THESIS

EXAMINING THE UNPREDICTABLE NATURE OF YELLOW TOADFLAX

Submitted by

Nicholas Jon Krick

Department of Bioagricultural Sciences and Pest Management

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Masters Committee:

Advisor: K. George Beck

Scott Nissen
Sarah Ward
Paul Meiman

ABSTRACT

EVALUATING THE UNPREDICABLE NATURE OF YELLOW TOADFLAX

Yellow toadflax (*Linaria vulgaris* Mill.) is an aggressive creeping perennial forb that was introduced to North America in the 1600's. It is now naturalized throughout the United States and Canada and is a serious weed in the Intermountain West. This plant threatens cropping systems, rangelands, and natural areas. Colorado State University Weed Science has conducted research on yellow toadflax control for more than 20 years yielding variable and inconsistent results. Short term success has been achieved; however, recommendations for acceptable long term control are unavailable. Dramatic site-to-site variation has been observed, but the source of that variation has not been determined. Yellow toadflax is an obligate outcrossing species that exhibits much genetic and phenotypic variation and may inhabit a wide range of ecosystems. The success of managing yellow toadflax might hinge on application timing; therefore, an observational study of root bud phenology was conducted for 2 years at two sites in Colorado. This study showed that yellow toadflax exhibited a pattern of root bud development and that pattern had an important relationship to flowering. Root buds were present throughout the growing season, but their numbers fluctuated. Following bud emergence in the spring, bud numbers declined until they reached their lowest counts around the full bloom/seed set growth stage. Following this growth stage, bud numbers

increased suggesting the root system was a demanding carbohydrate sink. This observational study supported results from herbicide field trials.

Identical herbicide efficacy trials were conducted at five separate locations in Colorado where four rates of chlorsulfuron and imazapyr were each applied in September 2008. Plants were harvested from these same sites and were subjected to a common garden experiment and an ALS enzyme bioassay. Analysis of field experiments 1 year after treatment (1 YAT) showed site variation using low herbicide rates (40 g ae ha^{-1} chlorsulfuron and 127 g ae ha^{-1} imazapyr), but most variation was overcome by increasing herbicide rates. Chlorsulfuron applied at 94 g ae ha^{-1} controlled more than 76% of yellow toadflax at all sites; whereas, 380 g ae ha^{-1} of imazapyr was necessary to overcome site to site variation, but control was $\geq$73% at four of five sites. Evaluations 2 YAT showed that yellow toadflax recovered at two sites. The common garden study and ALS enzyme bioassay revealed that these populations were susceptible to herbicides on a whole plant level and on a mechanistic level; confirming that herbicide resistance is not responsible for spatial variation. It appears that yellow toadflax recovery was largely driven by length of growing season and the growth stage of a population at which applications occurred. Lower elevation sites had a higher percentage of shoots flowering at the time of application and were more difficult to control. Comparing results from the field experiments to the pattern of root bud development, applications at the lower elevation sites occurred when bud numbers were decreasing or at their lowest counts. Higher elevation sites were in a more advanced growth stage, which resulted in better control likely based on the phenology of root bud development. It is likely that more buds present or developing on a plant resulted in better control because bud are strong

sinks and herbicides were likely translocated to those tissues and had a greater negative effect on their root systems.

Managers can utilize what has been learned though these experiments to improve their programs and management success. Management can be improved by increasing herbicide rate, but more importantly by targeting populations at their most susceptible growth stage. Although a rate increase of herbicide is necessary to overcome site variation, this should result in less overall herbicide use (decrease due to fewer tank mixes, fewer application events, and elimination of high rates of ineffective compounds), reduced costs for managers, and will decrease environmental exposure to herbicides.

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ORIGIN AND HISTORY

Yellow toadflax (*Linaria vulgaris* Mill.) commonly referred to as yellow toadflax, "butter and eggs", common toadflax, toadflax, and wild snapdragon (USDA, ARS 2007) is an exotic perennial forb of the Antirrhinae tribe in the Plantaginaceae family (formerly of the Scrophulariaceae family) (The Angiosperm Phylogeny Group 2003). Yellow toadflax belongs to a group of species that originated in the steppes of south-eastern Europe and south-western Asia (Saner et al. 1995). In its region of origin, yellow toadflax evolved in plant communities that were grazed moderately to intensely, primarily by sheep and goats, and by cattle to a lesser extent. Due to the arable landscape of this region, many populations evolved with and are adapted to, the periodic disturbances of agriculture (Lajeunesse 1999).

