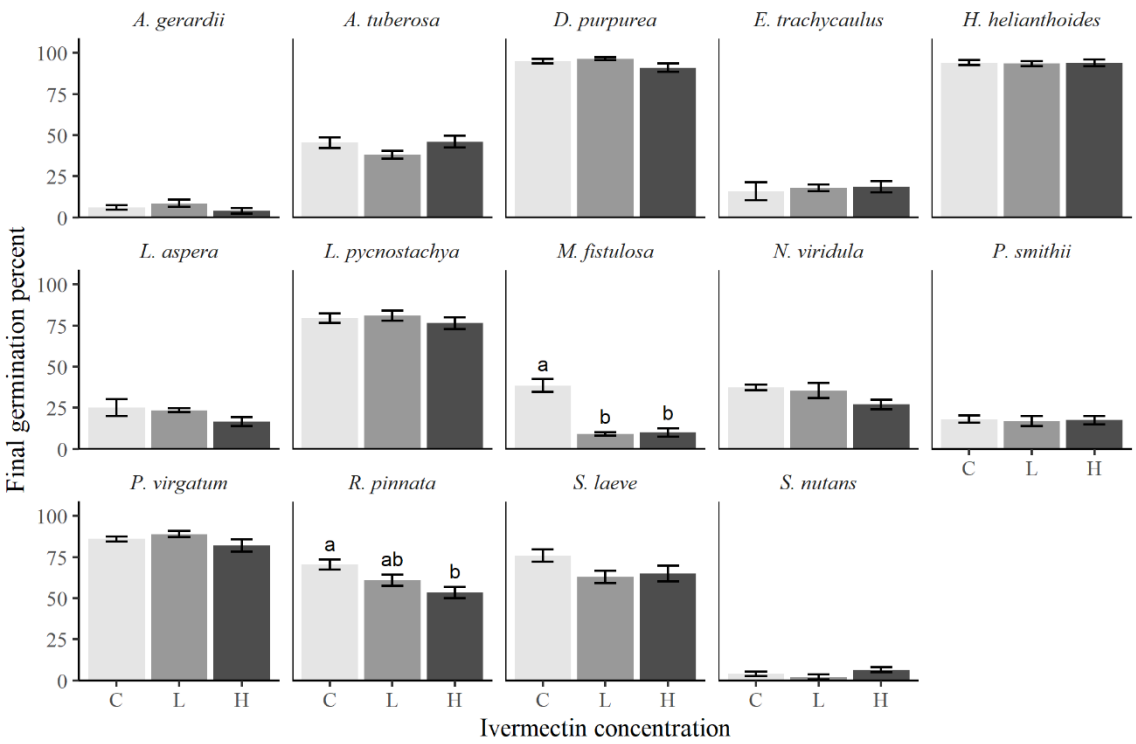

Figure 22. Effect of different ivermectin concentrations on final germination percent. C = control, L = low, H = high. Different letters indicate statistically different values (α = 0.05). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among ivermectin concentrations. Error bars represent one SE.

