ESTABLISHMENT AND SEED PRODUCTION OF NATIVE FORBS USED IN RESTORATION

by

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ABSTRACT

The importance of incorporating native wildflowers into seed mixtures for disturbed land revegetation projects is widely known and accepted. However, further assessment of weed management approaches is a necessary step to successfully establish and produce native wildflower seed. We examined the impact of pre and post-emergence herbicides alone and in combination with hand weeding on 5 wildflower species [slender white prairie clover (*Dalea candida*(Michx). ex Willd), blanketflower (*Gaillardia aristata* Pursh), fuzzy tongue penstemon (*Penstemon eriantherus* Pursh var. *eriantherus*), silverleaf phacelia (*Phacelia hastata* Douglas ex Lehm.), and prairie coneflower (*Ratibida columnifera* (Nutt.) Woot. & Standl)] under greenhouse and field conditions. Herbicides evaluated included Treflan (trifluralin) 189 l/ha, Lorox (linuron) 1.121 kg/ha., Permit (halsulfuron) 91 g/ha., Plateau (imazapic) 560 g/ha, and Prowl (pendimethalin) 4.2 l/ha. The objectives of this study were to 1) determine wildflower seedling tolerance to post-emergence herbicides, 2) evaluate the effect of pre-and post-emergence herbicides on native wildflower seedling establishment, weed control, and wildflower seed production.

For objective 1 a randomized block design was used and repeated twice. A Monte Carlo resampling assessed herbicide damage and a randomized block design analysis of variance (ANOVA) assessed herbicide impact on fresh and dry biomass. Results indicated that the *D. candida* and *R. columnifera* were minimally affected by herbicide treatments, while *G. aristata* and *P. hastata* were strongly affected, the first by linuron and halosulfuron and the last by halosulfuron and imazapic.

Objective 2 assessed hand weeding and pre and postemergence herbicide effects on native wildflowers. A randomized block design was used to assess wildflower establishment, percentage cover, yield, and seed germination and viability, along with weed community composition and cover as a function of weed management approach. Data were analyzed with a randomized block design analysis of variance (ANOVA) to test for differences in wildflowers seedling emergence, percent cover of wildflowers, and seed yield. Wildflower species responded uniquely to weed management, indicating caution should be used when applying herbicides to the tested species. Specifically, emergence of *P. eriantherus, D. candida* and *P. hastata* were negatively affected by trifluralin, indicating this herbicide may not be suited for the tested wildflowers.

CHAPTER 1

PROJECT BACKGROUND AND OBJECTIVES

Introduction

The native seed industry responds to a highly competitive and fluctuating market with prices driven by imbalances between supply and demand. On one hand, adequate supply has proven difficult because establishment and production of a seed field of native perennials is often tenuous (Jones and Young 2005). On the other hand, increased demand is driven by the interest in incorporating native wildflowers into seed mixtures for conservation plantings and land revegetation projects, as diversity has been suggested to be important in restoration and invasive plant management (Sheley and Half 2006, Davis et al. 2000).

Despite a growing interest in restoring the rangelands of the Intermountain West with native forbs (Shock et al. 2006), and unlike the marketing of non-native species by the commercial floriculture industry, wildflower seed producers need to gain scientific, technical, and practical knowledge. Specifically, there is a lack of species-specific information in many aspects of native forb seed production such as seed dormancy, seeding techniques, seedling emergence, plant growth and development, biomass and seed production characteristics, stand management, weed management including tolerance to herbicide, seed handling, and seed storage (Bailey and Martin 2007, Jones and Hayes 1999).

This project assess herbicide weed management approaches in wildflower seed production fields. The objectives listed below aim at providing a better understanding of native wildflower growth and management in commercial production settings. Understanding the impact of weed management approaches on wildflower establishment and seed production will provide a framework for initiating appropriate agronomic management, thereby meeting the increased demand for seeds.

Objectives

1. Determine wildflower seedling tolerance to post-emergence herbicides.
2. Evaluate the effect of pre-and post-emergence herbicides on native wildflower seedling establishment, weed control, and wildflower seed production.
3. Quantify best weed management programs based on production assessments.

Habitat Restoration, Biodiversity, and Ecosystem Services

Ecosystems generate a range of goods and services, for example, processes as diverse as pollination, erosion control, and reduced invasibility, all of which are important for human wellbeing and collectively called ecosystem services (Nelson et al. 2009). The inclusion of ecosystem services in conservation planning has great potential to provide opportunities for biodiversity protection (Chan et al. 2006) and increased ecosystem functioning (Loreau et al. 2001). Biodiversity is defined in this paper as diversity at all organized levels ranging from genetic diversity within a population to diversity of ecosystems across landscapes.

The number and kind of species present alters ecosystem processes because many species traits have functional consequences in their environment (Chapin et al. 2000). Although there are many theories on the effects of biodiversity on ecosystem properties, it is generally agreed that the rate of ecosystem processes increases with species richness when each species has a complimentary niche (Chapin et al. 2000). Species richness is easier to measure than functional biodiversity, however, functional biodiversity may be a better indicator of ecosystem resilience (Loreau et al. 2001). Functional groups have been defined as sets of species showing either similar responses to the environment or similar effects on major ecosystem processes. (Gitay and Noble 1997).

Native wildflower species are a morphogical group that is often underrepresented in restoration projects. Increasing functional diversity by establishing native forbs into degraded landscapes is crucial for invasive plant management and plant community restorations (Sheley and Half 2006). There is substantial evidence that, under similar environmental conditions, functionally distinct landscapes with high species diversity can influence the resilience and resistance of ecosystems to environmental change (Chapin et al. 2000) and inhibit weed invasion (Hooper et al. 2005, Naeem et al. 2000, Williams et al. 2007, Tilman et al. 1997, Zimdahl 2004) due to a lower chance for species redundancy in ecosystem functions.

In addition to increasing ecosystem functioning, diversity can provide many additional ecosystem services (Taylor et al. 2006). For example, plant functional diversity is crucial for the survival of beneficial organisms such as pollinator species. To enhance the survivorship and effectiveness of pollinators, the Natural Resource Conservation

Service (NRCS) recommends planting variety of flowering plants and shrubs, including native wildflowers, on farmlands and in natural settings (NRCS, USDA 2007). Planting species that have differing flowering times that occur throughout the growing season decreases competition for available flowers and provides pollinators with a constant supply of food, a critical factor for ecosystem functioning (NRCS, USDA 2007). Along with increasing beneficial organisms, native wildflowers can anchor soil with deep dense rooting and stabilize erodible slopes, rivers or stream banks, (Alario 2000). Native wildflowers also have the potential of decreasing community invasability in ecosystems by increasing functional diversity (Pokorny et al. 2005).

Restoration Using Native Wildflowers

There is an increased effort to restore degraded ecosystems worldwide (Chapin et al. 2000). In the past, reclamation of degraded land has usually been accomplished with a few number of non-native species that were quick to establish ground cover rapidly (Lesica and Allendorf 1999). However, one goal of restoration is creating a self supporting community that will provide ecosystem functions and processes such as preventing erosion and providing habitat for diverse native species (Bradshaw 1987). While it is essential to quickly establish vegetation to prevent erosion, it is equally important to protect the ecological interactions present in the native mosaic of locally adapted populations (Lesica and Allendorf 1999).

Despite the growing interest to include native wildflowers into restoration projects (Aldrich 2002), very little research has been conducted on species composition of grass and wildflower mixtures needed for restoration in semiarid regions (Dewey et al. 2006).

Many “native plantings” projects conducted from the 1960’s to the 1980’s had few or no native forbs in the mixes because seeds of these species were not commercially available (Williams et al. 2007). Cost of seed, failed establishment, and lack of available seed has made it impractical to use native wildflowers in habitat restoration. Before mixtures containing competitive native wildflower species can be developed, biological and ecological requirements such as competitive ability need to be further studied (Dewey et al. 2006).

The extent to which wildflowers establish and grow when replanted into an area where they are not naturally found it is not fully understood. Many restoration projects fail because they use a seed production source too different from the area being restored (Aldrich 2002). For example, several studies indicate that native wildflower species may be more competitive with weeds in local growing conditions (Gallitano and Skroch 1993, Halsey and Lilly 1998, Sherman 1995, Aldrich 2002), especially if the seed source is local and adapted to the area. Although research has been conducted on wildflower species and how they behave in natural environments, not enough is known about how these species will behave in a crop setting and which species are too vulnerable for restoration or seed production.

Seed Production and Agronomy of Native Wildflowers

The commercial production of wildflower seeds is an emerging science with management and species selection in its nascent stages. In recent years, there has been increased demand for native plant production for restoration projects and personal use (Martin 1986, Harper-Lore and Wilson 2000, Jones and Foote 1997). To meet the current

demand, wildflower species need to be grown in production fields to make a diverse ecotypic seed mix more readily and affordably available to restoration biologists and private contractors (Norcini et al. 2006, Aldrich 2002).

Although demand is a driver for the kind of species producers will grow, many considerations such as price of seed, local consumer demand, and competitive ability must be made when choosing which species to grow and where to obtain the seed. Few native wildflower species have management protocols in seed production fields. For example, planting time for most species is a late fall dormant seeding, proper seedbed preparation is smooth, free of hard clots, and firm, and it is recommended to apply a non selective post emergence herbicide prior to planting, once before tilling and irrigation, and then repeated as necessary before emergence (Aldrich 2002). In general, seeds need to be drill seeded 6-12 mm below the soil surface or broadcast seeded during a dormant period. Seeding at 75 PLS (pure live seed) per meter with at least a 60 cm row spacing is suggested for weed control and seed development. (Susan Winslow, NRCS personal communication), where PLS is defined as

PLS = (percent purity) * (percent germination rate) / 100 (Eq 1)

Percent purity = amount of seed vs. amount of chaff, other non-viable plant material, and weed seeds (Liskey 2001). Native perennial species rarely produce a seed crop the establishment year and a minimum of two to three years lead time must be factored in to achieve production goals (NRCS, USDA 2007). Seeds are harvested by hand or by using a leaf vacuum. The seed is rubbed on a rub board to scarify any achenes and remove

excess plant material and filtered through a series of sieves. The seed is then cleaned by using an air blown screen seed cleaner (NRCS, UDSA 2007).

Weed Competition

Competition is an important factor that affects individual plants, plant populations, and plant community dynamics both in wildland and in crop settings (Zimdahl 2004). It is defined as an individuals' demand on a common pool of resources (e.g. water, nutrients, space) that are limited (Booth et al. 2003). Prior to and during the 1950s when Elton (1958) introduced the term ''ecological resistance'', ecologists and land managers have been interested in managing for plant communities that are competitive with undesirable species (Krueger-Mangold et al. 2006).

There are two main theories for what makes a species most competitive in an environment. One states that competition is an important factor at all resource levels (Tilman 1997), postulating that late seral species have lower R*s for nutrients than early seral species (R* defined as the concentration of a limiting resource that a species requires to survive in a habitat). This lower R* allows late seral species to become dominant as secondary succession progresses and nutrient availability (especially nitrogen) decreases. This theory is countered by the concept that competition is more intense in high resource areas because there are more resources to compete over (Grime 1977). The exact method used to quantify competition intensity can fundamentally affect the interpretation (Grace 1993).

Many agronomic field studies have found that crop yield decreases with increased weed competition for resources (O'Donovan and Sharma 1983, Whish et al. 2002, van

Heemst 1985). Cousens (1985) developed a simple model to describe the relationship between crop yield loss and weed density (Equation 2)

$$Y = Y_{max}\left[1 - \frac{i \cdot Nw}{1 + i \cdot Nw / a}\right] \qquad \text{(Eq. 2)}$$

where Y is the crop yield per plot (g/m^2 or scaled up to kg/ha), i is the initial slope (proportional yield loss as weed density approaches zero) and a is the asymptote (maximum proportional yield loss as weed density approaches infinity), Nw is the weed density in the plot (plants/m^2) and Y_{max} is maximum yield observed in the experiment (Jasieniuk et al. 2001). Because competition is a spatially explicit process, this relationship is stronger when plants are close to their neighbors (Crawley 1997).

One measure that accounts for the efficiency of biomass accumulation of a plant, is its Relative Growth Rate (RGR), defined as:

$(\ln W_2 - \ln W_1) / (t_2 - t_1)$ where W_1 and W_2 are plant dry weights at times t_1 and t_2. (Eq.3)

RGR is a good approximation of the competitive ability of a plant (Wang et al. 2006) and differences in RGR between native and invasive plant species is thought to be a major factor contributing to invasion (James and Drenovsky 2007). Because plant growth rate has a strong influence on competitive ability (Zimdahl 2004), and wildflowers have a much slower growth rate than many weeds (Bazzaz 1979), weeds have the potential to decrease the establishment and growth of native wildflowers.

Weed competition has been cited as the most important cause of failure in prairiegrass and wildflower establishment (Aldrich 2002, Albright Seed 1998). The early growth stages of native wildflowers are threatened by slow germination, emergence,

establishment, and heavy weed competition for water, sunlight, and nutrients (Norcini and Aldrich 2004). Furthermore, weed competition is strongest when species are similar and make the same demands on the habitat. (Clements et al. 1929). Therefore, functionally similar species such as weedy forbs are most competitive with native forbs in seed production settings.

Weed Management

Weed management is critical for minimizing interspecific competition. It is accepted that crop yield reductions are generally in proportion to the amount of light, water, or nutrients weeds use at the expense of the crop (Roush and Radosevich 1985). However, wildflowers require a unique weed management approach from many crops due to their slow growth rate and low nutrient needs (Shock et al. 2006). Thus, a wide array of weed management options need to be assessed and further understood in the context of wildflower seed production and often the most effective approach is an integration of several methods (Aldrich 2002).

Hand weeding, mulching, and chemical weed management are discussed below, as well as the impact that moisture through irrigation, and nutrient additions via fertilizers, has on native wildflower competitive ability. Adding resources such as water and fertilizer to a field of native perennials can intensify the differences between invasive and native species RGR's (James and Drenovsky 2007) because native species have high nutrient use efficiencies and often cannot utilize the resources.

Traditional Weed Management Some traditional weed management techniques such as mowing could have negative or mixed effects on wildflower crops. For example, mowing, irrigation, and fertilizing have the potential to favor weeds in wildflower production fields as most wildflowers grow slowly and have low nutrient needs (Harper-Lore and Wilson 2000, Ahern and Boughton 1994) Effects of mowing are site specific, as are many of the factors in wildflower establishment (Gallitano and Skroch 1993). Timing and frequency of cutting appears to be a critical factor for increased seedling establishment (Jones and Hayes 1999). Mowing has been suggested as a weed control practice after weeds grow to 20-30 cm (mow to 10-15 cm) (Matzke 1998, Aldrich 2002).

It is important to know the life cycle of specific wildflower species to utilize a mowing regime that will facilitate a sustainable planting (Norcini and Aldrich 2004). Suggested mowing frequencies range from twice annually (EPA 1999), to four times annually, (Lickorish et al. 1997), and to once a week (Williams et tal. 2007), all recommendations being experiment and site dependent. Williams et al. (2007) found that while native wildflowers mowed once a week had significantly greater root and shoot biomass than those in unmowed control plots, number of living seedlings increased steadily through the summer in mowed plots where in control plots they peaked in late June and then decreased as the summer progressed. Williams et al. (2007) study also found that eight species of wildflowers bloomed in the mowed treatment by the second year and only one species in control plot. The one species that bloomed, blackeyed susan (*Rudbeckia Hirta* L.) had a relative abundance of 6,660 for flowering plants in mowed

plots compared to 210 in the control. This implies greater seed production in the mowed plots because of the number of wildflowers that were able to establish and flourish.

Irrigation in Wildflower Seed Production Fields Water does not have a role of equal magnitude in all crop-weed interactions (Zimdahl 2004) and high water use efficiency makes plants less competitive with abundant water (Booth et al. 2003). In traditional cropping systems, where many crops have low water use efficiencies relative to native wildflowers, irrigation can be critical for plant establishment. However, farm soils can have a large reservoir of both summer and winter annual weed seeds in the topsoil, which readily germinate and outcompete native species for space and water when irrigated (Banerjee et al. 2006).

The impact of irrigation on native seed production is controversial. While some studies indicate that irrigation is an important success factor in the establishment of native wildflowers and their seed production (Aldrich 2002, EPA 1999), others studies suggest that irrigation could favor weeds (Shock et al. 2006). Anecdotal evidences suggest that during the seedling stage of wildflower establishment irrigation may be critical, but afterward weeds could benefit from the irrigation (Albright Seed Company 1998a). Irrigation using water concentrating techniques such as contour furrows (Banerjee et al. 2006) and subsurface drip irrigation (Shock et al. 2006) have been suggested to concentrate water specifically on wildflower seedlings and minimize the potential of weed competition during seedling development. The water is placed deep in the soil, below the root zones of most weeds, but within most wildflower's root zones.

Impacts of Nutrient Management on Weed Competition in Wildflower Fields

Fertilizing at planting provides little benefit for wildflowers and could increase weed pressure (Aldrich 2002), likely due to later successional species' low nutrient requirements. Jones and Hayes (1999) found that it was possible to successfully introduce a common wildflower species lesser knapweed (*Centaurea nigra* L.), narrowleaf plantain (*Plantago lanceolata* L.), common yarrow (*Achillea millefolium* L. var. millefolium), wood betony (*Pedicularis canadensis* L.Trevis.) and common selfheal (*Prunella vulgaris* L.) into previously intensively managed grassland in the absence of fertilizer inputs. Nitrogen is one of the most mobile and absorbable nutrients, therefore adding fertilizer may benefit weeds that have low nutrient use efficiencies at the cost of the wildflower crop (Booth et al. 2003). Nitrogen inputs have also been cited as causing inhibition of forb seedling establishment in natural settings (Foster and Gross 1998) as well as seed production (Aldrich 2002).

Chemical Weed Management Herbicide use is one of the most prevalent management techniques in crop settings. However, there is a growing body of evidence that suggests herbicides have undesirable effects on ecosystems such as herbicide resistance, reduced groundwater quality and off target effects on insects and mammals (Liebman and Dyck 1993). They can also harm native species in addition to the weed species targeted (Rinella et al. 2009). Not only can native wildflowers be harmed by herbicides, they are usually more likely to be removed from the population with an herbicide spraying, often allowing invasive forbs to become established in the area. Despite these concerns, others argue that herbicides and crops genetically modified to be

herbicide tolerant will help feed the world (Borlaug 2000, Borlaug and Dowswell 2004). There are many options outside of herbicide use such as row spacing, tillage, polycultures, rotations, and fertility. (Zimdahl 2004), all of which need to be assessed in wildflower seed production fields.

There is an absence of successful management practices tailored for weed control in wildflower production fields. Testing wildflower species tolerance to herbicides under different management scenarios including pre- and post-emergence herbicides treatments represents a necessary step to facilitate the successful establishment and seed production of native wildflowers. Most herbicide use in wildflower seed production has been limited to graminocides including sethoxidim, clethodim, fluazifop, and fenoxaprop ethyl (Dickens 1992, Johnson 1995; Aldrich 2002, Grabowski 2005) with no post-emergence broadleaf products labeled for use in production fields.

Currently, imazapic at the rate of 0.07 kg ai/ha is the only herbicide labeled for use on non crop sites that have been recently seeded with native wildflowers. Imazapic is labeled for both pre- and post-emergence applications to establish and maintain plantings of black eyed susan, plains coreopsis *(Coreopsis tinctoria* Nutt), and partridge pea *(Chamaechrista fasciculata* (Michx.) Greene var.) (Grabowski 2005). Although the native wildflower species annual phlox (*Phlox drummondii* Hook. ssp. *drummondii* Hook), lanceleaf coreopsis (*Coreopsis lanceolata* L), standing cypress (*Ipomopsis rubra* (L.) Wherry); and sundial lupine (*Lupinus perennis* L.) are moderately to highly tolerant of a pre-emergence imazapic application (0.07 kg ai/ha), blanketflower (*Gaillardia pulchella* Foug.), sage (*Salvia coccinea* Buc'hoz ex Etl.) and black-eyed susan could only tolerate

0.0175– 0.035 kg ai/ha, however, this rate may be too low to control weeds, if they are present (Norcini et al. 2003, Aldrich 2002).