Humans have a long history with this plant; it has been adored and respected, at the same time cursed and despised. It has been appreciated for its ornamental and medicinal properties (Mitich 1993), as well as its association with mystical folklore (LeStrange 1977). During the Middle Ages, yellow toadflax was used to cure figs or piles (hemorrhoids) and remedy throat ailments such as scrofula (an enlargement of the lymph glands of the neck) (Mitich 1993). During the 16th century, it was used medicinally to treat hot swellings of buboes (swelling of lymph nodes), ulcers, skin conditions including pimples, scurf, and leprosy; and water distilled from the herb was

used to treat redness and inflammation of the eyes (LeStrange 1977). It was also believed at this time, that a spell cast upon a person could be removed by walking thrice around a yellow toadflax in full bloom and that three seeds strung on a linen thread would protect one from all evil (LeStrange 1977). During the late 1500s, yellow toadflax was a popular garden plant and herbalist John Gerard (1545-1612) referred to it as "a most glorious and goodly flower" (Gerard 2008). A generation later it was recommended by John Rea for flower beds for color and because it required little care (Haughton 1978). Soon after Gerard's and Rea's praises, Geoffery Grigson warned gardeners about "the Devil of a yellow toadflax that crowds out valuable plants" (Haughton 1978). In the 17th century, Sussex farmers called the plant gallwort and added it to the drinking water of poultry to rid them of galls (Culpeper 1802).

Yellow toadflax was introduced to the United States from Wales in the 1600's by the Welsh Quaker, Ranstead (Mitich 1993). Like many present day weedy species, yellow toadflax was introduced as an ornamental garden flower and early settlers were pleased to find the plant already growing in the New World (Haughton 1978). Settlers cultivated it for dyes, made lotions from it to treat insect bites (Mitich 1993), and also boiled it with milk and put it in saucers to poison flies (Haughton 1978).

Historically people have used yellow toadflax for its astringent, detergent, and hepatic principles. More modern science has discovered that yellow toadflax has two glycosides, linarin and pectolinarin, and the plant is still prescribed today to treat jaundice, liver troubles, and various skin conditions (LeStrange 1977).

DISTRIBUTION

Yellow toadflax is native to southeastern Europe and southwestern Asia (Saner et al. 1995) and is naturalized throughout this region (Reed and Hughes 1970). It has been introduced to Japan, Australia, New Zealand, South Africa, Jamaica, Chile, and North America (Zilke 1954). Yellow toadflax is now naturalized throughout the United States, where it can be found in every state with the exception of Hawaii (Reed and Hughes 1970; LeStrange 1977; Lajeunesse 1999; USDA-NRCS 2010) and in every Canadian province (USDA-NRCS 2010) (Figure 1.1). It is a relatively hardy species that can be found at sea level to greater than 3000 m in elevation (Pauchard et al. 2003) with a northern limit between 55 and 65 degrees latitude (Saner et al. 1995).

In North America, yellow toadflax is most common throughout the northeastern states and southeastern Canada, and is localized in other parts of the continent, particularly the western Canadian provinces (Lajeunesse 1999). Its range has been expanding in the Intermountain West, particularly Colorado, Idaho, Montana, and Wyoming (Markin 2002; Beck 2010).

Figure 1.1: Distribution of yellow toadflax in North America and noxious range.

HABITAT

In its region of origin, yellow toadflax is commonly found in vineyards, woodland clearings, and clearcuts (Saner et al. 1995). In central Europe, its preferred substrates are dry to moderately humid sandy loam soils that are moderate to rich in nutrients (nitrogen in particular) and minerals (Saner et al. 1995).

In the United States and Canada, yellow toadflax normally occurs on gravelly or sandy soil along roadsides, railroads, abandoned areas, dry fields, grain fields, gardens, pastures, and other cultivated fields (Reed and Hughes 1970). It is also common on well-drained rocky or gravelly river banks (Lajeunesse 1999). Overgrazed pastures, abandoned areas, and disturbed plant communities are vulnerable to invasion (Arnold 1982); however, yellow toadflax will also invade diverse ecosystems with high species richness (Sutton et al. 2007). Although there is no typical vegetation type or ecosystem

that yellow toadflax prefers to invade, it is limited by shaded areas (Saner et al. 1995) and saturated ecosystems i.e. ponds and marshes (Zilke 1954).