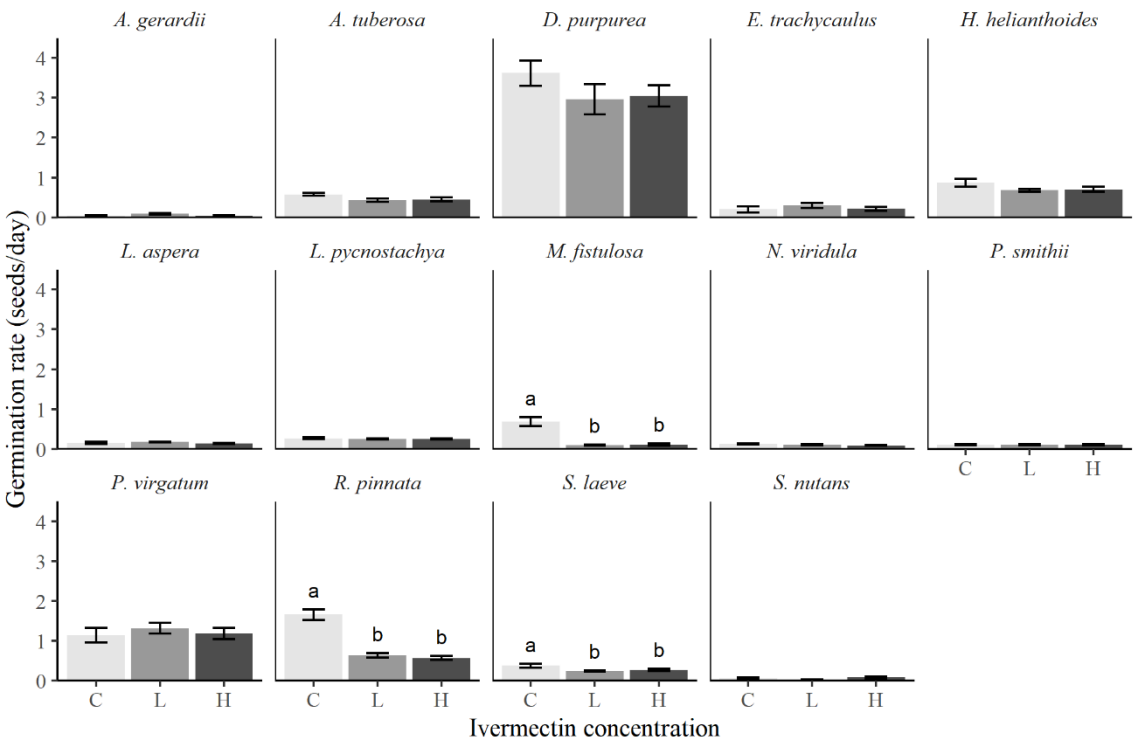

Figure 23. Effect of different ivermectin concentrations on germination rate. C = control, L = low, H = high. Different letters indicate statistically different values (α = 0.05). No letters indicate no significant difference among concentrations. Bars are shaded to distinguish among ivermectin concentrations. Error bars represent one SE.

CHAPTER 4: SUMMARY AND BROADER IMPLICATIONS

The goal of my research was to understand the effects of anthropogenic chemicals on NGP native plants and to make suggestions to improve the germination, emergence, and growth success of native plants in a fragmented landscape. The objective of Chapter 2 was to evaluate if native seed germination, emergence, and growth was affected by 2,4-D, atrazine, or trifluralin at different concentrations representative of herbicide drift. The objective of Chapter 3 was to evaluate if native seed germination was affected by ivermectin presence at low and high concentrations representative of levels found in treated livestock dung.

In Chapter 2, I found that herbicides at various concentrations negatively affected the germination, emergence, and growth of many NGP native plant species. Germination of the greatest number of species was most negatively affected by 2,4-D, while emergence and growth of the greatest number of species were most negatively affected by atrazine. Germination of *D. purpurea* and *P. virgatum* were unaffected by the study herbicides at any concentration. Emergence and growth of each study species were affected by at least one herbicide.

The knowledge gained in Chapter 2 can be used to inform restoration practitioners of which species will perform best in an area that may experience herbicide drift. Another factor to consider in seed-based restoration is distance from herbicide application. A seed closer to a field border where herbicide is sprayed is more likely to experience negative effects on germination, emergence, and growth than a seed farther away from a field border. Herbicide applicators can reduce the amount of herbicide drift from their applications to nontarget areas by applying during ideal weather conditions and using drift control equipment/additives (Dexter 1993).

In Chapter 3 I found that ivermectin at high and low levels found in dung of ivermectin-treated livestock decreased the germination of some NGP native plant species. The final germination percent of *M. fistulosa* and *R. pinnata* decreased with exposure to high and low residual levels of ivermectin. The germination rate of *A. gerardii, M. fistulosa, R. pinnata,* and *S. laeve* decreased with exposure to high and low residual levels of ivermectin. The germination of all other study species was unaffected by high and low residual levels of ivermectin, making them desirable species to have in grazing lands with ivermectin-treated animals.