Some success with pre-emergence broadleaf herbicides has been observed, but more testing still needs to be done. For example, Jacobs et al. (2007) found that none of the wildflowers they tested (including all 5 species described here) tolerated atrazine and sulfentrazone at 367 g ai/ha and 32 g ai/ha, respectively. They also observed that pre-emergence applications of trifluralin at 184 g ai/ha and DCPA at 1100 g ai/ha were least injurious to the species tested. Aldrich (2002) also found trifluralin showed no phytotoxicity when used on blanketflower and black eyed susan and DCPA showed no phytoxicity when used on eastern purple coneflower (*Echinacea purpurea* (L.) Moench), and blanketflower.

Incorporating herbicides as a component of a weed management systems is a common, effective method to control weeds. However, although the knowledge of the competitive processes between weeds and crops is an integral part of weed control, the availability and widespread use of herbicides has supplanted this knowledge in favor of prescriptive management plans (Jordan 1993). While this reactionary approach has weed control benefits that are quickly attainable, questions have arisen with regard to its sustainability (Liebman et al. 2001). Herbicides have implications which go beyond wildflower tolerance and weed control. Research on the basic disciplines of ecology, evolution, genetics and physiology of weeds (Holt 1994), along with funding for alternative weed control options such as integrated weed management (IWM) (Swanton and Weise 1991), has lead to a flux of new ideas in the realm of weed management. IWM

considers herbicides a weed management tool but incorporates other ecological principles to lower herbicide off target effects. Future studies could be conducted using multiple integrated weed management in wildflower production fields.

Economics of Wildflower Seed Production

Marketing demand is increasing for regionally and locally specific plant materials used for restoration, particularly for those species that are adapted to specific locations (Jones and Young 2005). Although wildflower planting for production goals can be expensive, the use of native seed that leads to better establishment improves the chance that the plants will be at an advantage because they are adapted to the area (Montalvo and Ellstrand 2000, Kaye 2001). Currently, average price in the United States of native seed used in restoration ranges between $110 and $1,100 per kg, often higher than that of comparable non native plant materials (Jones and Young 2005). Even in the first year of wildflower seed production, when less seed is typically produced a gross return of up to $4,446 per ha can be expected (Kutka and Tinderholt 1996). For example, figures from the 2006 USDA Plant Materials Center records show antelope white prairieclover (*Dalea candida* (Michx). ex Willd) producing 1723 kg per ha at $189,200 net return per year and stillwater prairie coneflower (*Ratibida columnifera* (Nutt.) Woot. & Standl) producing 728 kg per ha at $64,000 per year.

Wildflower Species and Seed Source Selection for Restoration Projects

The use of appropriate plant materials for revegetating natural ecosystems is critical for the successful establishment of native plant cover (Bailey and Martin 2007).

Criteria for selecting restoration species include regional adaptability across several regions, potential for providing erosion control, and potential to become established and dominant in a native plant community (Bailey and Martin 2007). Seed production of native wildflowers is further complicated by increased interest in using localized seed sources and concerns regarding genetic integrity (Rogers 2004, Krauss and He 2006, Hufford and Mazer 2003, McCully 1999).

A contentious issue in conservation biology and ecological restoration is whether seed sources should be mixed at a restoration site (Kaye 2001). One side of this debate contends that plant materials should be brought only from the closest, most ecologically and or genetically similar site. In this context, ecotypes from local provinces are recommended as they are better adapted to local site conditions, facilitating vegetation establishment (Bischoff et al. 2006). In contrast, other research supports free movement of materials from distant sources as long as the species is native (Kaye 2001) as natural selection will remove less fit hybrids (Wilkinson 2001).

Recent studies have shown that there is a significant effect on establishment due to seed collection source but non significant effect when germination time was altered suggesting that the source of seed is more important for seed production and restoration than environmental factors (Bischoff et al. 2006). The locality of the seed source has been shown to affect germination and establishment with local ecotypes blooming longer compared to non local ecotypes (Halsey and Lilly 1998). Another positive ramification of planting local genotypes of native wildflowers is that they may be more competitive with weed species in local growing conditions (Gallitanio and Skroch 1993). Nevertheless,

many genetic studies have difficulty isolating the relative importance of local sources in determining the success of native wildflower establishment, growth, and reproduction (Bischoff et al. 2006, Wilkinson 2007).

Studied Species

For this study, we have chosen 5 wildflower species, silverleaf phacelia (*Phacelia hastata* Douglas ex Lehm.), fuzzytounge penstemon (*Penstemon eriantherus* Pursh var. *eriantherus)*, prairie coneflower, slender white prairie clover, and blanketflower. These species were chosen based on their known ability to establish in the area and their success in previous seed production and restoration settings (Susan Winslow personal communication). The species have been grown at one of the study sites for several years and the seed source for that crop was collected in a nearby similar environment. Several of these species are also known to provide ecosystem services by providing habitat to many pollinator species in areas where human activities have destroyed and fragmented pollinator species habitats.

Phacelia hastata is a member of the Hydrophyllaceae family with prostrate branched woody stems. It naturally occurs from Washington to North Dakota and as far south as Southern California. It is found in open places, often in sand. The leaves are covered in silvery silky white hairs. The inflourescences are curled to coiled and appear bristly due to extended stamens. The petals are fused in a five lobe funnel, typically dull or white lavender. This species usually blooms in mid to late spring and the seed matures in mid to late August. The fruits are in capsules, two chambered with two to several seeds per chamber. (USDA 2009) (Fig. 1).

Penstemon eriantherus is a native perennial forb in the Scrophulariaceae family and is found in dry open terrain from the prairies into the mountains in Montana and Wyoming, southern British Columbia and Alberta, Canada. Individuals have between one to seven stems reaching 10 to 40 cm high with short leafy, sterile stems at the base. The leaves are entire or sharply toothed narrowly lance shaped to oblanceolate, glandular to finely pubescent. Penstemons' flower is lavender with a short narrow tube at the base expanding to a broad mouth and has a long dry papery fruit capsule filled with dark angular seeds. Coarse yellow hairs protrude from the tube, which is nearly closed by a crest on the lower lip. It blooms in late spring to early summer (USDA 2009) (Fig. 2).

Ratibida. columnifera (prairie coneflower) is a late season herbaceous perennial from the Asteraceae family. It is commonly found from suthern cntral Canada to nrthern Mexico and west from Manitoba and Minnesota to southeastern Idaho. It grows upright from 30 to 60 cm and has pinnate leaves which are deeply cut into linear or lance shaped segments along alternately branched stems. Showy yellow ray flowers droop and surround the columnar shaped, brown, central disk. Individual plants bloom late June until August with seed ripening completing in early August to September. The fruit is a one seeded, gray black achene. It is palatable and nutritious to all classes of domestic livestock and big game. It also may fill a structural cover and nesting niche for multiple upland birds. (USDA 2009) (Fig. 3).

Dalea candida (slender white prairie clover) is a long lived perennial legume (Fabaceae family) that ranges from the southern portions of the Canadian prairie provinces through the prairies of the Dakotas, south to Kansas, and is found in the

foothills of Montana, Wyoming and Colorado. It generally grows to a height of 45 to 60 cm. Multiple stems rise from a woody base. The leaves are alternate and odd pinnate. The flowers are in terminal spikes that are compact and cylindrical. The flowers develop in July and August with seed maturation occurring in September. It is palatable and nutritious forage for all classes of livestock and is an important browse species for antelope, deer, elk, and upland game birds, particularly sharp-tail grouse. D. candida also fixes nitrogen, thereby being an important component of revegetation projects. Scarification will improve total germination and speed of germination (USDA 2009) (Fig. 4).

Gaillardia aristata (blanketflower) is found from Minnesota to British Columbia (Canada), New Mexico, Arizona, and Oregon. Hot, dry sandy, conditions are best for this species. It is a tap rooted perennial from the Asteraceae family that is 20 to 60 cm tall with stems that have a short crinkly pubescence. The leaves are simple, alternate, scattered along the stem, mostly toward the base, oblong and oblanceate, mostly 5 to15 cm long, entire to irregularly toothed or shallowly pinnately lobed, conspicuously short hairy. The flowers are 4 to 8 cm across, radiate, with a round purple disk, yellow but with purplish disk at the base. The fruit is globulose and bearing achenes or seeds which have a pappus of lanceolate papery, bristly scales. The seed matures mid to late August (USDA 2009) (Fig. 5).

This study evaluates the impact weed management practices have on wildflower establishment, growth, and yield by combining greenhouse and field studies. This

knowledge will enhance the ability of farmers to locally produce native plant seeds, thereby maintaining the seed source for land restoration projects.

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Figure 1. Vegetative and reproductive structures of silver leaf phacelia (*Phacelia hastata)* from the UDSA plants database (USDA, NRCS 2009).

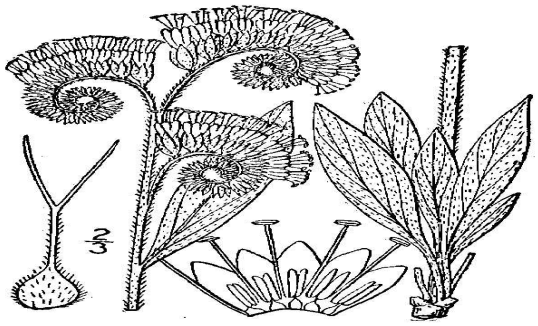


Figure 2. Vegetative and reproductive structures of fuzzytounge penstemon (*Penstemon eriantherus)* from the UDSA plants database (USDA, NRCS 2009).

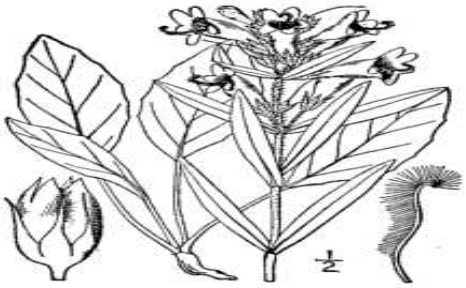


Figure 3. Vegetative and reproductive structures of slender white prairie clover (*Dalea candida)* from the UDSA plants database (USDA, NRCS 2009).

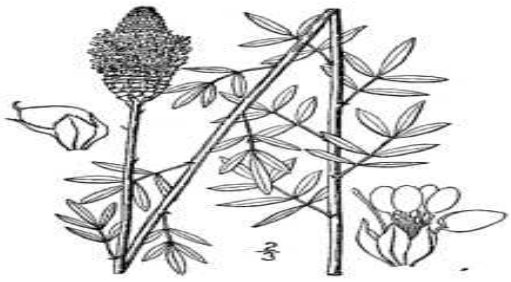


Figure 4.Vegetative and reproductive structures of prairie coneflower (*Ratibida columnifera)* from the UDSA plants database (USDA, NRCS 2009).

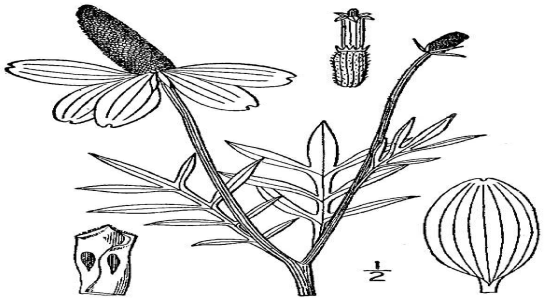
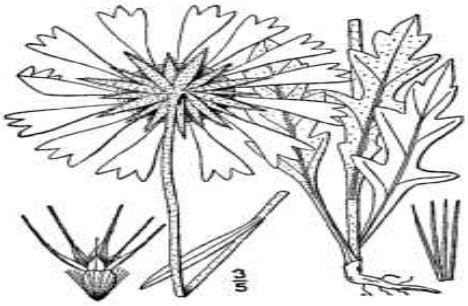


Figure 5. Vegetative and reproductive structures of blanketflower (*Gaillardia aristata)* from the UDSA plants database (USDA, NRCS 2009).



CHAPTER 2

DETERMINING WILDFLOWER SPECIES SEEDLING TOLERANCES TO POSTEMERGENCE HERBICIDES

Introduction

The commercial production of wildflower seeds for revegetation and restoration purposes is a relatively new agronomic enterprise for which optimal weed management practices are still being researched. Traditionally, non-indigenous seed mixtures have been used in revegetation because they are relatively inexpensive, easy to obtain, and typically fast growing; providing quick ground cover to compete with undesired plant species and hold soil in place (Beyers 2004, Landis et al. 2005, Matesanz et al. 2006). However, it is difficult to increase species and functional diversity when the seed mixes being used in restoration or revegetation projects consist mostly of rhizomatous grasses and non-indigenous species (Matesanz et al. 2006). Interest in native wildflower seed production has come about due to an increasing awareness of the importance of diversity (Hooper et al 2005), especially functional diversity (Loreau et al. 2001), in providing ecosystem services.

To meet the current demand of native wildflower seeds, many species need to be grown in production fields to make diverse seed mixes more readily available and affordable. Increased demand for wildflower seeds has elucidated the lack of management knowledge in crop settings. Specifically, little information is available on

wildflower stand establishment and weed management (Jacobs et al. 2007). This lack of information can, in turn, lead to high rates of establishment failure (James and Drenovsky 2007).

Native wildflowers early growth stages are affected by slow rates of germination, emergence, and establishment as well as by weed competition for sunlight, water, and nutrients (Norcini and Aldrich 2004). Weed management is crucial during the establishment year as resource competition is the primary cause of failure in prairie grass and wildflower establishment both in seed production settings and when restoring degraded areas (Aldrich 2002, Albright Seed 1998). Specifically, the management of broadleaf weeds is critical to securing successful wildflower establishment, as weed competition is strongest between functionally similar species with analogous environmental requirements (Clements et al. 1929). Functionally similar species perform similar roles in an ecosystem, such legumes fixing nitrogen, or trees that provide food and nesting place for certain kinds of birds.

Testing wildflower species' tolerance to crop herbicides is a necessary step to facilitate the successful establishment and seed production of native wildflowers. Several studies have investigated the response of wildflowers to broadleaf herbicides (Norcini et al. 2003, Barnes 2007, Boutin and Rogers 2000, Jacobs et al. 2007), but results are not always consistent. Also, multiple studies on the same herbicide/wildflower combinations do not exist. In Oregon, a series of postemergence testing over a two-year period indicates species tolerance to some herbicides may be associated with plant family (Shock et al. 2006). For example, hotrock penstemon *(Penstemon deustus* Douglas ex

lindl), sand penstemon (*P. acuminatus* Douglas ex lindl) and royal or sagebrush penstemon (*P. speciosus* D.D. Keck and Cronquist), all members of the Scrophulariaceae family, were significantly damaged by bromoxynil (0.14 kg ai/ha) and linuron (0.56 kg ai/ha). However, fernleaf biscuit root [*Lomatium dissectum* (Nutt.0 Mathius and Constance], nineleaf desert parsley [*L. triternatum* (Pursh) J.M. Coult. & Rose var. *brevifolium* (J.M. Coult. & Rose) Mathias] and Gray's lomatium [*L. grayi* (J. M. Coult and Rose) J. M. Coult and Rose], all members of the Apiaceae family, were damaged only by bromoxynil at 0.14 kg ai/ha. All species were tolerant to pendimethalin at 1.2 kg ai/ha, indicating herbicide mode of action may be critical to species susceptibility to broadleaf herbicides.

Although imazapic has been tested for crop safety on wildflower seedlings and is labeled for use on native wildflowers not growing in production fields (Aldrich 2002), wildflower species have varied sensitivity to this product, depending on family and life stage. Norcini et al. (2003) determined that Asteraceae species blanketflower (*Gaillardia aristata* Pursh), black eyed susan *(Rudbeckia hirta* L.), and lanceleaf coreopsis *(Coreopsis lanceolata* L.) were more susceptible to imazapic applications compared to the species tested within the Polemoniaceae, Lamiaceae and Fabaceae families. Barnes (2007) found that most of the 100 wildflower species tested that were uninjured by imazapic belonged to the Asteraceae, Fabaceae and Laminaceae families. In accordance, Vollmer and Vollmer (1999) determined that the tested Asteraceae species were tolerant to imazapic. Finally, although Norcini et al. (2003) found lewis flax (*Linum lewisii* Pursh) (Linaceae) at seedling stage sustained a low phytotoxicity rating by imazapic, the

Aberdeen Idaho Natural Resource Conservation Service (NRCS) Plant Materials Center (Tilley 2007) found that treated plants failed to produce seeds due to delayed blooming. These studies indicate that caution should be used when applying herbicides on native wildflowers because the family and species of forbs tolerant to herbicides, as well as the early and late signs of damage are not documented thoroughly enough (Washburn et al. 2002).

Herbicide greenhouse screening represents a logical first step in the understanding of weed management in wildflower seed production fields, as seedling death or damage is more likely to be caused by herbicide injury than by weed competition or environmental conditions when tested under controlled environmental conditions (Jacobs et al. 2007). The overall goal of this study is to enhance our understanding of postemergence herbicidal weed management on seedlings of wildflower species grown for seed production. Specifically, this study evaluated four wildflower species visual injury, wet weight and dry weight under four postemergence herbicide treatments. We hypothesized

1. Postemergence herbicides would injure wildflower species beyond an acceptable level, and that injury would differ depending on species and chemistry
2. Wet and dry biomass would be reduced by herbicide treatment

Materials and Methods

This study was conducted in a temperature and light controlled greenhouse at the Montana State University Plant Growth Center, Bozeman, Montana. Greenhouse day/night temperatures were set up for approximately a 15/25 C° cycle with a 16/8 h (light/dark) artificial photoperiod. Natural light was supplemented by 1000 watt GE Multi-Vapor MVR1000/C/U metal halide lamps. The experimental design followed a completely randomized design with five replications, conducted first from January 12th to April 30th, 2008 and again from May 24th to September 22nd, 2008.

The postemergence herbicides treatments included imazapic, halosulfuron, linuron, pendimethalin, and a no herbicide control (Table 1). Herbicides were selected based on the weed spectrum commonly found in Montana and recommendations provided by weed specialists in the region. These four herbicides were also used in a field study assessing the effect of pre and postemergence herbicides on native wildflower seedling establishment, weed control, and wildflower seed production (see Chapter 3).

These herbicides were tested on four wildflower species; silver leaf phacelia (*Phacelia hastata* Douglas ex Lehm), prairie coneflower (*Ratibida columnifera* (Nutt.) Woot. and Standl.), slender white prairie clover (*Dalea candida* Michx. ex Willd.) and blanketflower (Table 2). These species were chosen based on their known ability to establish in the area and their success in previous seed production and restoration settings (Aldrich et al. 1998, Aldrich 2002). Specific species information is available in Chapter 1. The seeds were planted at a 1 mm depth into rectangular pots 28 cm in length, 6 cm in

width, and 15 cm in depth. In each pot, two rows of one wildflower species were planted at 15 pure live seed (PLS) where PLS is defined as:

PLS = (percent purity) * (percent germination rate) / 100 (Eq 1)

and percent purity was estimated as the ratio between amount of seed and amount of chaff, other non-viable plant material, and weed seeds (Liskey 2001).

The soil used in this study consisted of equal parts (by volume) of loam soil and washed concrete sand. Canadian Sphagnum peat moss and AquaGro 2000 G wetting agent was blended in at 454 grams per 0.9 m^3 of soil mix. Prior to planting, the soil was aerated and steam pasteurized at 80° C for 45 minutes. Pots were watered twice daily until seedlings reached the three-leaf stage, then once a day. Pots were thinned to a targeted density of five plants per pot when they reached the three-leaf stage to remove any potential impact of density dependence on herbicide performance (Dieleman et al. 2000). The first experimental run was sprayed on February 21st, 2008 and the second on July 1st, 2008. All herbicide applications were conducted utilizing a spray table fitted with a TeeJet® Flat Fan 8002E nozzle. Prior to application, the spray table was calibrated based on a 35 L/ha volume applied at 4.8 kilometers per hour and a nozzle height of 0.3 m. Twenty days after spraying, leaf injury estimated as percent damaged relative to the untreated control were visually assessed. All plants were harvested 30 days after spraying and fresh and dry weights of all plants with each pot were recorded to the nearest 0.0001 gram.