BIOLOGY AND PHENOLOGY

Yellow toadflax is an erect perennial herb that has glabrous to sparsely pilose stems that can range in height from a few cm to over 1.5 m (McGregor et al. 1986; Sutton et al. 2007). Stems are mostly simple, but some branching occurs near the terminal end or when apical dominance has been removed (Lajeunesse 1999). Stems can be solitary or several may be clustered on a taproot. Leaves are linear, ranging from 2.5 to 5 cm long and 2 to 6 mm wide, and are alternate to nearly opposite (Zouhar 2003). Flowers are exhibited in a crowded terminal raceme (Zilke 1954). The corolla is typically zygomorphic, although there are frequent abnormalities that result in peloric flower formations (Saner et al. 1995). Peloric floral formation is explained by the methylation of the *Lcyc* gene in mutants (Cubas et al. 1999; Bird 2007). Petals are 1.0 to 1.8 cm long excluding the spur, which is approximately 7 mm long. Color shades vary, but flowers are generally bright yellow with an orange beard and they originate from the bases of upper leaves (Cronquist et al. 1984). The calyx is deeply 5-parted, lanceolate, and nearly equal (Haddock 2005). Flowering develops in an indeterminate manner where seed pods develop on the lower parts of the stem and flowers continue to develop further up the stalk. Seed pods dehisce upright and exhibit a cup-like shape (Saner et al. 1995).

When temperatures reach 5 to 10 C, ramets begin to grow; this is usually in early to middle April in Canada (Saner et al. 1995). In unpublished data (See Chapter 2), root buds emerged from the soil and transitioned into shoots when soil temperatures were greater than 4 C for a duration of 3 to 4 weeks in late March through April in Colorado

(Nicholas Krick pers. obs.). Vegetative shoots will emerge throughout the growing season, but many do not produce flowers or seed. Flowering can begin in late May and last through late September (Saner et al. 1995), or even into November (Mohlenbrock 1986). The variable phenology of flowering appears to be dependent on environmental factors rather than genetic factors. This is supported by the regular observations of geographically separated yellow toadflax populations for multiple growing seasons. Two yellow toadflax populations exhibited flowering periods different by 1 to 4 weeks from year to year (See Chapter 2). Variable flowering has also been observed by Saner (1994) and McClay (1992). Seed capsules begin to dehisce in September and October; however, flowering may still occur at this time (Saner et al. 1995).

Reproduction by Seed. Yellow toadflax is capable of reproducing sexually by seed and asexually by adventitious root buds. It is an obligate outcrossing species that is fertilized by insects (Bruun 1937; Arnold 1982; Docherty 1982). Darwin (1892) reported that a small portion of seeds developed without cross pollination; however, more recent evaluations found no self-fertilized seed developed among plants examined (Docherty 1982; Franklin et al. 1995; Xue et al. 1996). Self incompatibility within the Plantaginaceae and Scrophulariaceae families has been found to be the result of gametophytic self-incompatibility (Franklin et al. 1995).

Yellow toadflax has conspicuous yellow flowers with bright orange palates (nectar guides) that provide 0.09 µL h^{-1} nectar and attract the major pollinators: bumblebees (*Bombus* spp., *Psithyrus* sp.) and halictid bees (*Dialictus* spp., *Halictus* sp.) (Corbet et al. 1981; Arnold 1982). The flowers have restricted openings that require pollinators to push their way to the spur at the bottom of the homogamous flower to reach

the nectar (Thomson 1986). *Bombus terrestris* (L.) and *Vespula vulgaris* (L.) have been observed cutting holes in the spur of the corolla to rob nectar (Hill 1909; Corbet et al. 1981; Arnold 1982; Saner et al. 1995). In laboratory settings, Knoll (1922 in Saner et al. 1995) described pollination by night active butterflies. Ants have also been observed, in field and garden settings, travelling into and out of flowers (Nicholas Krick pers. obs.). Pollen grains have a strong tendency to be retained by pollinators (Thomson 1986).