The knowledge gained in Chapter 3 can inform NGP grazing managers of the effects of high and low residual levels of ivermectin on the germination of native plant species on their lands. If a grazing manager uses ivermectin on their animals, they may see a decrease in the presence of certain native plant species. Perennial plants that experience decreased final germination percent when exposed to residual levels of ivermectin, such as *A. gerardii* and *R. pinnata*, may still be able to reproduce vegetatively, but would have a decreased capacity for seed-based reproduction. Little research exists on the effect of ivermectin on native plants. Considering the negative effects seen in my research, I encourage further study of the effect of ivermectin on native plants to identify other potential effects of this chemical on grasslands.

Although I have demonstrated the potential effects of agricultural chemicals on individuals of certain native plant species, these chemicals can also impact native plant communities as a whole. Multiple theories attempt to explain community assembly such as unified neutral theory (neutral theory) and filtering (Ruffley et al. 2019). I will view the results of my experiments through the lens of these community assembly theories to

predict what changes may happen when native plant species are exposed to low concentrations of agricultural chemicals.

Neutral theory seeks to explain the dynamics of ecological communities. Neutral theory states that the probabilities of individuals in a community reproducing, dying, migrating, and speciating are essentially identical, and assumes that all individuals of all species are ecologically equivalent (Hubbell 2001). Further, neutral theory assumes that each community has a fixed number of individuals it will hold, and the species abundances will increase and decrease stochastically (Hubbell 2001; Etienne and Olff 2004). A main tenet of neutral theory is the idea of a zero-sum game, which describes the relationship among density of individuals (Hubbell 2001). This theory states that if density of individuals in a community is constant, then an increase in one species will be paired with a decrease in all other species in the community; the sum of all abundance changes will always be zero (Hubbell 2001). For example, if one individual dies, a new individual of any species will replace it.

Neutral theory can be used to predict the changes in community assembly when a community is exposed to agricultural chemicals. When a native plant community is exposed to agricultural chemicals, neutral theory would suggest that if individuals of a certain species died as a result of the chemical exposure, individuals of other species would take their place whether they occur in the same functional group or not (Hubbell 2001). This could cause the decrease or absence of certain species from the community but may also simultaneously cause the increase in abundance of other species (Hubbell 2001). A decrease or absence of some species may be beneficial if the given species was invasive, but the loss may be considered detrimental to the community if the species was

of conservation or cultural value. However, even as some species are lost, it may create the opportunity for new species to enter the community, therefore causing no loss of species richness (Volkov et al. 2003). If the replacement of chemical-intolerant species with chemical-tolerant species were to continue through time and space, communities may no longer experience negative effects from agricultural chemical exposure, other than the potential loss of biodiversity, as all of the chemical-intolerant species would no longer be present in the community. This concept is evident in herbicide resistant weed communities, which can demonstrate how herbicide resistance can occur when subjected to consistent agricultural chemical applications (Baucom 2019). If natural plant communities respond to agricultural chemicals in the same way as agricultural weed communities, one may expect to see herbicide resistant native plants in the future.

Agricultural chemical exposure may be a new ecological filter to consider when analyzing native plant community assembly. Filter-based community assembly theorizes that communities are created from the passing of species through various biotic and abiotic ecological filters (Keddy 1992; Cadotte and Tucker 2017; Nguyen et al. 2023). If a species is not able to pass through one or more of these filters, it will not be present in that community (Keddy 1992). If a native plant/seed is able to tolerate all other filter conditions such as dispersal, weather conditions, available resources, competition, etc. but dies or is unable to grow in the presence of agricultural chemicals, then the chemical(s) might be the only barrier to that species presence in a community. However, this barrier may not be impenetrable as there is also a temporal component to potential for agricultural chemical effects (McManamen et al. 2018). If the chemical has a short half-life or if the chemical spray time does not coincide with important life stage

transitions of native plants, the probability for chemical to act as a filter is low. If the agricultural chemical is not acting as a filter, then we would expect the community assembly to only be affected by the other ecological filters.

Agricultural chemicals affect individual native plant species but can also affect the community assembly of grasslands. When viewed through neutral theory, agricultural chemical exposure may shift species composition away from chemical-sensitive species, and towards chemical-tolerant species. Filter-based community assembly theory suggests that agricultural chemicals may act as an additional filter to affect species composition in native plant communities. Viewing my research through different community assembly theories provides insight into how agricultural chemicals may further present themselves on the greater landscape.

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