Data Analysis

In seed production settings, wildflowers are usually grown for seed production in monocultures. Therefore, species were analyzed separately to provide specific management recommendations. The herbicide damage data set violated normality assumptions and a Monte Carlo resampling of the data was used to simulate observed distributions and estimate probabilities of difference between distributions of the data from each treatment. (Metropolis and Ulam 1949). In this study, the Monte Carlo simulation was conducted one million times and used to estimate the probabilities of herbicide damage to be greater than 10% of the control. The data distribution from the control was compared to distribution from the treatments and in each random selection of datum from each distribution (control and herbicide treatments) a damage level 10% greater than the control was considered as unacceptable and were issued a 1. Herbicide applications with damage levels between 0% and 10% of the control were considered acceptable and were assigned a 0. Wet and dry biomass at harvest were analyzed using a randomized block design analysis of variance (ANOVA) with a Tukey's non-additivity test used to evaluate the existence of interactions between trial and herbicide treatments (Neter et al. 1990). No interactions were found and when significant herbicide effects ($P < 0.05$) were obtained, differences among herbicides where further explored with a Tukey's honest significant differences test (Copenhaver and Holland 1988). All data were analyzed using the R 2.7.2 software package (R Development Core Team 2009.

Results

For *D. candida,* the Monte Carlo simulation of the observed data indicated that halosulfuron injured this wildflower species the least (Table 3). Pendimethalin applications resulted in unacceptable injures on *D. candida* only 10% of the time and linuron and imazapic both over 50% of the time. Despite the high levels of herbicide injury, herbicide applications did not reduce *D. candida* fresh weights (Table 4). Dry weight was affected by herbicide treatment (P=0.032, Table 4), but a Tukey HSD comparison did not find significance at *P*=0.05 (Figure 2).

The Monte Carlo simulation indicated that all herbicides injured *G. aristata* at least 60% of the times (Table 3). In accordance, *G. aristata* plants sprayed with linuron, halosulfuron, and imazapic had lower wet biomass (Table 4, *P* = 0.001, *P* <0.001, and *P*=0.009, respectively) and dry biomass (Table 4, *P*<0.001, *P* <0.001 and *P*=0.0213, respectively) than the control (Table 4). *G. aristata* plants sprayed with pendimethalin had fresh and dry weights similar to the control, higher than halosulfuron (*P*=0.003, and *P*= 0.001, respectively) and linuron (*P*=0.021, and *P*=0.01, respectively) indicating that despite its potential to cause injury, this herbicide could be a used for weed control (Table 4) (Figure 1, Figure 2).

Phacelia hastata was susceptible to all tested broadleaf herbicides with only pendimethalin resulting in unacceptable injuries less than 50% of the time (Table 3). Herbicide affected wet and dry weight (Table 4). The Tukey HSD test indicated that while plants sprayed with linuron or pendimethalin had similar fresh and dry biomass to the control, imazapic and halosulfuron applications decreased fresh (*P*<0.001 and

P=0.009, respectively) and dry biomass (*P*=0.023 and *P*=0.001, respectively), as compared with the untreated control (Figures 1 and 2) . Plants sprayed with halosulfuron also had lower fresh biomass when compared to pendimethalin (*P*=0.024, Table 4, Figure 1). Plants treated with imazapic had a lower fresh biomass than pendimenthalin (*P*=<0.001) and linuron (*P*=0.013) (Table 4) (Figure 1). Furthermore, dry biomass for plants treated with imazapic was lower than for those treated with linuron or pendimethalin (*P*=0.011 and *P*<0.001, respectively) (Table 4) (Figure 2).

Finally, the Monte Carlo simulation of the observed data distributions indicated that for *R. columnifera*, herbicides caused damage above the acceptable level between 35% (pendimethalin) and 60% of the time (imazapic) (Table 3). While herbicides did not impact fresh weights of *R. columnifera* plants, (Table 4) (Figure 1), they impacted plant dry weights. Specifically, plants treated with imazapic produced significantly lower dry biomasses than the control (*P*=0.042, and *P*<0.001, respectively). Plants treated with pendimethalin and linuron produced similar dry biomass to the control plants, but significantly larger than those treated with imazapic (*P*=0.009) (Table 4) (Figure 2).

Discussion and Conclusions

To our knowledge, this study is one of the first to date that looks specifically at wildflower tolerance to broadleaf herbicides. Our results concur with previous studies indicating that wildflower species respond uniquely to herbicides (Shock et al. 2006, Boutin and Rogers 2000). To ease the interpretation of the results, the suitability of the

tested herbicides for wildflower seed production will be discussed individually. Then, the overall management implications of this study will be considered.

In accordance with McDonald et al. (1996), linuron, a photosynthesis inhibitor herbicide that causes interveinal chlorosis preferentially in older leaves, appeared to be a promising alternative for weed control in wildflower seed production settings. Although the initial injury levels to linuron ranged between 40 and 90 percent, only *G. aristata* fresh and dry biomass were reduced by this herbicide. Although marginally significant (P=0.08), *D. candida* fresh biomass may have been lowered by linuron. This could be because *D. candida* is a legume and linuron can cause lowered nitrogen fixation (Sandhu et al. 1991) or inhibited fungal growth and lowered root nodulation and nitrogensase production (Sawicka et al. 1996). When treated with linuron, *G. aristata* dry weight was reduced compared to the control but *R. columnifera* was not. These results conflict with previous observations for imazapic made by Norcini et al. (2003) and agree with Boutin and Rogers (2000) indicting that the species to species and within family responses to herbicides should be further examined.

Halosulfuron is in the family of sulfonylurea herbicides, which are acetylacetate synthase ALS inhibitors, causing symptoms such as rapid growth inhibition and chlorosis. Herbicides of this family are absorbed by foliage and roots, inhibiting growth at both locations. Once absorbed into the plant, sulfonylurea herbicides are generally translocated acropetally and basipetally from the shoot to the root in the xylem and phloem to the areas of active growth. Previous studies of halosulfuron on watermelon indicate various impacts through stunting, phytotoxicity or a combination of both

symptoms (Shrefler et al 2007). Similarly, our results indicate the use of halosulfuron on wildflowers should be limited due to severe injury and reduced fresh and dry biomass.

Previous studies indicate that caution should be used when applying imazapic, another ALS inhibitor herbicide, on native wildflowers (Washburn et al. 2002). Several studies observed that the Asteraeceae, Fabaceae and Laminaceae families are able to tolerate imazapic (Washburn et al. 2002, Barnes 2007), while a separate study indicated mixed effects on species in the Asteraceae family and tolerance in the Fabaceae family (Beran et al. 1999). While these studies showed promising results for the Fabaceae family, they did not evaluate seed production. Even if seedlings survive the treatment of imazapic, seed production could be affected by halting of growth at the meristem and delayed bud production. Even without visual injury, imazapic is known to cause delayed flowering and empty seed pods (Susan Winslow, personal communication). Furthermore, complete kill of plants may not occur for weeks or even months after application. In this study, *G. aristata* did not show tolerance to imazapic, consistent with Beran et al. (1999) and contrasting Aldrich et al. (1998) and Vollmer and Vollmer (1999) who found *G. aristata* tolerated imazapic applications. Imazapic and halosulfuron proved injurious to *R. columnifera* (also Asteraceae), consistent with Beran et al. (1999) but contrary to Vollmer and Vollmer (1999) that found *R. columnifera* to be tolerant to imazapic. These mixed responses are further evidence that more studies need to be conducted to clarify this herbicide's effects on wildflowers.

Pendimethalin, a mitosis inhibitor herbicide that causes swelling and stunting of the root tips, showed the least amount of wildflower injury across all species. In

accordance with this study, Smith (2004) observed that spinach crop emergence from a low dose of pendimethalin (0.066 ai kg/ha) was distinctly better than that from high doses and control, indicating that judicious use of pendimethalin or integrated management using low doses of pendimethalin could be possible. Tilley (2007) determined that pendimethalin could be used for seed production of lewis flax even though it had low weed control and some phytotoxicity. Due to the nature of the study conducted by Tilley (2007), all observations and measurements were taken at the seedling stage, potentially missing pendimethalin's long-term effects. This phytotoxicity may have been due to root swelling and stunting and could have long-term effects on plant fitness and growth (WSSA 2007).

In summary, this study showed that while imazapic and halosulfuron reduced *G. aristata*, *P hastata* and *D. candida* biomass, pendimethalin caused the least seedling visual injury across all species and may, as a general herbicide selection, help wildflower establishment. The most noted exception was the response of *G. aristata*. This study was conducted in a greenhouse setting without weed interference or competitive stress. Even though greenhouse studies are excellent tools for performing controlled experiments and evaluating first principles, caution should be used to extend their outcomes to field settings as this depends on the metrics measured and the growing conditions (Mokany and Ash 2008). For example, plant tolerance to herbicides may be affected by environmental stress and life stage. Many herbicides are formulated to work at the meristem of plants, and an actively growing seedling wildflower in greenhouse conditions may be more susceptible to damage. Furthermore, the damage that occurs at

seedling stage could have long-term effects on plant fitness and growth (WSSA 2007), thereby influencing seed production at plant maturity. Even without visual injury, herbicides are known to cause delayed flowering and empty seed pods (Susan Winslow, personal communication). Evaluation of injury at all life stages and at seed production is necessary and field experiments of longer duration should be conducted to determine the effect of herbicides on wildflower species under competition with weeds and under environmental stress (Erusha, et al. 1991).

Incorporating herbicides as a component of a weed management system is a common, effective method to control weeds. However, although the knowledge of the competitive processes between weeds and crops is an integral part of weed control, the availability and widespread use of herbicides has supplanted this knowledge in favor of prescriptive management plans (Jordan 1993). While this reactionary approach has weed control benefits that are quickly attainable, environmental, economic, and societal questions have arisen with regard to its sustainability (Liebman and Dyke 1993). Herbicides have implications which go beyond wildflower tolerance and weed control. Research on the basic disciplines of ecology, evolution, genetics and physiology of weeds (Holt 1990), along with knowledge for alternative weed control options such as integrated weed management (IWM) (Swanton 1991) has lead to a flux of new ideas in the realm of weed management. IWM considers herbicides a weed management tool but incorporates other ecological principles to lower herbicide off target effects. Future studies should be conducted to incorporate multiple integrated weed management in wildflower production fields.

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Table 1. Trade names, common names, modes of action, and application rates of herbicides tested in a greenhouse setting to determine native wildflower seedling tolerance.

Trade Name	Common Name	Mode of Action	Rate
Lorox®	Linuron	Inhibits photosynthesis in PS II	1.121 kg/ha
Permit®	Halosulfuron	ALS inhibitor	91 g/ha
Plateau®	Imazapic	ALS inhibitor	560 g/ha
Prowl® H_2O	Pendimethalin	Microtubule assembly inhibitor	4.2 liters/ha
Control	-	-	-

Table 2: Wildflower species, percentage seed viability, percentage purity, and percentage pure-live-seed used in a greenhouse screening to test tolerance to postemergence herbicides.

Species	Family	Common Name	% Viability	% Purity	PLS
Dalea candida	Fabaceae	White prairie clover	90	99.65	89.6
Gaillardia aristata	Asteraceae	Blanketflower	83	86.81	72.1
Phacelia hastata	Hydrophyllaceae	Silverleaf phacelia	84	97.41	81.8
Ratibida columnifera	Asteraceae	Prairie coneflower	98	99.11	97.1

† PLS = Pure-Live-Seed estimated as (percent purity) * (percent germination rate) / 100 where percent purity was estimated as the ratio between amount of seed and amount of chaff, other non-viable plant material, and weed seeds.

Table 3. Probabilities of postemergence herbicide injury levels 10 % greater than the injury levels in the control on wildflower seedlings. (i.e. P values are 1 – value presented in the table).

	Dalea candida	*Gailardia aristata*	*Phacelia hastata*	*Ratibida columnifera*
Linuron	0.5981	0.9005	0.6006	0.3982
Halosulfuron	0.0000	0.9009	0.8500	0.4509
Imazapic	0.5037	0.6020	0.9003	0.5909
Pendimethalin	0.1003	0.8033	0.4248	0.3497

Table 4. F-statistics reported for randomized block design ANOVA tests assessing differences in fresh and dry weight of wildflowers under four postemergence herbicide treatments. The model included herbicide treatment (d.f. = 4), and trial. (df = 1) with 44 residual df. Herbicide treatments include linuron 1.121 kg/ha, halosulfuron 91 g/ha, imazapic 560 g/ha and pendimethalin 4.2 L/ha.

	Dry biomass		Wet biomass	
	Treatment	trial	treatment	trial
Dalea candida	2.8342*	5.75 **	NS	NS
Gaillardia aristata	7.9213***	37.67***	7.4912***	55.43***
Phacelia hastata	6.8937***	NS	8.2832***	NS
Ratibida columnifera	5.3882**	NS	NS	NS

NS = not significant *P < 0.05, **P < 0.01, ***P < 0.001.

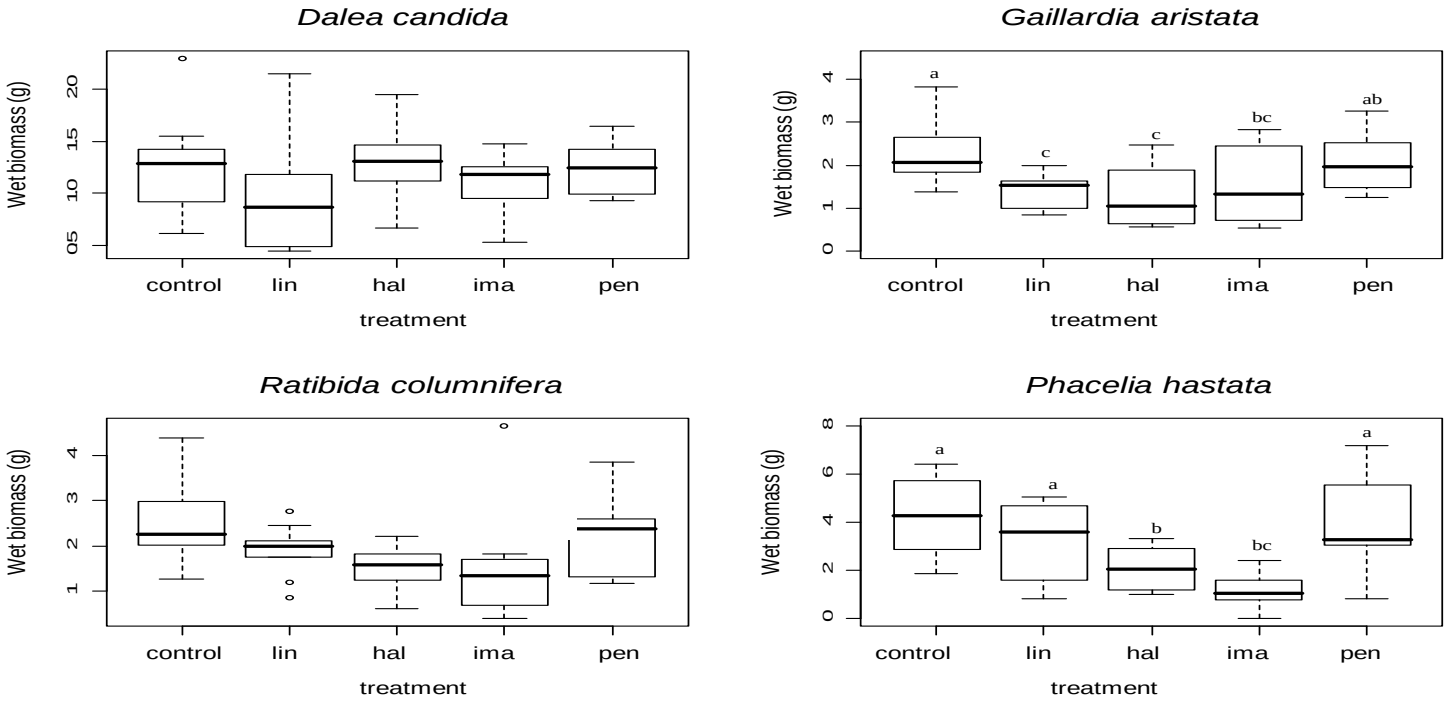

Figure 1: The wet weight of five wildflower species under five postemergence herbicide treatments. The horizontal lines on the box plots, from bottom to top, represent the lowest value that is not an outlier, the first quartile, the median, the third quartile, and the largest value that is not an outlier. Any points outside the range of whiskers are outliers. Lower case letters represent significant differences in mean fresh weight based on multiple Tukey Honest Significant Difference (HSD) test ($P<0.05$) Three letter codes for the herbicide common names are listed along the x axis and are defined as lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, and control = no herbicide application.

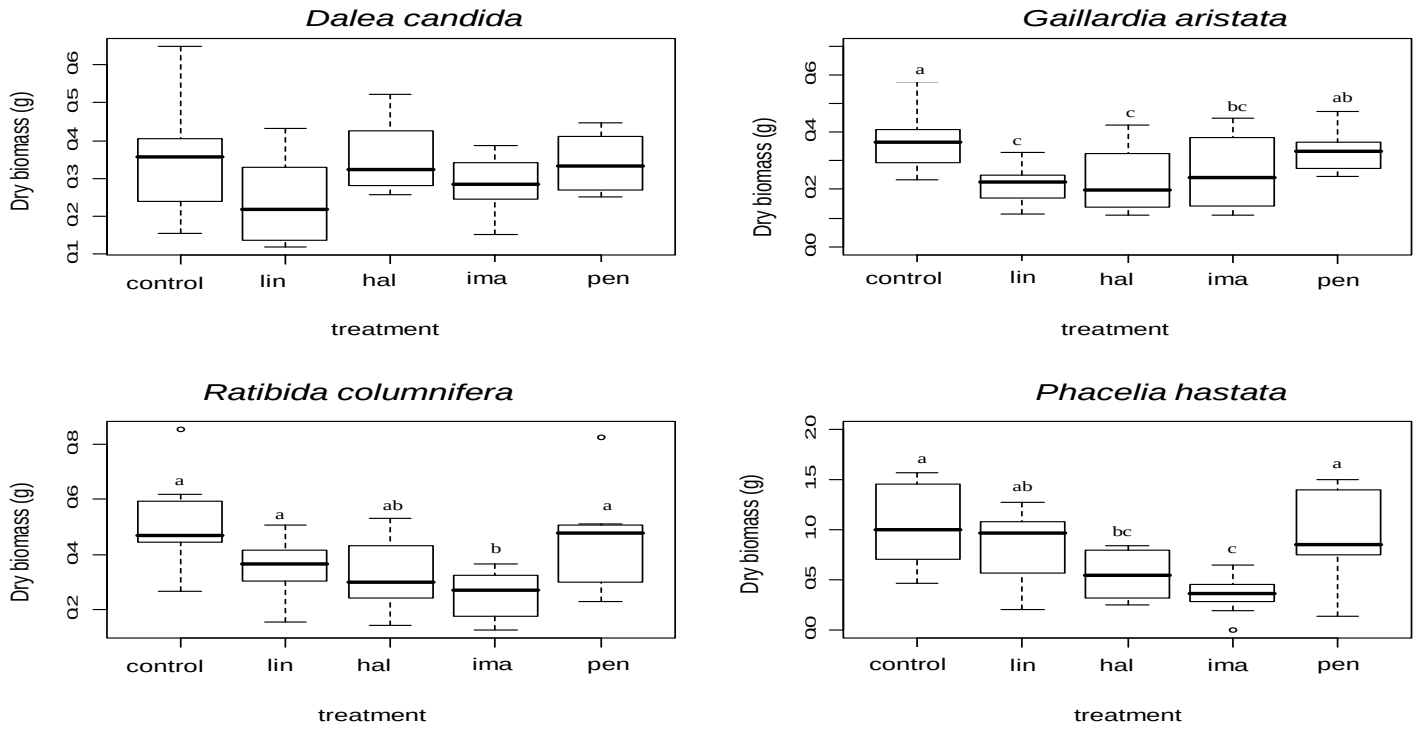

Figure 2 The dry weight of five wildflower species under five postemergence herbicide treatments. The horizontal lines on the box plots, from bottom to top, represent the lowest value that is not an outlier, the first quartile, the median, the third quartile, and the largest value that is not an outlier. Any points outside the range of whiskers are outliers. Lower case letters represent significant differences in mean dry weight based a multiple Tukey Honest Significant Difference (HSD) test ($P<0.05$). Three letter codes for the herbicide active ingredients are listed along the x axis and are defined as lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, and control = no herbicide application.