The seed of yellow toadflax is brown to black in color, 1.4 to 2.1 mm in diameter, disk shaped and flattened (Saner et al. 1995). The central portion of the seed is tuberculate with a notched chartaceous wing (Cronquist et al. 1984). Seed production per individual is highly variable due to clonal propagation, indeterminate inflorescence (Nadeau and King 1991), and variable flowering phenology (Saner et al. 1995). Authors have reported 10 to 110 seeds per capsule and 1,500 to 30,000 seeds per plant (Saner et al. 1995). A single flowering stem growing in a barley production system produced an average of 5,584 seeds (Zilke 1954) and other researchers counted 210,000 seeds within 0.5 m radius around a parent plant (Nadeau and King 1991). The production of viable seed is also highly variable due to pollinator limitation (Arnold 1982), resource limitation (Clements and Cavers 1990), predation by beetles *Brahypterolus pulicarius* L. and weevils *Gymnetron antirrhini* Payk. (Darwent 1975; Arnold 1982), and possibly from lack of outcross pollen (Saner et al. 1995). Intense competition with other plants also appears to lower or prevent seed production (Sutton et al. 2007).

Germination and Establishment. At maturity, most seeds of yellow toadflax are dormant and can be viable for up to 8 years (Carder 1963). Viability of seeds collected in Alberta was between 40 and 50% based on tetrazolium test and germination ranged from

0.13 to 0.23% (Nadeau and King 1991). Germination success is usually poor and highly variable (Zilke 1954; Lewis 1954; Nadeau and King 1991). A cool, moist period (cold stratification) leads to an increase in gibberellic acid in seeds, and this is likely the physiological mechanism that can break dormancy (Saner et al. 1995). Seeds treated with gibberellic acid increased the success of germination and light was found to have no effect on germination (Saner et al. 1995). However, other studies (Mitchell 1926; Lewis 1954; Nadeau and King 1991) suggest that light stimulates germination of seeds that have not had a cool, moist period.

Germination occurs on the soil surface and to a depth of 3 cm (Korsmo et al. in Saner et al. 1995; Nadeau and King 1991). Seedlings emerge in spring or early summer (Nadeau and King 1991) and some may also emerge in the fall (Lajeunesse 1999). Seedlings are vulnerable to dehydration and competition from other species, particularly areas with good ground cover (Lajeunesse 1999).

Chromosome Counts. There are some conflicting records with respect to chromosome counts in yellow toadflax. In (1927), Tjebbes reported that yellow toadflax was a diploid species where 2n=12. This report is supported by Darlington and Wylie (1955) and Tandon and Bali (1957). However, more a more recent report determined that yellow toadflax is diploid with 2n=24 (Moore 1982). In unpublished results, researchers at Colorado State University determined yellow toadflax plants from Montana had 2n=24 (Dr. Sarah Ward, Colorado State University, Soil and Crop Science, pers. comm.).

Vegetative Reproduction. Yellow toadflax can reproduce vegetatively from the formation of adventitious shoots from both the lateral and tap roots (Salisbury 1942; Bakshi and Coupland 1960). Furthermore, lateral roots retain the capability to develop

secondary roots developing juxtaposition with shoots arising from adventitious buds (Bakshi and Coupland 1960). Morphological studies showed that yellow toadflax produces long roots and short roots, but most of the reproductive buds develop from the short roots (Charlton 1966). The rate of bud formation is highly variable and can be stimulated by exposure to light (Charlton 1966; Saner et al. 1995), but somewhat inhibited by soil disturbance (Bakshi and Coupland 1960). Bud initiation does not occur until some secondary growth has occurred in the parent root (Charlton 1965).

Vegetative reproduction from root buds can occur as early as 2 to 3 weeks after germination (Zilke 1954; Nadeau et al. 1992), and is possible from root fragments as short as 1 cm in length (Nadeau et al. 1992). These buds can grow their own root and shoot systems and become independent plants the following year (Saner et al. 1995). First-year plants can produce 90 to 100 secondary shoots from roots and 200 to 250 shoots by the second season (Salisbury 1942; Zilke 1954).

Rapid patch expansion from vegetative reproduction can occur, but patch expansion can be limited by environment (Lehnhoff et al. 2008). Single clippings (20 cm in length, half root and shoot) spread in one season to a radius of 1 to 2 m and produced 75 to 694 shoots in barley or on fallow land, respectively (Nadeau et al. 1991). A first-year seedling produced a patch with a radius of 1 m and established patches expanded 1.2 m y^{-1} in Saskatchewan (Zilke 1954). The rate and scale of patch expansion is variable and influenced by differences in environment (Lehnhoff et al 2008). In barley and fallow land, average shoot densities of 300 and 700 m^{-2} were observed in mid-summer (Nadeau et al. 1991). Shoot densities were higher in sites or years that had increased precipitation and lower competition (Zilke 1954; Nadeau et al. 1991).

Vegetative propagation of yellow toadflax presumably allows the plant to persist in a wide range of environments including subarctic areas (Staniforth and Scott 1991) or in pastures and orchards where regular fire or herbicide applications occur (Saner et al. 1995). Vegetative propagation is also important for reproduction because seedling establishment is low (Nadeau and King 1991; Nadeau et al. 1992; Saner et al. 1995). Salsibury (1942) summarizes the importance of vegetative reproduction as follows: "The distribution of the species and its local abundance suggest rather poor dispersal but efficient multiplication, once colonization has taken place, and this is probably an example of a species whose maintenance in the northern and western extremes of its range has depended rather on successful vegetative multiplication than upon seed production."

Despite the importance of vegetative reproduction, Ward et al. (2008) sampled 220 individuals from 11 yellow toadflax populations in Colorado, Wyoming, Montana, and North Dakota and found 30.6% genetic variation among populations and 69.4% within populations. These data reveal that there is a high degree of genetic variation within populations, thus supporting the hypothesis that persistence and patch expansion is occurring by seedling recruitment.

Dispersal. Seed dispersal can occur by wind, water, ants, birds and rodents (Ridley 1930; Lewis 1954; Zilke 1954; Häfliger and Brun-Hool 1976). The chartaceous wing on the small disc-shaped seeds may provide a mechanism for wind and water dispersal; however, Nadeau et al. (1991) found that 80% of seeds were dispersed within 0.5 m of the parent plant and very few were collected from 0.5 m to 2 m from the parent plant. Wind dispersal may play an important role during the winter months by dispersing seed

across snow surfaces (Saner et al. 1995). The morphology of the seeds, in addition to an oily surface, allow them to float on water for an extended period of time (Lewis 1954), which supports Zilke's (1954) observations of migrations along water courses. Ants, birds, and rodents that eat yellow toadflax seeds may be responsible for dispersal (Saner et al. 1995). Farming operations are likely an important dispersal mechanism in agricultural land (Zilke 1954; Coupland et al. 1963)

Other dispersal mechanisms observed during field data collection, which are not found in the literature, include "hitchhiking" and "catapulting" (See Chapter 2). Hitchhiking seeds use animals and humans as dispersal agents. Yellow toadflax seeds were observed clinging to shoes, clothing, and skin; particularly the hairy areas of the forearms (Nicholas Krick, Pers. Obs.). It is conceivable that this same mechanism could occur easily on the undersides of large mammals including livestock and wildlife, but no studies have been conducted to support this hypothesis. "Catapulting" refers to a dispersal mechanism that also involves physical interaction with a plant. The seed capsules dehisce in an upward direction and dry seeds are held loosely in the open capsules. When walking through a patch of yellow toadflax, some stalks bend over with contact, and when that pressure is released, the stalks spring back into an upright position, launching seed in a 'catapult-like' fashion.

Hybridization. Yellow toadflax has been found to hybridize with closely related species. In nature, yellow toadflax will hybridize with the exotic striped toadflax (*Linaria repens*) forming hybrid swarms (*Linaria* X *sepium*) (Dillemann 1953; Olsson 1974, 1975; Sutton 1988; Stace 1991 in Saner et al. 1995). Fortunately, only isolated populations of striped toadflax have been recorded in the Northeast of the United States

and no hybrids (*Linaria* X *sepium*) have been recorded (The Biota of North America Program 2010). Hybridization between yellow toadflax and Dalmatian toadflax (*Linaria genistifolia* spp. *dalmatica.)* will occur in nature and under controlled conditions (Ward et al. 2009) and hybrid swarms have been recorded in Montana. Potential impacts including heterosis ("hybrid vigor") have yet to be quantified (Ward et al. 2009); however, preliminary results from a common garden study suggest that hybrids exhibit greater growth and reproductive potential when compared to non-hybrids (Marie Turner, Colorado State University, pers. comm.).