CHAPTER 3

EFFECT OF WEED MANAGEMENT ON NATIVE WILDFLOWER SEEDLING ESTABLISHMENT, COVER, AND SEED PRODUCTION IN A COMMERCIAL FIELD SETTING

Introduction

Many native plantings projects conducted from the 1960's to the 1980's used few or no native wildflowers (dicots) because seeds of these species were not commercially available (Williams et al 2007). In recent years, there has been increased use of native plants and seeds throughout the United States as nurseries and seed providers begin to supply them for restoration projects, personal use, and use in reclamation areas and roadsides (Martin 1990, Harper-Lore and Wilson 2000, Jones and Foote 1997). The reason for this increased demand is twofold. First, the demand is driven by an understanding that biodiversity could play a critical role for ecosystem health and functioning as well as providing multiple ecosystem services (Loreau et al. 2001). For example, including native wildflowers in agroecosystems increases biodiversity and can buffer pest invasions by enhancing the survivorship and effectiveness of natural enemies (Moonen and Barbieri 2009). In wildlands, native wildflowers can increase microorganism species richness, enhance soil nitrogen retention, and may lower invasion by non indigenous species (Knops 1999). Also, native wildflowers can anchor soil with deep dense rooting and stabilize erodible slopes, rivers, or stream banks (Alario 2000). Due to these services, the Natural Resource Conservation Service (NRCS) recommends

planting a variety of flowering plants and shrubs, including native wildflowers, on abandoned farmlands and in natural settings (NRCS, USDA 2007).

Second, increased land development and disturbance, along with new restoration protocols for creating self-supporting communities that provide ecosystem services (Bradshaw 1987, USDA 2008) are causing national agencies and private firms to seek native wildflowers for use in their restoration seed mixes. In the past, reclamation of degraded land was accomplished with few non-native species that were quick to establish ground cover (Lesica and Allendorf 1999). While it is essential to quickly establish vegetation to prevent erosion, it is equally important to protect the ecological interactions present in the native mosaic of locally adapted populations (Lesica and Allendorf 1999). Moreover, non-native species are increasingly recognized as one of the most serious ongoing causes of species declines and native habitat degradation (D'Antonio and Myerson 2002).

Despite the growing demand on native wildflower seeds for restoration purposes, few research studies have been conducted on approaches to manage them in production settings (Aldrich 2002). Specifically, agricultural questions regarding protocols that will promote good establishment, lower weed pressure, and secure yield remain unanswered. Increasing our agricultural understanding of native wildflower propagation is essential as the early growth stages of these species are threatened by heavy weed competition for water, sunlight, and nutrients due to their slow germination, emergence, and establishment (James and Drenovsky 2007, Norcini and Aldrich 2004). Plant growth rate has a strong influence on competitive ability (Zimdahl 2004) and due to perennial

wildflowers' slow growth rate (Norcini and Aldrich 2004), weed management protocols are necessary for the successful commercial production of these species.

Weed competition is the primary cause of failure in commercial prairie grass and wildflower establishment (Aldrich 2002, Albright Seed 1998). A wide array of weed management options, such as chemical control, mowing, hand weeding, and weed fabric needs to be assessed and further understood for the commercial production of native wildflowers (Aldrich 2002). Among them, wildflower tolerance to broadleaf herbicides is a critical management component that has been understudied. Although glyphosate is often used as a precursor to wildflower seed production (Norcini et al. 2003), most herbicide use has been limited to selective graminocides such as sethoxidim and clethodim (Grabowski 2005). Currently, imazapic is the only pre- and postemergence herbicide labeled for use on non-crop sites that have been recently seeded with native wildflowers including blackeyed susan (*Rudbeckia Hirta* L.), plains coreopsis (*Coreopsis tinctoria* Nutt), and partridge pea (*Chamaechrista fasciculata* (Michx.) Greene var.) (Grabowski 2005, Norcini et al. 2003, Aldrich 2002).

Although success with preemergence broadleaf herbicides has been observed, results have not always been satisfactory. For example, a greenhouse study conducted by Jacobs et al. (2007) observed that preemergence applications of trifluralin at 184 g ai/ha and DCPA at 1100 g ai/ha, although least injurious to the species tested, injured white prairie clover (*Dalea candida* (Michx). ex Willd), blanketflower (*Gaillardia aristata* Pursh), and prairie coneflower (*Ratibida columnifera* (Nutt.) Woot. & Standl). Contrastingly, Aldrich et al. (1998) observed that firewheel (*Gaillardia pulchella* Foug.)

was able to recover from applications of either trifluralin + isoxaben (4.48 + 1.12 kg a.i./ha) or oryzaline + benefin (3.36 + 3.36 kg a.i./ha,). Boutin and Rogers (2000) reviewed two large databases (the Canadian Regulatory Industry Authority on Sponsored Data on Weed Efficacy and Crop Margin of Safety and the United States Environmental Protection Agency) to examine the pattern of species sensitivity to pesticides and found consistencies within plant families responses to preemergence herbicides. This coincides with Erusha et al. (1991) finding that the Asteraceae species tested (prairie coneflower, firewheel and common yarrow *(Achillea millefollium* L.) tolerated EPTC/trifluralin treatments at 2.3 + 0.6 kg ai/ha applied preemergence.

Native wildflower species response to broadleaf postemergence herbicides has been examined, but multiple studies on the same species and herbicide treatment have not been conducted. In Oregon, a series of postemergence testing over a two-year period indicated that species tolerance to herbicides may be associated with plant family (Shock et al. 2006). For example, hotrock penstemon (*Penstemon deutus* (Douglas ex lindl)), sand penstemon (*P. acuminatus* (Douglas ex lindl)), and royal penstemon, (*P. speciosus* D.D. Keck and Cronquist), all members of the Scrophulariaceae family, were significantly damaged by bromoxynil (0.14 kg ai/ha) and linuron (0.57 kg ai/ha). Also, fernleaf biscuit root (*Lomatium dissectum* (Nutt.) Mathius and Constance), nineleaf desert parsley (*L. triternatum* (Pursh) J.M. Coult. & Rose var. *brevifolium* (J.M. Coult. & Rose) Mathias) and Gray's lomatium (*L. grayi* J. M. Coult and Rose) members of the Apiaceae family, were damaged only by bromoxynil at 0.14 kg ai/ha. However, all species were

tolerant to pendimethalin at 1.1 kg ai/ha, indicating herbicide mode of action may be also critical to species susceptibility to broadleaf herbicides.

Although imazapic is labeled for use on native wildflowers not growing in production fields (Aldrich 2002), wildflower species have varied sensitivity to this product, depending on family and life stage. Norcini et al. (2003) determined that the Asteraceae species blanketflower, black eyed susan and lanceleaf coreopsis (*Coreopsis lanceolata* L.) were more susceptible to imazapic applications compared to the members of the Polemoniaceae, Lamiaceae and Fabaceae families tested. Barnes (2007) found that most of the 100 wildflower species that were uninjured by imazapic belonged to the Asteraceae, the Fabaceae, and the Laminaceae families. Finally, Norcini et al. (2003) found that lewis flax (*Linum lewisii* Pursh) (Linaceae) at seedling stage sustained a low phytotoxicity rating by imazapic. Although a study conducted at the Aberdeen Idaho Natural Resource Conservation Service (NRCS) Plant Materials Center (Tilley 2007) concurred with this result, plants failed to produce seeds due to delayed blooming. These studies indicate caution should be used when applying herbicides on native wildflowers because the family and species of forbs tolerant to herbicides as well as the early and late signs of damage have not yet been documented thoroughly (Washburn et al. 2002).

The goal of this study was to evaluate the impact of several weed management approaches including pre- and postemergence herbicides and mechanical weed removal on wildflower seedling establishment, weed control, and seed production in the field, under cropping conditions. Specifically, it tested the following hypotheses:

Hypothesis 1: Herbicide applications injure the select set of wildflower species, thereby lowering their cover, establishment, and yield.

Hypothesis 2: Weed competition lowers the select set of wildflower species cover, establishment, and yield relative to hand weeded, herbicide applied fields.

Hypothesis 3: The select set of wildflower species establishment, cover, and yield will be higher in herbicide treated fields than in untreated fields.

Materials and Methods

This study was conducted at two sites across Montana to assess establishment and weed management for five wildflower species in contrasting environmental conditions (Table 1). The first site was located at the Montana State University Post Research Farm near Bozeman, Montana. The second site, at the USDA NRCS Bridger Plant Materials Center, in Bridger, MT.

At each site, the experiment consisted of a 35 m by 36.6 m area following a split-split-plot randomized block design with three replications (Fig. 1). In this experiment, wildflower species were the main plot factor and herbicide treatments the split-plot factors. Each split-plot was further subdivided into hand weeded and non-weeded split-split plots. Two types of no herbicide control plots were established. One was hand weeded control and used to assess crop tolerance to herbicide applications (Hypothesis 1). We predicted that due to herbicide injury, weed free fields treated with herbicide would have lower establishment, cover, and yield than the weed free fields without herbicide. The other control consisted of a no weeded and no herbicide treatment and

used to assess weed effects on wildflower establishment, cover, and yield (Hypotheses 2 and 3). Specifically, hypothesis 2 is tested by the comparison of weed competition versus herbicide damage to wildflower crops on plant establishment, cover, and yield. We predicted that fields treated with herbicides and hand weeding would have a higher establishment, cover, and yield than the unweeded control plots. Finally, hypothesis 3 is tested by the comparison of herbicide treatments and the unweeded control to determine if herbicides when used alone are useful tools for wildflower seed producers. We predict fields with herbicide applications will have a higher yield than the unweeded control plots.

Prior to seeding, the sites were prepared on September 22nd, 2006 at Bridger, MT and on October 17th, 2006 at Bozeman, MT by roller harrowing, which mixes and levels the soil for a clod-free, firm seedbed. Good seed bed contact is important to secure proper wildflower establishment (Aldrich 2002). The wildflowers were seeded at 75 pure live seed (hereafter PLS) per meter, where PLS is defined as:

PLS = (percent purity) * (percent germination rate) / 100 (Eq 1)

where percent purity = amount of seed vs. amount of chaff, other non-viable plant material, and weed seeds (Liskey 2001).

The seeds were planted at a depth of 2 cm on September 30th, 2006 at Bridger, MT and on October 25th, 2006 in Bozeman, MT. Eight rows spaced 0.35 m apart were planted for each species. Seeding rates and agronomic practices reflect common approaches for this region and soil type (Susan Winslow, personal communication).

The preemergence herbicide trifluralin and the postemergence herbicides imazapic, halosulfuron, linuron, and pendimethalin (Table 2) were tested on five wildflower species; silver leaf phacelia (*Phacelia hastata* Douglas ex Lehm.), fuzzytounge penstemon (*Penstemon eriantherus* Pursh var. *eriantherus*), prairie coneflower, slender white prairie clover, and blanketflower (Table 3). These species were chosen based on their known ability to establish in the studied area and their success in previous seed production and restoration settings (Norcini et al. 2003). Within each species, trifluralin was selectively applied to specific subplots to generate a set of pre- and postemergence herbicide treatments. Trifluralin, a preemergence herbicide, was applied on September 30th, 2006 in Bridger, MT and October 26th 2006 in Bozeman, MT at a rate of 189 liters/ha with 6 Flat Fan 8002 nozzle with 45.72 cm spacing at a rate of 109 kilograms per square centimeter, moving at 4 kilometers/hour. Trifluralin was incorporated with a rake at a depth of 1.5 cm. Environmental conditions at the time of pre and postemergence applications are listed in Table 4. All postemergence herbicides were applied with a Flat Band 80-01 Tee Jet at 6 kmph and 109 kilograms per square centimeter. These products and rates were chosen after a greenhouse herbicide screening of several herbicides at multiple rates (Chapter 2, Jacobs et al. 2007).

Hand weeding was conducted multiple times throughout the growing season, starting at weed emergence and continuing any time weeds became larger than seedling size. We made the assumption that mechanical practices have no negative effects on wildflower establishment, cover, and yield as we were diligent at wildflower species identification and minimized soil disturbance.

During the summer and fall of 2007 and 2008, wildflower seedling emergence, wildflower percent cover, weed abundance and cover, and herbicide damage were evaluated on the middle two rows of each sub-sub-plot by placing a 55 by 66 cm frame 40 cm from the east edge and 20 cm from the west edge. Measurements were obtained every three weeks from May of 2007 through September 2007 and June 2008 through October 2008 with wildflower seedling establishment assessed as a discrete variable and wildflower percent cover and weed percent cover as values ranging between 0 and 100. For each sampled area, wildflower seed production and seed viability were assessed at the end of the second growing season. Wildflower seeds were harvested by hand. After collection, all seeds were stored at 7.2° C for approximately 60 days until they were tested for germination. Testing for germination included seed cleaning using a Seed Buro® air blower and multiple sieves and rub boards to remove chaff and achenes. For each species, seed germinability was assessed by taking 20 seeds from each split-split-plot of collected seed and placing them on damp blotting paper in plastic trays in a growth chamber at 25° C with a 16:8 h light:dark photoperiod. Seeds that did not germinate were tested for dormancy using the tetrazolium technique (Miller 2004). Extremely high initial weed pressure and unfavorable climate led to very low wildflower establishment at the Bozeman, MT site (see Tables 1 and 5), therefore only data collected from Bridger, MT was analyzed.

Data Analysis

For each hypothesis, species were analyzed separately as we were interested in species specific responses to management scenarios for wildflower seeds grown in monocultures. Data were analyzed with a randomized block design analysis of variance (ANOVA) to test for significant differences among treatments in wildflowers seedling emergence, percent cover of wildflowers, and seed yield. Second year measurements of establishment and cover obtained on June 24th were used for the analysis, as this timeframe was most representative of all species peak growth. Yield was a one-time measurement as species produced seed only in the second year. The yield data was transformed to differences from the control. Plant establishment data was normalized using a square root transformation, and percent cover values were normalized using an arc sin square root transformation. Plant establishment, percent cover and yield were further analyzed using a post-hoc Tukey's honest significant differences test (Copenhaver and Holland 1988) at the 0.05 level of confidence when *P* values from *F*-tests were lower than 0.05 in the overall test of treatment effect. Due to the large number of contrasts conducted, *P* values that are marginally significant (P=0.05-P=0.1) should be interpreted with caution. R statistical software (version 2.8.2) was used for statistical tests(R Development Core Team 2009).

Results

Average weed density and standard deviation for each wildflower species, unweeded herbicide treatment, and control plots are listed in Appendix B. Five weed species were

prevalent in all wildflower plots, Canada thistle (*Cirsium arvense* L. Scop), grasses and sedges (grouped as *Graminoid* species), kochia (*Kochia scoparia* L. Roth), common lambsquarter (*Chenopodium album* L.), prickly lettuce (*Lactuca seriola* L.) and common dandelion (*Taraxacum officinale* F.H. Wigg). Kochia and prickly lettuce were most abundant and reduced by some herbicide treatments, such as halosulfuron, pendimethalin, and all treatments of trifluralin.

Hypothesis 1 was tested by a comparison of hand weeded fields versus hand weeded, herbicide treated fields. We hypothesized herbicide applications would injure wildflowers, thereby lowering wildflower establishment, cover, and yield.

Establishment of *P. hastata*, *G. aristata*, *P. eriantherus*, and *D. candida* were affected by weed treatments (P=0.02 , P<0.01, P<0.01, and P<0.01, respectively) (Table 6). *P. hastata* established more in plots sprayed with linuron than in plots sprayed with imazapic (P=0.01) and trifluralin/halosulfuron (P=0.01) (Figure 2). *G. aristata* establishment was lowered in plots sprayed with trifluralin/linuron when compared to imazapic (P<0.01), pendimethalin (P=0.04), and trifluralin/halosulfuron (P=0.02). For *P. eriantherus*, plots sprayed with trifluralin/imazapic had lower establishment when compared to the control (P=0.006), halosulfuron plots (P<0.001), and pendimethalin plots (P<0.001) (Figure 2). Trifluralin/pendimethalin also reduced establishment of *P. eriantherus* when compared to the control (P=0.02), halosulfuron (P=0.002), and pendimethalin (P <0.001) (Figure 2). Pendimethalin treated plots had a higher establishment when compared to trifluralin/linuron plots (P=0.003) and higher when compared to trifluralin/halosulfuron plots (P=0.04). For *D. candida*, linuron treated plots

had reduced establishment compared to the control (P=0.009), halosulfuron (P=0.025), imazapic (P=0.006), and pendimethalin (P=0.024) (Figure 2). Plots sprayed with trifluralin/pendimethalin had lower establishment than the control (P=0.008), halosulfuron (P=0.02), imazapic (P=0.006) and pendimethalin (P=0.025) (Figure 2).

Herbicide applications impacted *P. hastata*, *G. aristata* and *P. eriantherus* cover (P=0.003, P=0.032, and P=0.033, respectively) (Table 7, Figure 3). *P. hastata* plots sprayed with linuron had a greater percent cover than those sprayed with imazapic (P=0.014) or trifluralin/halosulfuron (P=0.015). *G. aristata* plots sprayed with trifluralin/linuron had lower percent cover than the trifluralin/pendimethalin and pendimethalin treated plots (P<0.01 and P<0.01, respectively). *P. eriantherus* plots sprayed with trifluralin/imazapic had lower percent cover than the control (P=0.022), linuron (P=0.025), and halosulfuron (P<0.001) plots. Plots sprayed with trifluralin/pendimethalin had lower percent cover than halosulfuron (P=0.0012) and pendimethalin (P=0.006) plots (Figure 3).

Herbicide applications affected *P. hastata* yield (P=0.028) (Table 8). The Tukey multiple comparison showed that plots treated with linuron had a higher yield than imazapic (P=0.01), trifluralin/halosulfuron (P=0.039) and trifluralin/imazapic (P=0.02) treated plots (Figure 4).

Hypothesis 2 addressed the question of weed competition effects compared to herbicide crop safety on native wildflower establishment, cover, and yield. Specifically, it involved a comparison between the hand weeded herbicide treatments and the unweeded control plots. We hypothesized that fields without weed control will have lower

wildflower cover, establishment, and yield relative to herbicide applied, hand weeded fields.