INVASIVENESS

Yellow toadflax meets most of the ideal weed characteristics proposed by Baker (Baker 1974). The plant is able to quickly colonize open sites (large seed production, potential long distance dispersal, variable germination, early vegetative reproduction), adapt its growth form to the site conditions (rapidly spreading roots, shoots branch, all branches flower), and persist (deep roots, perennial habit) (Saner et al. 1995). Exhibiting these factors, yellow toadflax can be a serious problem in cropping systems, recreational areas, and on rangelands where it has a reputation for being difficult to control. It can invade from sea level to over 3000 m and can be found as far north as Edinburgh, Scotland and Fairbanks, Alaska (55 to 65 degrees latitude) (Saner et al. 1995; Pauchard et al. 2003).

Large populations have been observed in Europe where competition was suppressed by fire, grazing, or regular application of non-selective herbicides (Saner et al. 1995). Although yellow toadflax will readily invade disturbed sites such as roadsides, near dwellings, vacant lots, cemeteries, gravel pits, abandoned areas, and overgrazed

pastures (Reed and Hughes 1970; Parker and Peabody 1983), it will also establish in pristine areas on rangeland in excellent condition (Lajeunesse 1999). This is confirmed by Sutton et al. (2007) when surveying Colorado rangelands with high species richness.

Large scale invasion surveys (4,933 quarter sections) were conducted in Saskatchewan in the 1950s. The authors found that there were 57,749 infested hectares; 86% occurring on cultivated ground, 5% occurring on abandoned cultivation, and 8% in natural areas (Coupland et al. 1963). Fourteen of these areas were re-surveyed and these same researchers found that there was an average increase of 47% increase in the number of hectares infested with yellow toadflax, with a range between -67% and +186%. The authors note that there was an increase in precipitation during this time period that also increased the cover of grass species; therefore, the increase in yellow toadflax may have been in response to the precipitation.

According to the USDA Plants Database (2010), yellow toadflax is legally designated as a noxious weed in seven states (Idaho, Montana, Nevada, New Mexico, Oregon, Washington, and Wyoming) and a regulated non-native plant species in one state (South Dakota). However, additional resources (USDA, ARS 2007; Wrage 2009; Colorado Department of Agriculture 2010; North Dakota Department of Agriculture 2010) indicate that yellow toadflax is a noxious weed in South Dakota as well as in Alaska, Colorado, and North Dakota. Distribution of yellow toadflax and where it is considered noxious can be seen in Figure 1.1.

IMPACTS

Ecological. Yellow toadflax threatens natural ecosystems and rangelands (McClay 1992; Lajeunesse 1999). It can also displace existing plant communities and associated animal

life (Lajeunesse 1999). Yellow toadflax is not highly palatable to livestock and is not known to be heavily utilized by any native species including big game. Where sod-forming or bunch grass communities have been replaced by yellow toadflax, soil erosion, surface runoff, and sediment yield can increase; however, in sparsely vegetated sites, yellow toadflax can help stabilize soil (Lajeunesse 1999). In North America, there are no native members of the *Linaria* genus and the threat of genetic pollution is non-existent (The Biota of North America Program 2010).

Economic. Infestations of yellow toadflax can displace desirable vegetation, thereby decreasing the carrying capacity and the appraised value of infested ranch land (Lacey and Olsen 1991). Cattle will occasionally browse flowering shoots of yellow toadflax (Harris and Carder 1971), but they most often avoid the plant. Toxic compounds in the plant include alkaloids (peganin and choline) and iridoid glycosides (antirrhinoside and glycosylaucubin) (Saner et al. 1995; Beninger et al. 2009). The ingestion of these compounds by cattle may be mildly toxic (Mitich 1993); however, they do not negatively impact sheep and goats and yellow toadflax can be used by them as a food source (Walker 1994).