Treatments affected establishment for *P. hastata* (*P*=0.013), *G. aristata* (*P*=0.027), *P. eriantherus* (*P*=0.001), and *D. candida* (*P*=0.027) (Table 6). *P. hastata* plots that were sprayed with linuron had higher establishment than plots treated with imazapic (*P*=0.007), trifluralin/halosulfuron (*P*=0.008), and trifluralin/imazapic (*P*=0.046) treated plots. Pendimethalin treated plots also had greater establishment than plots treated with imazapic (*P*=0.043), and trifluralin/halosulfuron plots (*P*=0.043) (Figure 5). *G. aristata* plots sprayed with trifluralin/linuron had marginally lower establishment than the control (*P*=0.047), and lower establishment than imazapic (*P*=<0.001), pendimethalin (*P*=0.034) and trifluralin/halosulfuron (*P*=0.018) (Figure 5). *P. eriantherus* plots that were sprayed with pendimethalin had higher establishment than the plots sprayed with trifluralin/linuron (*P*=0.002), trifluralin/halosulfuron (*P*=0.028), trifluralin/imazapic (*P*<0.001), and trifluralin/pendimethalin (*P*<0.001). Trifluralin/imazapic treated plots had lower establishment than the control (*P*=0.002) and halosulfuron and pendimethalin plots (*P*<0.001). Trifluralin/linuron sprayed plots had lower establishment than ones sprayed with halosulfuron (*P*=0.037) (Figure 5). *D. candida* plots had higher establishment when sprayed with imazapic than plots that were sprayed with linuron (*P*=0.033) and trifluralin/pendimethalin (*P*=0.033) (Figure 5).

Herbicide treatment affected cover for *P. hastata* (*P*=0.026), *G. aristata* (*P*=0.032) and *P. eriantherus* (*P*=0.002) (Table 7). *P. hastata* plots treated with linuron had greater cover than those treated with imazapic (*P*=0.011) and trifluralin/halosulfuron

(P=0.01) (Figure 6). *G. aristata* plots treated with trifluralin/linuron had lower cover than plots treated with pendimethalin (P=0.002) and trifluralin/pendimethalin (P=0.003) (Figure 6). *P. eriantherus* plots sprayed with trifluralin/imazapic had lower cover than plots treated with linuron (P=0.0123), halosulfuron (P<0.01) and pendimethalin (P<0.01). Trifluralin/pendimethalin also had lower cover than halosulfuron (P<0.01) and pendimethalin (P<0.01) (Figure 6).

Trifluralin/imazapic and imazapic treated plots had lower yield differences to the control for *P. hastata* (P=0.0295 and 0.0296 respectively) when compared to linuron yield (Table 8, Figure 7).

Hypothesis 3 addressed the question, are their differences between the herbicide treated plots and the unweeded control, to test if herbicides, when used without any additional weed management practice, are a useful tool for wildflower seed producers. We hypothesized that, despite their injury potential, herbicides will provide sufficient weed control and result in increased wildflower establishment, cover, and yield in comparison to untreated fields.

Establishment of *P. hastata* and *P. eriantherus* were affected by herbicide applications (P=0.003 and P=0.016, respectively) (Table 6). *P. hastata* establishment was lowered with applications of imazapic (P<0.01) and trifluralin/imazapic (P<0.01) when compared to the control (Figure 8). Pendimethalin treated plots had higher establishment than imazapic and trifluralin/imazapic (P<0.01, Figure 8) treated plots. *P. eriantherus* plots treated with trifluralin/pendimethalin and trifluralin/imazapic had fewer established

plants than the control or linuron plots ($P<0.05$, Figure 8). Trifluralin/halosulfuron treated plots also had lower establishment compared to linuron ($P=0.037$) (Figure 8).

Herbicides affected percent cover only in *P. eriantherus* plots ($P=0.028$) (Table 7). Trifluralin/pendimethalin-treated plants had a lower percent cover than the control ($P=0.04$), linuron ($P=0.008$), and halosulfuron ($P=0.033$) (Figure 9). Despite our prediction, weed competition did not impacted yield in any of the tested wildflower species (Table 8).

Discussion

The tested weed management practices in this study had varied implications for wildflower seed production. Herbicide treatments had mixed effects on wildflower plots, with some products causing serious crop injuries and others increasing wildflower cover and yield relative to the control. These effects appeared to vary across wildflower species and herbicide mode of actions and may have been affected by weed density. For ease of interpretation, this discussion will first assess site to site variation in the observed results. Second, it will evaluate the management implications of the observed results for wildflower crop production.

Potential Impact of Environmental and Biological Variables on Wildflower Establishment and Growth

Contrasting patterns of wildflower stand establishment and growth were observed across the two studied sites. Weed competition, along with climate and seed source, may have caused the Post Research Farm site to have very low wildflower establishment and

yield. O'Donovan and Sharma showed crop yield decreases with increased weed density or biomass (1983) and, if the density is high enough, can lead to total crop failure. Although this study did not formally evaluate weed seedbank densities at the studied sites, it is possible that high seedbank density led to dense weed populations observed at the Post Research Farm during the two studied growing seasons. The field used for this experiment had been used for over a decade as an experimental plot for weed research, and its weed seedbank could have adversely affected our experiment.

The time of weed seedling emergence influences plant competitive ability as well as plant susceptibility to specific a crop production practices (Buhler 1999). Also, weed density has been negatively associated with herbicide efficacy (Winkle et al. 1981). In this study, spraying during active growth on the dense weedy community present at the Post Research Farm site may have been too injurious to the wildflower crop. These factors could explain the reduced weed control observed at this site, leading to increased weed competition throughout the growing season. The NRCS Bridger Plant Materials Center weed density was lower and species composition was different, resulting in a better weed control, reduced weed competition, and adequate wildflower crop establishment.

The Post Research Farm's temperature and moisture levels were possibly on the climate evelope edge for suitability of the seeds planted. A seed's conditions are considered local when abiotic factors such as climate or topography, as well as biotic factors, such as identity of the other species found in the community are within the species suitability range (Wilkinson 2001). Not only does local seed source often lead to

greater plant establishment (Hufford and Mazer 2003), it also lowers the risk of genetically altering the species that are already in the area (Krauss and He 2006), and may reduce the competitive impact of weeds (Gallitanio et al. 1993).

The seeds used in both study sites originated at the Bridger Plant Materials Center. It is difficult to isolate the relative importance of the effects of genetic and non-genetic sources on the success of native wildflower establishment, growth, and reproduction (Bischoff et al. 2006). Reciprocal transplanting can help to isolate these effects, but was not conducted in this study. Nevertheless, it is possible that seed source led to increased establishment, cover and yield at the Bridger Plant Materials Center.

Weed Management in Native Wildflower Production Settings Weed management practices impacted weed abundance and wildflower establishment and performance. Previous studies reported wildflower injury due to broadleaf herbicides (Norcini et. al 2003, Erusha et al. 1991), however, confounding effects such as weed interference and environmental conditions make these claims difficult to substantiate. To isolate the relative impact of crop loss due to herbicide application and crop loss due to weed interference, separate hypotheses were tested. The potentially confounding effect of weed interference on wildflower establishment, percent cover and yield was eliminated in hypothesis 1 to assess herbicide crop injury. This was tested by comparing weed free plots to herbicide treated weed free plots. Hypothesis 2 assessed the effect of weed competition on establishment, cover and yield by comparing weedy control plots to weed free herbicide sprayed plots. The effectiveness of herbicides as the sole weed control in wildflower production settings was the question addressed in Hypothesis 3. When

herbicide injured wildflowers, the injury was generally consistent across hypotheses, so will be discussed together, except in the cases that species response to weed management was different between hypotheses.

The preemergence treatment of trifluralin, in the dinitroaniline family, in combinations with the four tested postemergence herbicides was evaluated in the field but not the greenhouse experiment (Chapter 2). However, Jacobs et al. (2007) found that although a preemergence application of trifluralin at 184 g ai/ha reduced wildflower seedling density, height, and shoot dry biomass of *G. aristata* and *R.columnifera,* it was the least deleterious of all preemergence herbicides tested. In this study, *P. eriantherus* was negatively affected by all treatments of trifluralin. This observation agrees with Erusha et al. (1991), who observed that two species similar to those tested in this study, desert bells and rocky mountain penstemon, were susceptible to EPTC + trifluralin at 2.3 +0.6 kg ai/ha. However, our results contrasts with Lieth et al. (2004), where red rocks beardstongue (*Penstemon X Mexicali* 'Red Rocks') was uninjured by a mixture of isoxoben and trifluralin at 0.48 kg ai/ha, indicating further research on trifluralin effects on penstemon species is necessary. *R. columnifera* and *G. aristata* had a mixed response to trifluralin, but generally were tolerant, agreeing with Erusha et al. (1991) where annual Indian blanket *(Gaillardia puchella* Foug). and *R. columnifera* were tolerant to EPTC + trifluralin at 2.3 +0.6 kg ai/ha, although delayed flowering and slight stunting occurred for Indian blanket. Multiple studies document Asteraceae species tolerance to trifluralin (Barnes 2007, Rosenberg 1997), indicating its use may improve crop establishment for this family of plants.

In accordance with McDonald et al. (1996), linuron, a photosynthesis inhibitor herbicide that causes interveinal chlorosis preferentially in older leaves, had mixed effects on wildflowers in seed production settings. In this study, linuron treatments negatively affected *D. candida* and *G. aristata*, and to our knowledge, this is the only study testing this herbicide on this species. However, treatments increased or had no effect on *P.eriantherus* or *P. Hastata* plots establishment, percent cover and yield. Shock et al. (2006) studied hotrock penstemon *(Penstemon deustus* Douglas ex lindl), sand penstemon (*P. acuminatus* Douglas ex lindl) and royal or sagebrush penstemon (*P. speciosus* D.D. Keck and Cronquist), with contrasting results, all species being injured by a rate of 0.57 kg ai/ha of linuron.

Although imazapic, an ALS inhibitor herbicide, is labeled for use in mixed grass and wildflower revegetation projects, previous studies indicate that caution should be used when applying this herbicide on native wildflowers (Washburn et al. 2002). Tolerance to imazapic is not well documented and varies across plant families. Even if native wildflowers seedlings survive the treatment of imazapic, seed production could be affected by halting of growth at the meristem and delayed bud production. For *D. candida*, imazapic was consistently not injurious, consistent with Barnes (2007) and Beran et al. (1999) who found tolerance to imazapic in the Fabaceae family. *G. aristata* was consistently tolerant and establishment was sometimes increased by imazapic, agreeing with Barnes (2007) who found that most of the 100 wildflower species that were uninjured by imazapic belonged to the Asteraceae, the Fabaceae, and the Laminaceae families. Contrastingly, Aldrich et al. (1998) found stunting and damage to firewheel

(*Gaillardia puchella* Foug.) with imazapic applications of 0.014 kg ai/ha. In our study, *P. hastata* was consistently negatively affected by imazapic, possibly due to inhibition of branched chained amino acids on early spring growth. Symptoms of injury were cholorosis and necrosis days after application. Therefore caution should be used if applying an herbicide on a mixture of wildflower species as species responded contrastingly to applications.

Halosulfuron is in the family of sulfonylurea herbicides, which are acetolactate synthase (ALS) inhibitors, causing symptoms such as rapid growth inhibition and chlorosis. Herbicides of this family are absorbed by foliage and roots, inhibiting growth at both locations. Once absorbed into the plant, sulfonylurea is rapidly translocated acropetally from the root to the shoot, and basipetally from the shoot to the root in the xylem and phloem to the areas of active growth. In this study, no species was negatively affected and *P. eriantherus* sometimes had increased establishment with treatments of halosulfuron probably due to high weed control and little herbicide activity on this species. These results indicate that halosulfuron could be used on monocultures of wildflowers and possibly on mixed species assemblages because none of the species were negatively affected.

Pendimethalin, a mitosis inhibitor herbicide that causes swelling and stunting of the root tips, had little effect on establishment, cover, and yield for all studied species. *P. eriantherus* was uninjured by pendimethalin, agreeing with Lieth et al. (2005) that found no effect of pendimethalin on *P. Mexicali* at 3.62, 7.25, or 14.5 kg ai/ha. Our result also agree with Shock et al. (2006) where hotrock penstemon, sand penstemon, and royal

penstemon were uninjured by an application of pendimethalin at 1.1 kg ai/ha. Pendimethalin was consistently not injurious to *D. candida*, agreeing with crop labels for pendimethalin use on clover and alfalfa production, also in the Fabaceae family, indicating a possible plant family response to herbicide.

There were no differences in *G. aristata*, *P. eriantherus*, and *R.columnifera* establishment between unmanaged control plots and herbicide and mechanically treated plots. Similarly, establishment of four of the five tested species was unaffected by herbicide treatment (*D. candida*, *G. aristata*, *R. columnifera*, and *P. hastata*) when no mechanical weeding was done. This could be because relatively low weed density observed at the USDA NRCS Bridger Plant Materials Center, or because weeds sheltered the wildflower species from wind, providing shade that helped maintain moisture. However, many herbicide treatments resulted in greater percent cover of native wildflowers than the control, suggesting that although wildflower plants were able to establish in untreated plots, weed competition can still reduce yields. If herbicides are the only weed management used and weeds are large enough to protect wildflower plants, selecting an herbicide based on effectiveness for controlling the weed species present in a field may be more important than selecting an herbicide based on potential wildflower injury and related yield loss.

Herbicide crop safety was tested in a greenhouse experiment using these same species (see Chapter 2). Although some differences were observed among field and greenhouse settings, *P. hastata* was tolerant to linuron in both studies, *G. aristata* and *R. columnifera* were consistently tolerant to pendimethalin across studies, although

pendimethalin injury was observed in the greenhouse, no reduction in biomass resulted. Linuron and halosulfuron were consistently the least injurious herbicides, except for *D. candida*, which was not tolerant to linuron, potentially due to the root growth inhibition caused by this herbicide, possibly leading to lower root nodulation and nitrogen fixation.

Conclusions

Wildflower species responded uniquely to weed management. This indicates that caution should be used if treating these species in both a cropland or a wildland setting.

Although the impact of weed management on traditional agricultural crop yield has been studied (Cousens 1985), few studies have examined influence of weed management on wildflower growth and yield. While incorporating herbicides as a component of weed management systems is a common, effective method to control weeds, caution should be used in wildflower seed production, as not all wildflower species show similar tolerance to herbicides. Further research should be conducted evaluating multiple and integrated weed control tactics, such as hand weeding, weed fabric, herbicide, and mowing. Integrated weed management could be a more effective weed control technique because a balanced approach to weed management can control a broader spectrum of weeds and is often more economical and ecologically sound.

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Table 1: Field site properties of Bozeman, MT and Bridger, MT showing 30 year mean precipitation (cm) and temperature (°C) as well as cumulative precipitation (cm) and mean temperature (°C) for 2007 and 2008.

	Bozeman, MT	Bridger, MT
Soil type	Amsterdam silty loam, loess loamy glacial till and clayey lacustrine deposits underlain by sandstone and shale	Sandstone shale and alluvium deep, well drained, loamy, and clayey textured soils formed in residuum from sandstone
Elevation	~1524 m	~1127 m
Mean Temperature	30 year 3.3° C 2007 - 7.5° C 2008 - 5.6° C	30 year 7° C 2007 - 8.5° C 2008 - 7.4° C
Yearly Precipitation	30 year - 38 cm 2007 - 45.2 cm 2008 - 27.2 cm	30 year - 30 cm 2007 - 10.5 cm 2008 - 12 cm
Latitude/Longitude	45.679N/111.037W	45.295 N/108.913 W

Table 2. Trade names, active ingredients, modes of action, and application rates of herbicides tested in a field setting to determine native wildflower seedling tolerance.

Trade Name	Active Ingredient	Mode of Action	Rate
Prowl® H_2O	Pendimethalin	Microtubule assembly inhibitor	4.2 liters/ha
Treflan™	Trifluralin	Microtubule assembly inhibitor	189 liters/ hectares
Plateau®	Imazapic	ALS inhibitor	560 g/ha
Permit®	Halosulfuron	ALS inhibitor	91 g/ha
Lorox™	Linuron	Inhibits photosynthesis in ps 2	1.121 kg/ha

Table 3: Wildflower species, percent seed viability, percent purity, and percentage pure-live-seed planted at Montana State University Post Farm and Bridger Plant Materials Center.

Genus and Species	Family	Common Name	% Viability	% Purity	PLS†
Dalea candida	Fabaceae	White prairieclover	90	99.6	89.6
Gaillardia aristata	Asteraceae	Blanketflower	83	86.8	72.1
Penstemon eriantherus	Scrophulariaceae	Fuzzytongue penstemon	64	91.2	58.3
Phacelia hastata	Hydrophyllaceae	Silverleaf phacelia	84	97.4	81.8
Ratibida columnifera	Asteraceae	Prairie coneflower	98	99.1	97.1

† PLS = Pure-Live-Seed estimated as (Percent purity) * (Percent germination rate) / 100 where percent purity was estimated as the ratio between amount of seed and amount of chaff, other non-viable plant material, and weed seeds.

Table 4. Environmental conditions at the time of treatment application of pre and postemergence herbicide application.

Herbicide	Location	Date	Temp	Wind (kmph)	Humidity	Cloud cover
Preemergence	Bozeman	October 26th 2006	14C	4	40%	60%
Preemergence	Bridger	September 30th 2006	13C	18	40%	100%
Postemergence	Bozeman	June 12nd 2007	18C	0	53%	0%
Postemergence	Bridger	June 22th 2007	25C	0	46%	0%
Postemergence	Bozeman	May 13th 2008	13C	5-7	33%	85%
Postemergence	Bridger	May 15th 2008	14C	5	40%	100%

Table 5. Percent cover of all species found in all control plots of native wildflower fields at Bozeman, MT and Bridger, MT field sites

Species	Bozeman	Bridger
Amaranthus albus	3	-
Chenopodium album	5	23
Malva neglecta	5	5
Amaranthus retroflexus	10	-
Thlaspi arvense	11	
Poaceae spp	12	16.8
Tragopogon dubius	13	4.5
Salsola tragus	17	-
Taraxacum officinale	19	-
Capsella bursa-pastoris	21	-
Wildflower spp	25	36.3
Cirsium arvense	26	16.4
Galium aparine	27	-
Lactuca serriola	39	31
Kochia scoparia	50	34

Table 6. F values from an ANOVA assessing herbicide affects on establishment of five wildflower species. Model df are 8 for treatment 2 for replication, and 16 for residuals. See text for description of the specific hypotheses tested.

Species	Hypothesis 1		Hypothesis 2		Hypothesis 3	
	treatment	rep	treatment	rep	treatment	rep
Phacelia hastata	3.3 *	1.62	3.67 *	1.5	4.94 **	2.15
Gaillardia aristata	3.03 *	0.087	3.06 *	0.11	0.98	0.86
Penstemon eriantherus	6.02 **	0.925	6.3 **	0.57	3.49 *	2.04
Dalea candida	4.57 **	7.54**	3.04 *	4.46*	0.83	0.06
Ratibida columnifera	0.897	0.15	0.44	0.27	0.89	0.1

*P < 0.05, **P < 0.01, ***P < 0.001.

Table 7. F values from an ANOVA assessing herbicide and affects on wildflower cover for five wildflower species. Model df are 8 for treatment, 2 for replication, and 16 for residuals. See text for description of the specific hypotheses tested.

Species	Hypothesis 1		Hypothesis 2		Hypothesis 3	
	treatment	rep	treatment	rep	treatment	rep
Phacelia hastata	2.9*	3.26	3.03 *	3.13	1.91	0.78
Gaillardia aristata	2.9*	1.02	2.93 *	1.08	1.4	1.44
Penstemon eriantherus	4.97 **	3.4	5.36**	4.52*	3.04 *	8.2**
Dalea candida	2.35	2.36	2.2	2.87	0.64	0.25
Ratibida columnifera	0.79	0.15	0.77	0.19	0.67	0.30

*P < 0.05, **P < 0.01, ***P < 0.001.

Table 8. F values from an ANOVA assessing herbicide effects on the yield of five wildflower species. Model df are 8 for treatment, 2 for replication, and 16 for residuals. See text for description of the specific hypotheses tested.

Species	Hypothesis 1		Hypothesis 2		Hypothesis 3	
	treatment	rep	treatment	rep	treatment	rep
Phacelia hastata	3.01 *	0.012	2.84*	0.86	1.15	0.83
Gaillardia aristata	0.5626	4.41*	0.22	2.69	0.56	0.3
Penstemon eriantherus	0.74	14.8***	1.11	4.45	1.72	8.94**
Dalea candida	1.99	21***	0.54	0.83	1.28	11.1***
Ratibida columnifera	0.85	0.078	1.69	1.10	0.84	0.02

*P < 0.05, **P < 0.01, ***P < 0.001.

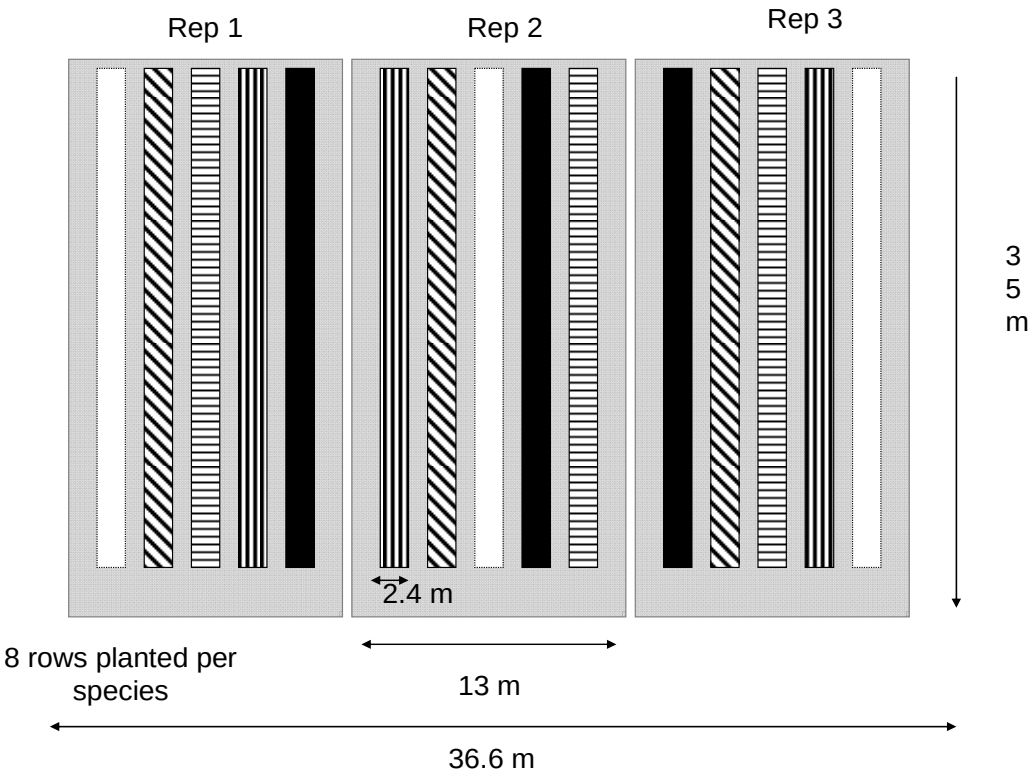


Figure 1: Experimental design to evaluate the effect of pre-and postemergence herbicides on native wildflower seedling establishment and seed production. Each patterned area represents one of the five forb species tested. Wildflower species were randomized within replications. Ten herbicide treatments and a control were randomized within species (first split) and weeding techniques were randomized within herbicide applications (second split).

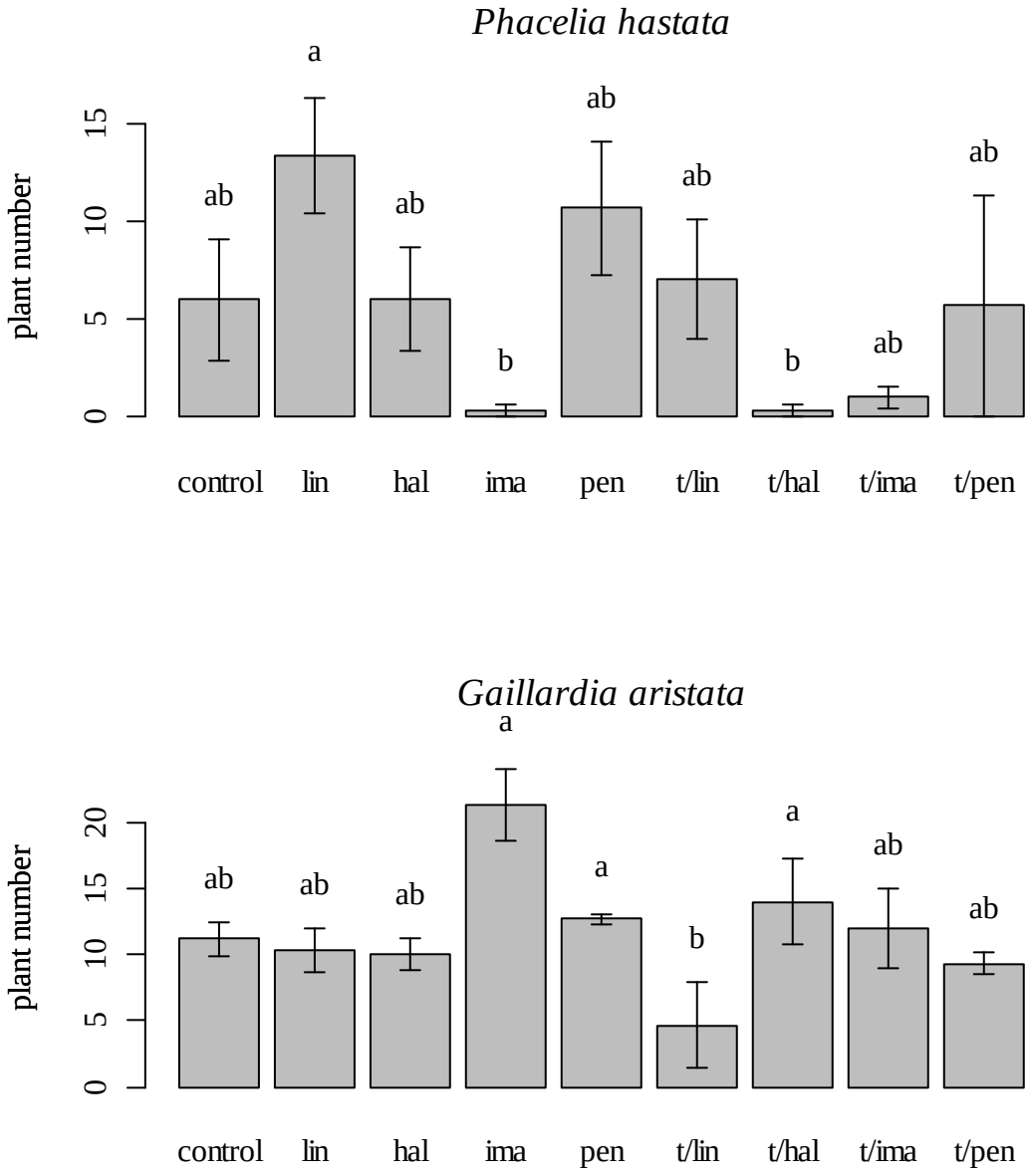


Figure 2. Effect of herbicide and hand weeding treatments on native wildflower species establishment. Control plots were hand weeded. Bar plots with different letters indicate significant differences in plant establishment between treatments from a Tukey's HSD test ($P < 0.05$). Error bars are standard errors of data. Plant number is per 55 x 66 cm plot Herbicide active ingredient listed in the x axis and abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.

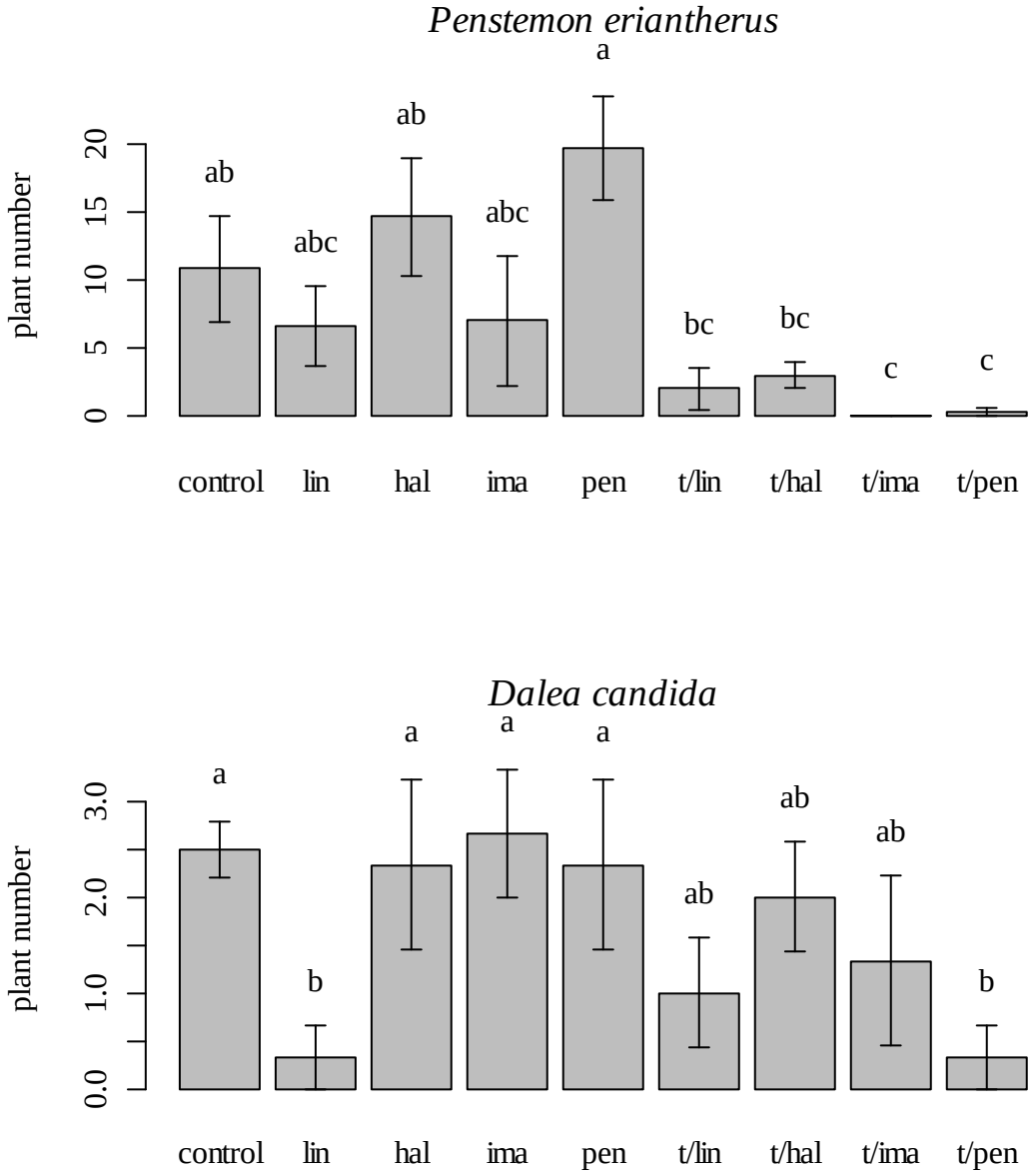


Figure 2, continued.

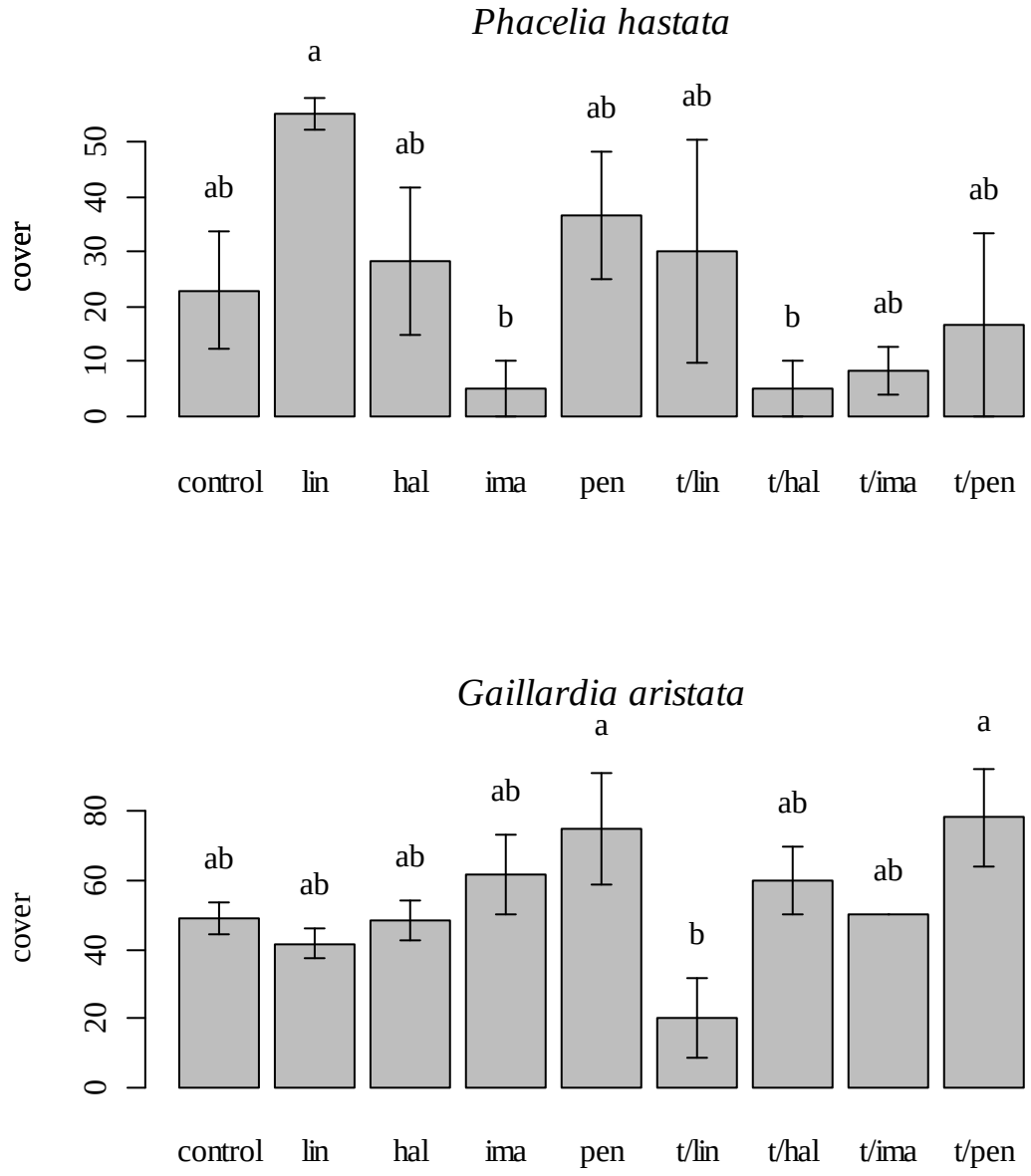
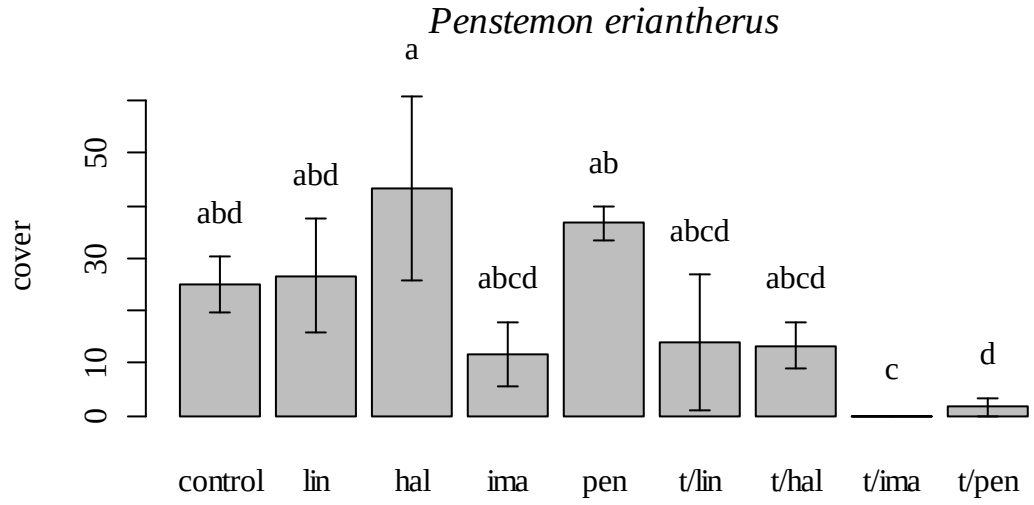


Figure 3. Effect of herbicide and hand weeding treatments on native wildflower species percent cover. Control plots were hand weeded. Bar plots with different letters indicate significant differences in plant establishment between treatments from a Tukey's HSD test ($P < 0.05$). Error bars are standard errors of data. Percent cover is per 55 x 66 cm plot Herbicide active ingredient listed in the x axis and abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.



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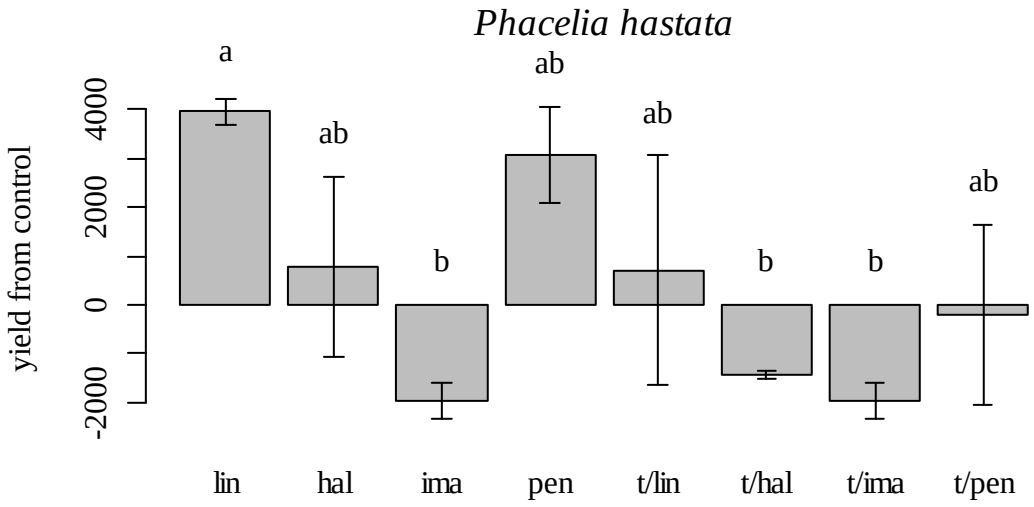

Figure 4. Barplot of yield as differences from a hand weeded control when herbicide and hand weeding were applied. Bar plots with different letters indicate significant differences in plant establishment between treatments from a Tukey's HSD test ($P < 0.05$). Error bars are standard errors of data. Yield difference is per 55 x 66 cm plot. The y axis is seed number and set to have 0 represent no difference from the control. Herbicide active ingredient listed in the x axis and abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.

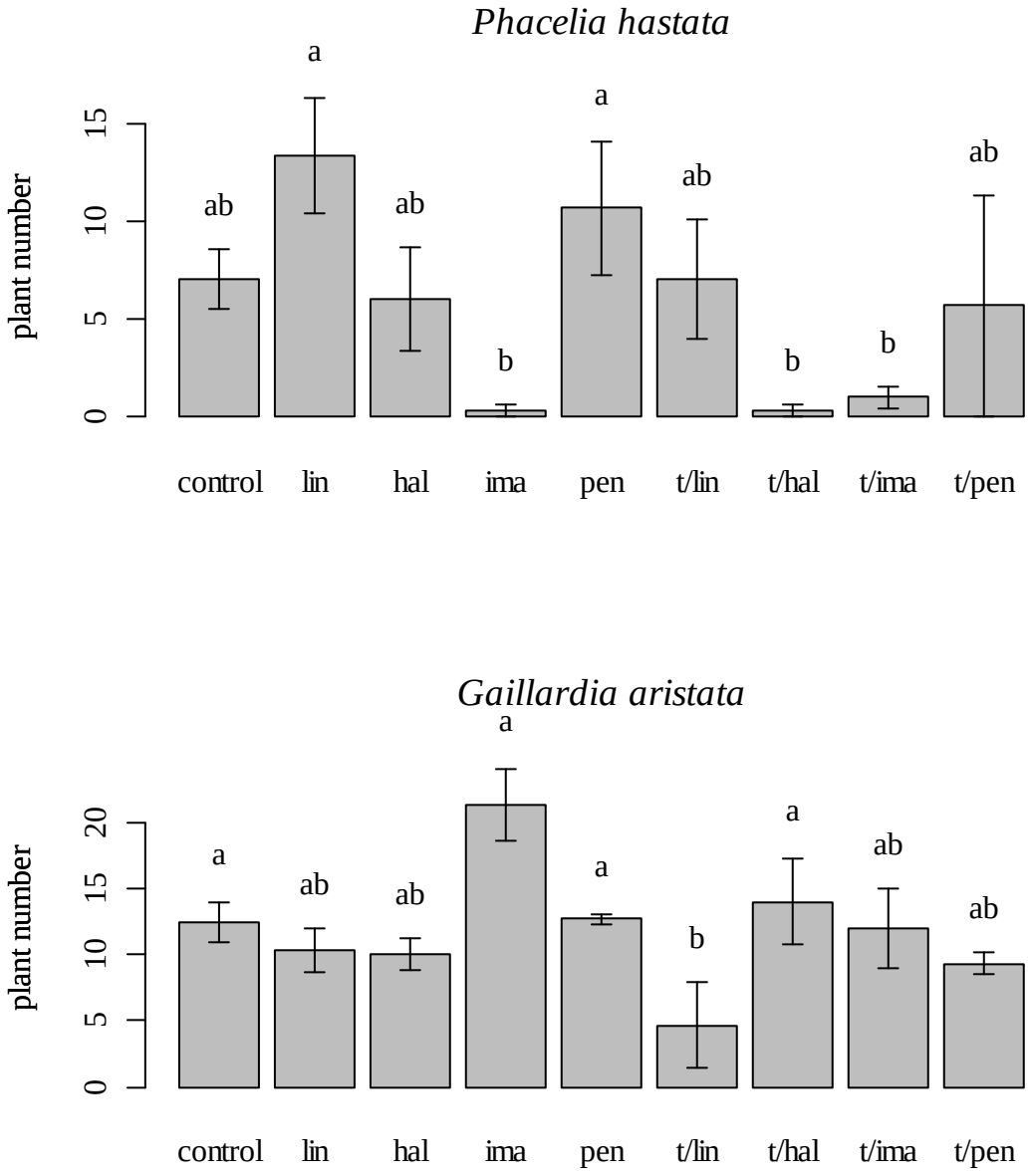

Figure 5. Effect of herbicide and hand weeding treatments on native wildflower species establishment. Control plots were not hand weeded. Bar plots with different letters indicate significant differences in plant establishment between treatments from a Tukey's HSD test ($P < 0.05$). Error bars are standard errors of data. Plant number is per 55 x 66 cm plot. Herbicide active ingredient listed in the x axis and abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.

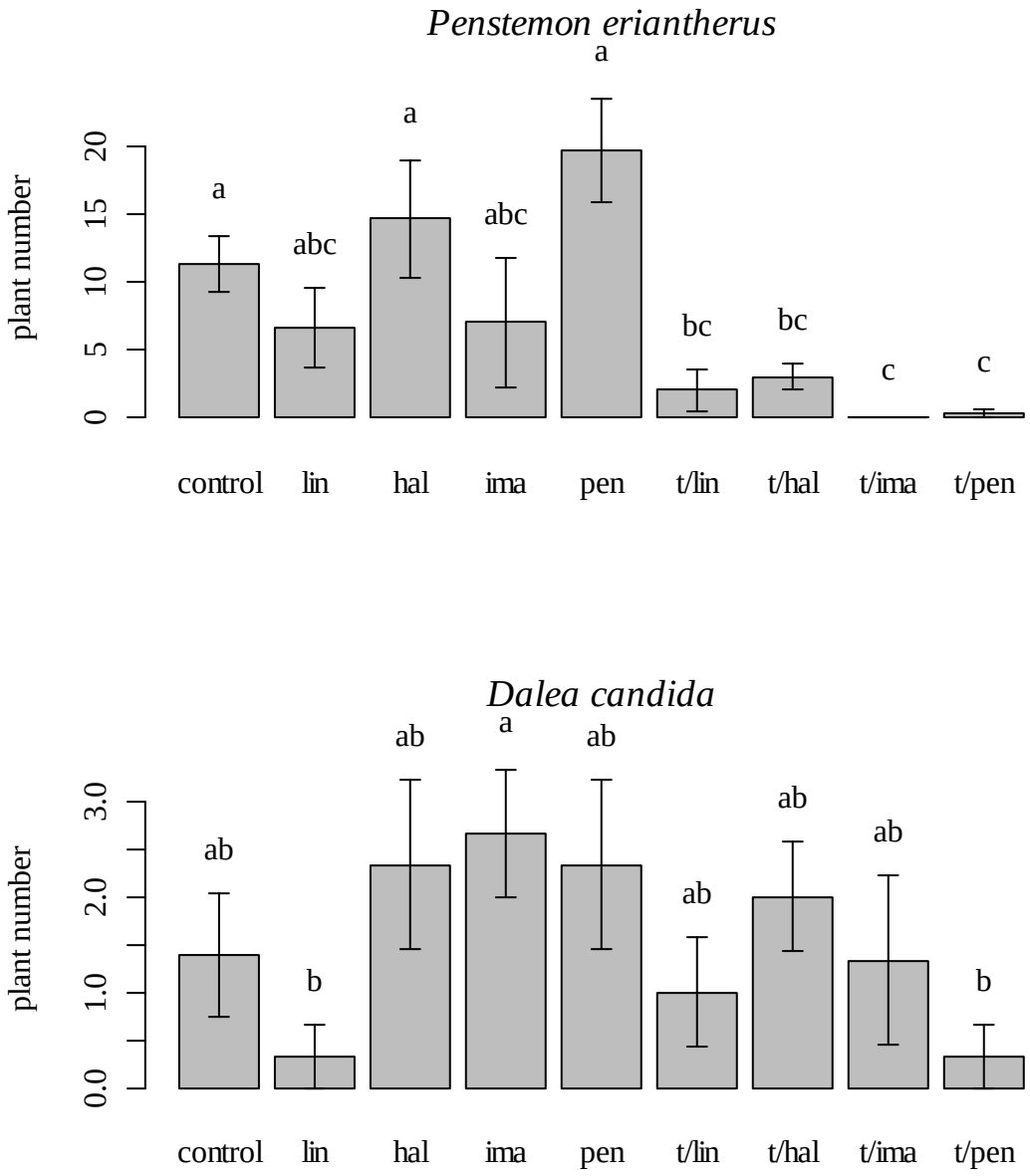

Figure 5, continued.

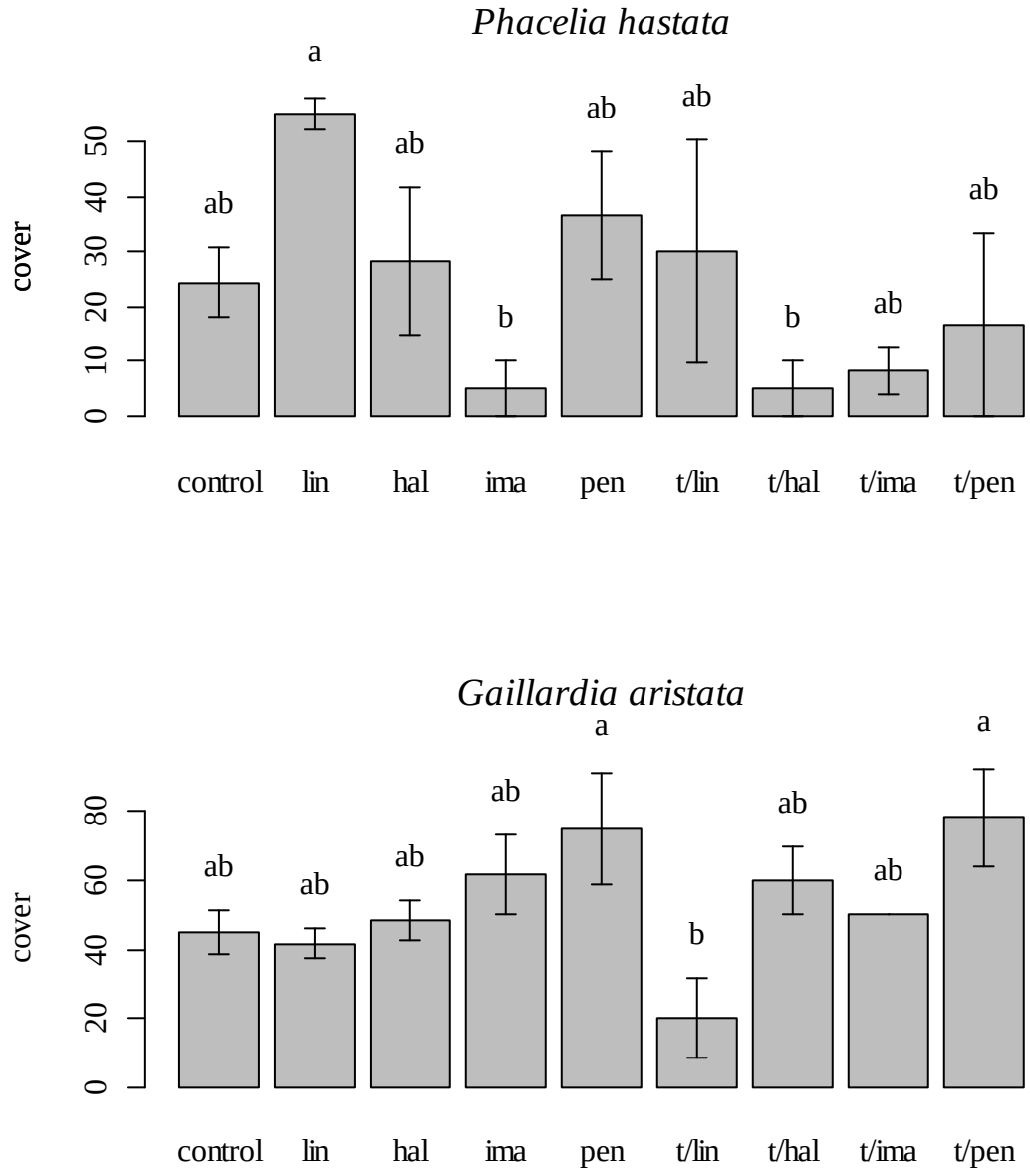

Figure 6. Effect of herbicide and hand weeding treatments on native wildflower species percent cover. Control plots were not hand weeded. Bar plots with different letters indicate significant differences in plant establishment between treatments from a Tukey's HSD test ($P < 0.05$). Error bars are standard errors of data. Percent cover is per 55 x 66 cm plot. Herbicide active ingredient listed in the x axis and abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.

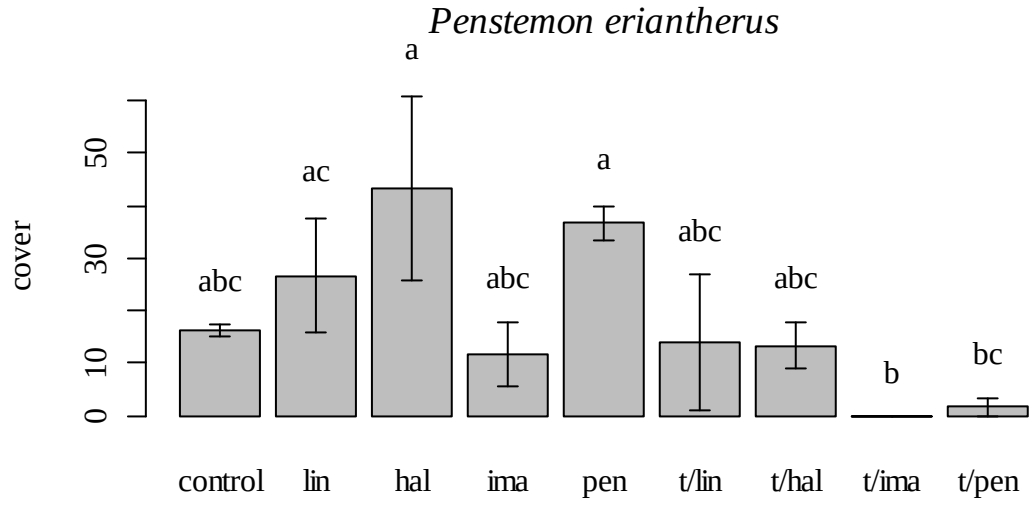

Figure 6, continued.

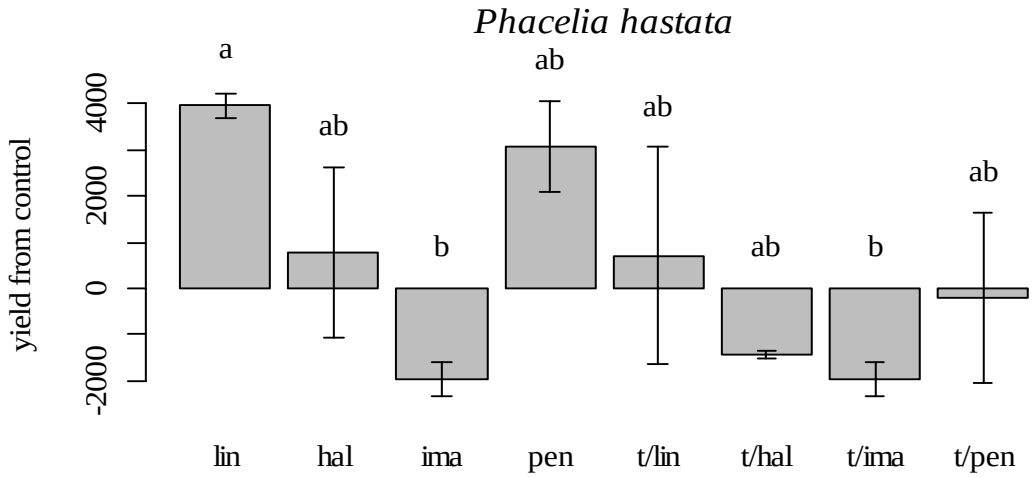

Figure 7. Barplot of seed yield as differences from an unmanaged control when herbicide and hand weeding were applied. Bars with different letters indicate significant differences between treatments from a Tukey's HSD test. Yield differences are per 55 x 66 cm plots. The y axis is number of seeds and set to have 0 represent no difference from the control. The x axis is herbicide treatment, active ingredient is abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin

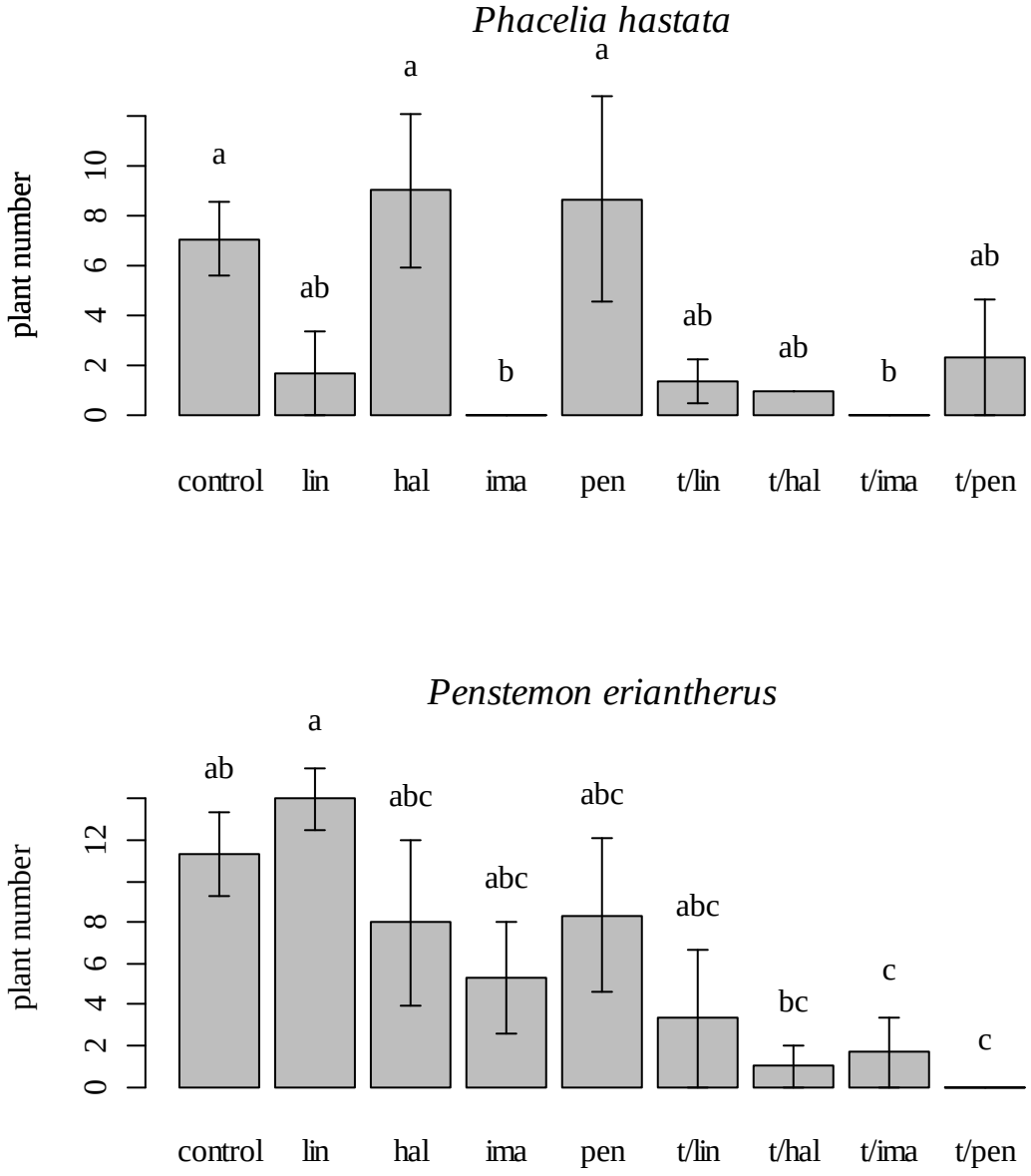

Figure 8. Effect on species establishment when herbicide treatments were applied. Control plots had no weed management. Bar plots with different letters indicate significant differences in plant establishment between treatments from a Tukey's HSD test ($P < 0.05$). Error bars are standard errors of data. Plant number are per 55 x 66 cm plots. Herbicide active ingredient listed in the x axis and abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.

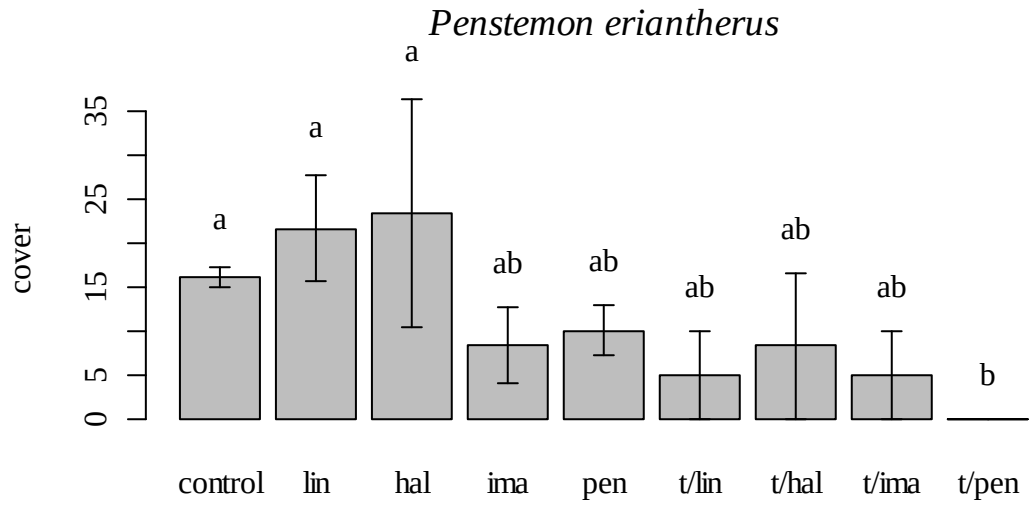

Figure 9. Effect on species cover when herbicide treatments were applied. Control plots had no weed management. Bar plots with different letters indicate significant differences in plant establishment between treatments from a Tukey's HSD test ($P < 0.05$). Error bars are standard errors of data. Percent cover is per 55 x 66 cm plot. Herbicide active ingredient listed in the x axis and abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.

CHAPTER 4

SUMMARY OF FINDINGS / FUTURE RESEARCH

The study of native wildflowers as a crop is a new and complex science. This study measured the impact of two weed management approaches; herbicides and mechanical weeding, on five wildflower species [slender white prairie clover (*Dalea candida*(Michx). ex Willd), blanketflower (*Gaillardia aristata* Pursh), fuzzy tongue penstemon (*Penstemon eriantherus* Pursh var. *eriantherus*), silverleaf phacelia (*Phacelia hastata* Douglas ex Lehm.), and prairie coneflower (*Ratibida columnifera* (Nutt.) Woot. & Standl)] establishment, growth, and seed production. Herbicides tested included Treflan(trifluralin) 189 l/ha, Lorox (linuron) 1.121 kg/ha., Permit (halsulfuron) 91 g/ha., Plateau (imazapic) 560 g/ha, Prowl (pendimethalin) 4.2 l/ha.

Multiple factors, such as competitive ability, consistency in establishment, and diffuse seed production, affected the ability of the studied species to behave as a crop. Results pointed out the relative importance of weed competition, climate, and seed source on wildflower growth and seed production as evidenced by their low performance at the Montana State University Post Research Farm.

In addition, this study showed that results from greenhouse studies may not correlate strongly with patterns observed in field situations.Some consistencies across studies were observed and possible reasons for the differences observed include field settings having various and sometimes adverse environmental conditions, greenhouse observations being taken at the seedling stage, and temperature and light differences from

greenhouse to field. However, common patterns were observed in greenhouse and field settings. Among them, *P. hastata* was tolerant to linuron in both studies, *R. columnifera* was consistently tolerant to pendimethalin, and *G. aristata* was also tolerant to pendimethalin, with the exception of high visual injury levels. Overall, linuron and pendimethalin were consistently less injurious to wildflowers than the other herbicide treatments, except for *D. candida*, which was not tolerant to linuron.

Although previous research has focused on individual weed control tactics in wildflower production settings, this study utilized a dual approach to weed management by incorporating hand weeding in combination with pre and postemergence herbicides. Our results indicated that combining hand weeding with herbicide can improve wildflower establishment, cover and yield. Preemergence herbicides may be useful, however, incorporation of trifluralin negatively affected plant growth for *P. hastata*, *P. eriantherus*, and *D. candida* in this study. This elucidates a need for further testing of this preemergence herbicide, along with others, for possible use in wildflower seed production fields.

Differences in wildflower and weeds sizes made it difficult to properly time chemical weed management practices. However, the choice of herbicide was equally a critical factor determining wildflower performance and recommendations vary by species. Also, wildflower responses to the herbicides appeared to be influenced by the chemical used and level of weed interference. For example, establishment of *P. hastata* was improved after application of linuron under all hypotheses tested and *G. aristata* was consistently tolerant to imazapic, and tolerant to pendimethalin (except for high visual

injury levels). Finally, when weed interference was present, *P. eriantherus* was consistently tolerant to linuron. This may be due to weeds acting as a protective barrier from herbicide for wildflowers.

In field conditions, we hypothesized that weed interference would cause lower establishment when compared to herbicide treated, hand weeded plots. However, control plots in *G. aristata*, *P. eriantherus* and *R. columnifera* all established equally as well as herbicide and mechanically treated plots, indicating weed management was not necessary for these species establishment. Results were similar in plots that were only herbicide treated. Establishment and yield in untreated control plots was the same as treated plots for *P. hastata*, *G. aristata*, *D. candida* and *R. columnifera*. This could be because weed competition was not a factor in plant establishment due to low weed pressure. Additionally, when only herbicide was applied to wildflower production fields, less crop impact was observed than where plots were hand weeded, indicating the weeds could have served as a barrier to herbicide contact for the crops probably because weeds were larger at the time of spraying, possibly creating a canopy over the wildflower seedlings. However, many herbicide treatments, wildflowers had greater percent cover than the untreated control, indicating wildflowers in control plots were able to persist through heavy weed competition, but may not grow competitively, or produce as high of a yield.

Future studies could evaluate if, for mechanically weeded and herbicide treated plots, a reduced rate of herbicide could cause less crop injury and provide enough management for areas that have lower weed density. Further work should also focus on testing how these herbicides affect wildflowers' long term seed production and how they

affect population dynamics in restoration. For example, can wildflower species recover from herbicide applications and produce a crop in the third and fourth years of production? Is weed management only critical in the establishment year? Would applying the herbicides used in this study have the same effect in a restoration scenario as it did in the crop production field? How would these herbicides affect and potentially shift native populations? The answers to these questions would give a more detailed understanding of the production of wildflowers' as a crop and, in a broader context, facilitate weed management in restoration ecology.

Integrated weed management could be an important strategy for controlling invasive annual and perennial weeds in wildflower fields. An integrated approach to weed management should be investigated, including weed fabric, charcoal banding and new cultural methods, such as broadcast planting wildflowers to minimize bare ground and weed invasion opportunities. Since hand weeding, herbicide treatment, and weed density all affect individual plant establishment and seed production, these variables should be further studied to provide a more informative picture of native wildflower's management as a crop. In addition, the effect of seed source on establishment should be further examined by doing reciprocal transplant studies. Furthermore, an economic analysis of the value of a variety of management strategies should be conducted and would be useful for wildflower crop producers.

This study represents a building block in the foundation of weed management in native wildflower fields. We have determined that environmental and management factors can impact the growth and seed production of the five studied species. We have

also elucidated some of the establishment requirements and weed management for each species. Additionally, this study aids restoration ecologists in understanding which wildflower species can be planted together and tolerate similar weed management practices. This information is valuable because diverse mixtures of native wildflowers can enhance the likelihood of establishment in various and unpredictable environments as they have a variety of traits that may match year to year and site to site conditions and persist longer than a monoculture.

In conclusion, this study crosses scientific disciplines by providing management recommendations for wildflower seed producers and restoration biologists alike. This information could lead to more wildflower seed production, and in turn, reduce cost and difficulty in establishment of these species for restoration biologists, thereby increasing use of native wildflowers in seed mixes used for restoration.

APPENDICES

APPENDIX A

R CODE FOR WILDFLOWER SEEDLING TOLERANCE TO POSTEMERGENCE HERBICIDES

(# explains action)

```
##Reading in the Tukey test
tukey.add.test <- function(y,A,B){
  ## Y is the response vector
  ## A and B are factors used to predict the mean of y
  ## Note the ORDER of arguments: Y first, then A and B
  dname <- paste(deparse(substitute(A)), "and", deparse(substitute(B)),
            "on",deparse(substitute(y)) )
  A <- factor(A); B <- factor(B)
  ybar.. <- mean(y)
  ybari. <- tapply(y,A,mean)
  ybar.j <- tapply(y,B,mean)
  len.means <- c(length(levels(A)), length(levels(B)))
  SSAB <- sum( rep(ybari. - ybar.., len.means[2]) *
            rep(ybar.j - ybar.., rep(len.means[1], len.means[2])) *
            tapply(y, interaction(A,B), mean))^2 /
             ( sum((ybari. - ybar..)^2) * sum((ybar.j - ybar..)^2))
  aovm <- anova(lm(y ~ A+B))
  SSrem <- aovm[3,2] - SSAB
  dfdenom <- aovm[3,1] - 1
   STATISTIC <- SSAB/SSrem*dfdenom
   names(STATISTIC) <- "F"
   PARAMETER <- c(1, dfdenom)
   names(PARAMETER) <- c("num df", "denom df")
   D <- sqrt(SSAB/  ( sum((ybari. - ybar..)^2) * sum((ybar.j - ybar..)^2)))
   names(D) <- "D estimate"
   RVAL <- list(statistic = STATISTIC, parameter = PARAMETER,
      p.value = 1 - pf(STATISTIC, 1,dfdenom), estimate = D,
      method = "Tukey's one df F test for Additivity",
      data.name = dname)
   attr(RVAL, "class") <- "htest"
   return(RVAL)
  }
#### Tukey's additivity test: THIS SHOWS RESULTS FOR EACH SPECIES
Ghcom is the data set I used
by(ghcom,ghcom$species,function(x) tukey.add.test(x$WET,x$treatment,x$run))
ghcom$species: daca

### output:

        Tukey's one df F test for Additivity

data:  x$treatment and x$run on x$WET
F = 0.0352, num df = 1, denom df = 43, p-value = 0.852
```

```
sample estimates:
D estimate
  1.835366

----------------------------------------------------
ghcom$species: gaar

        Tukey's one df F test for Additivity

data:  x$treatment and x$run on x$WET
F = 0.2736, num df = 1, denom df = 42, p-value = 0.6037
sample estimates:
D estimate
 0.3929779

----------------------------------------------------
ghcom$species: phha

        Tukey's one df F test for Additivity

data:  x$treatment and x$run on x$WET
F = 0.9153, num df = 1, denom df = 43, p-value = 0.3441
sample estimates:
D estimate
  1.691110

----------------------------------------------------
ghcom$species: raco

        Tukey's one df F test for Additivity

data:  x$treatment and x$run on x$WET
F = 0.055, num df = 1, denom df = 43, p-value = 0.8156
sample estimates:
D estimate
  1.849128

MONTE CARLO SIMULATION
# A function to do a simulation on % damage:

nonpardmg<- function(spc,n,cutoff=0,datatable=ghcom,...){

# spc:              species name in quotes
# n:                        number of simulations
```

```
# cutoff:         cut off value for % damge default is 0
# datatable:     the data set, default is ghcom

d<- subset(datatable,species==spc)    # get the desired species from ghcom
t<- tapply(d$damage,d$treatment,function(x) x)       # get damage by treatment
tt<- t[levels(d$treatment)%in%'control'=='FALSE']                    # get treatments only
res<- sapply(tt,function(y){
sum(sample(y,n,replace=T)-sample(t$control,n,replace=T)>cutoff)/n         # compare to
control

# or results of 10000 simulations with a cutoff value of 10:

sapply(levels(ghcom$species),nonpardmg,n=10000,cutoff=10)
        daca   gaar   phha   raco
lorox   0.2047 0.9069 0.2674 0.2158
permit  0.0000 0.7066 0.6975 0.2485
plateau 0.3978 0.6084 0.8578 0.5383
prowl   0.0000 0.2950 0.1932 0.0757

###ANOVAs to determine if the herbicides had an effect.   Z is the object I created to
show me the aov output
###Multiple comparisons follows-had to test if herbicide had an effect first

z<-aov(WET~treatment+Error(run), data=subset(ghcom, species=='phha'))
summary(z)
z

z<-aov(WET~treatment+Error(run), data=subset(ghcom, species=='gaar'))
summary(z)
z

z<-aov(WET~treatment+Error(run), data=subset(ghcom, species=='daca'))
summary(z)
z

z<-aov(WET~treatment+Error(run), data=subset(ghcom, species=='raco'))
summary(z)
z
z<-aov(DRY~treatment+Error(run), data=subset(ghcom, species=='phha'))
summary(z)
z
z<-aov(DRY~treatment+Error(run), data=subset(ghcom, species=='gaar'))
summary(z)
z
```

```
z<-aov(DRY~treatment+Error(run), data=subset(ghcom, species=='daca'))
summary(z)
z
z<-aov(DRY~treatment+Error(run), data=subset(ghcom, species=='raco'))
summary(z)
z
```

##TUKEY HSD test

###This is a multiple comparison using a linear mixed effects model with trial as a random effect and treatment as a fixed effect. Ghcom is the name of the data set and Wet indicates wet weight. I have the same code for the dry weight.

```
require(multcomp)
require(nlme)

combwet<- by(ghcom,ghcom$species,function(x){
fit<- lme(WET~treatment-1,random=~1|run, data=x)
mc<- glht(fit,linfct = mcp(treatment = "Tukey"))
s<- summary(mc)
print(s)
invisible(list(fit=fit,mc=s))
```

APPENDIX B

R CODE FOR THE EFFECT OF WEED MANAGEMENT OF SEEDLING ESTABLISHMENT, GROWTH AND YIELD IN COMMERCIAL PRODUCTION FIELDS

(# explains action)

```
########ANOVA’s for each of the hypotheses: diff 2 is my data set in this case
summary(aov(weight~treat+Error(factor(reps)),data=subset(diff2,spp=='peer')))
summary(aov(weight~treat+Error(factor(reps)),data=subset(diff2,spp=='phha')))
summary(aov(weight~treat+Error(factor(reps)),data=subset(diff2,spp=='raco')))
summary(aov(weight~treat+Error(factor(reps)),data=subset(diff2,spp=='gaar')))
summary(aov(weight~treat+Error(factor(reps)),data=subset(diff2,spp=='daca')))

summary(aov(transplants~treat+Error(factor(reps)),data=subset(diff2,spp=='peer')))
summary(aov(transplants~treat+Error(factor(reps)),data=subset(diff2,spp=='phha')))
summary(aov(transplants~treat+Error(factor(reps)),data=subset(diff2,spp=='raco')))
summary(aov(transplants~treat+Error(factor(reps)),data=subset(diff2,spp=='gaar')))
summary(aov(transplants~treat+Error(factor(reps)),data=subset(diff2,spp=='daca')))


summary(aov(transcover~treat+Error(factor(reps)),data=subset(diff2,spp=='peer')))
summary(aov(transcover~treat+Error(factor(reps)),data=subset(diff2,spp=='phha')))
summary(aov(transcover~treat+Error(factor(reps)),data=subset(diff2,spp=='raco')))
summary(aov(transcover~treat+Error(factor(reps)),data=subset(diff2,spp=='gaar')))
summary(aov(transcover~treat+Error(factor(reps)),data=subset(diff2,spp=='daca')))
########Transformations
diff2$transcover<-asin(sqrt(diff2$cover/100))
fit<-lm(transcover~treat, data=diff2)
summary(fit)
plot(fit)
summary(aov(weight~spp+Error(factor(reps)),data=diff2))
)
diff2$transplants<-(sqrt(diff2$plants))
fit<-lm(transplants~treat,random=~1|reps, data=diff2)
summary(fit)
plot(fit)
##########Multiple comparisons-examples
require(nlme)
require(multcomp)
multi<- by(diff2,diff2$spp,function(subset.by.species){
fit<- lme(transcover~treat-1,random=~1|reps, data=subset.by.species)
mc<- glht(fit,linfct = mcp(treat = "Tukey"))
s<- summary(mc)
print(subset.by.species$spp[1])
print(s)
invisible(list(fit=fit,mc=s))
})

require(multcomp)
```

```
multi<- by(diff2,diff2$spp,function(subset.by.species){
fit<- lme(transplants~treat-1,random=~1|reps, data=subset.by.species)
mc<- glht(fit,linfct = mcp(treat = "Tukey"))
s<- summary(mc)
print(subset.by.species$spp[1])

print(s)
invisible(list(fit=fit,mc=s))
})
require(multcomp)
multi<- by(diff2,diff2$spp,function(subset.by.species){
D<- subset(subset.by.species, treat!='Control')
D$treat<- factor(D$treat,levels=levels(D$treat)[2:9])
fit<- lme(weight~treat-1,random=~1|reps, data=D)
mc<- glht(fit,linfct = mcp(treat = "Tukey"))
s<- summary(mc)
print(s)
invisible(list(fit=fit,mc=s))
print(subset.by.species$spp[1])
print(anova(fit))
```

Species	Herbicide treatment								
Dalea candida	lin	hal	ima	pen	tri/lin	tri/hal	tri/ima	tri/pen	control
C. arvense	0 (0)	0(0)	0(0)	0(0)	15(15)	27(46)	0.33(0.57)	0(0)	0(0)
Graminoids	0(0)	20(36)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
K.scoparia	73(29)	38 (40)	40(45)	0(0)	13(15)	10(0)	16(12)	3(5)	42(34)
C. album	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	6(12)
L. serriola	16(28)	0(0)	55(33)	60(26)	11(20)	13(23)	21(25)	60(32)	20(22)
T. officinale	0(0)	0(0)	0(0)	0(0)	5(8)	0(0)	0.4(1.6)	0(0)	0(0)
Gaillardia aristata	lin	hal	ima	pen	tri/lin	tri/hal	tri/ima	tri/pen	control
C. arvense	0(0)	0(0)	0(0)	0(0)	0(0)	3(6)	13(15)	0(0)	2(5)
Graminoids	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.3(1)
K.scoparia	20(20)	13(23)	2(3)	3(6)	2(3)	8(14)	0(0)	0(0)	14(18)
C. album	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	6(12)
L. serriola	2(3)	0(0)	0(0)	0(0)	0(0)	6(7)	0(0)	7(8)	3(6)
T. officinale	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Penstemon eriantherus	lin	hal	ima	pen	tri/lin	tri/hal	tri/ima	tri/pen	control
C. arvense	25(43)	24(43)	3(5)	1(1)	10(17)	3(6)	0(0)	3(6)	1(2)
Graminoids	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
K.scoparia	23(20)	20(8)	27(25)	30(36)	31(7)	15(18)	40(36)	2(3)	32(8)
C. album	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
L. serriola	16(12)	2(3)	15(18)	25(43)	40(26)	36(40)	9(10)	48(10)	32(24)
T. officinale	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(4)
Phacelia hastata	lin	hal	ima	pen	tri/lin	tri/hal	tri/ima	tri/pen	control
C. arvense	8(14)	0(0)	0(0)	3(6)	2(5)	7(11)	15(26)	5(9)	2(3)
Graminoids	0(0)	0(0)	0(0)	0(0)	3(5)	5(9)	0(0)	3(6)	2(5)
K.scoparia	36(31)	33(28)	60(30)	12(20)	7(12)	42(28)	48(10)	6(12)	25(30)
C. album	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
L. serriola	18(3)	15(22)	22(20)	18(24)	20(20)	13(12)	25(25)	0(0)	10(12)
T. officinale	0(0)	3(6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3)
Ratibida columnifera	lin	hal	ima	pen	tri/lin	tri/hal	tri/ima	tri/pen	control
C. arvense	12(10)	8(10)	10(9)	18(28)	17(29)	0(0)	0(0)	0(0)	3(5)
Graminoids	0(0)	0(0)	3(6)	0(0)	0(0)	5(9)	0(0)	0(0)	1(4)
K.scoparia	5(5)	23(15)	10(17)	5(8)	10(17)	26(38)	22(16)	10(17)	23(19)
C. album	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
L. serriola	5(9)	27(28)	15(0)	5(9)	8(14)	8(8)	0(0)	30(52)	8(14)
T. officinale	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Table 1. Weed % cover for each wildflower species in Bridger, MT under different management treatments. Mean and standard deviation are shown for each species. Wildflower species are listed with weed species below. Herbicide active ingredient is abbreviated as follows: lin=linuron, hal=halosulfuron, ima=imazapic, pen=pendimethalin, t/lin=trifluralin/linuron, t/hal=trifluralin/halosulfuron, t/ima=trifluralin/imazapic, t/pen=trifluralin/pendimethalin.