In the mid-20th Century, yellow toadflax became recognized as a significant problem. In 1973 the Alberta Weed Advisory Committee recognized yellow toadflax as the most serious perennial broad-leaved weed in Alberta (Saner et al. 1995). In invaded cropping systems, economic impacts are the result of lower yields. Seed yields from creeping red fescue (*Festuca rubra* L.) were lowered by one-third when yellow toadflax densities were at 180 shoots m^{-2}, but in most sampled areas, densities of 20 shoots m^{-2} decreased yields (Darwent 1975). Yields in canola and wheat were reduced by 20% at

infestation rates of 12 shoots m^{-2} and 74 shoots m^{-2}, respectively (O'Donovan and McClay 1987; O'Donovan and Newman 1987). A survey conducted between 1971 and 1973 in Northwestern Alberta found 4,189 hectares to be moderately to heavily infested (Darwent 1975). In 1987 a study conducted in Alberta, researchers estimated that 28,000 hectares (30% annual crops, 30% forage crops, 20% pastures, 20% non-agricultrual land) were infested with yellow toadflax, which resulted in treatment costs of $360,000 per year (McClay 1987). In the United States, hundreds of thousands of dollars are spent annually in attempts to control yellow toadflax (Hal Pearce, USDA-Forest Service, pers. comm.).

MANAGEMENT

According to Alberta Agriculture (1988 in Saner et al. 1995) the keys to successful control of yellow toadflax are prevention of seed production and "root starvation." The extensive root system of this plant makes it difficult to control (Whitson et al. 2000). Previous attempts to manage yellow toadflax have produced variable results that might be explained by genetic variability (Lajeunesse 1999) or differential environmental factors in invaded systems.

Biological. The classical approach to biological control involves screening natural enemies of a target species found in its native range for effectiveness and host specificity, then releasing the natural enemies on target populations in the invaded range (Harris 1991). The objective of biological control agents is not to eradicate a non-indigenous species, but rather lower the population or reduce its competitive ability.

There are approximately 100 species of arthropods that attack yellow toadflax and several of them have been introduced to North America, accidentally or intentionally

(Lajeunesse 1999). The released species include a defoliating moth (*Calophasia lunula*), an ovary-feeding beetle (*Brachypterolus pulicarius*), two seed capsule-feeding weevils (*Gynmaetron antirrhini* and *Gymnaetron netum*), a stem-boring weevil (*Mecinus janthinus*), and a root-boring moth (*Eteobalea intermediella*) (Lajeunesse 1999).

Two of the biocontrol agents, *B. pulicarius* and *G. antirrhini*, were partially responsible for the decline of yellow toadflax in Canada in the 1950s (Harris and Carder 1971). The larvae of *B. pulicarius* and *G. antirrhini* develop in ovaries and fruits, respectively, where they limit seed production and viability of seed. Attacks on yellow toadflax by *B. pulicarius* reduced seed production by 90% (Harris and Carder 1971) and reduced the number of viable seeds by 74% in addition to lowering germination rates (McClay 1992). Harris (1961 in Saner et al. 1995) concluded that *G. antirrhini* was the most important agent responsible for yellow toadflax control and it appears that *G. netum* has little to no effect.

C. lunula, the leaf-feeding moth, was released in Canada in 1962 (Harris 1963), but failed to establish, which was likely due to intolerance to cold (McClay and Hughes 1995). Harris (1984 in Volenberg et al. 1999) found that *C. lunula* defoliated 20% of yellow toadflax attacked. *C. lunula* is now established on Dalmatian toadflax in Montana (McDermott et al. 1990 in Saner et al. 1995).

Larvae of *Mecinus janthinus* will bore stems of toadflax and adults will feed on shoots (Lohse 1983; Jeanneret and Schroeder 1992 in Saner et al. 1995). *M. janthinus* has been recommended for control of Dalmatian toadflax; however it will also attack yellow toadflax (Lohse 1983; Jeanneret and Schroeder 1992 in Saner et al. 1995). Greenhouse studies showed that *M. janthinus* decreased biomass of Dalmatian toadflax

(Saner et al. 1994). In 2002, *M. janthinus* adults were released on yellow toadflax in the White River National Forest of Colorado and *M. janthinus* larvae were found on the same yellow toadflax population 2 years later (Hal Pearce, USDA-Forest Service, pers. comm.). Established populations of *M. janthinus* were found on yellow toadflax near Orvando, MT in 2009 and transplanted insects from that population have successfully established yellow toadflax populations in Colorado, but the impact of the insects has yet to be determined (Dr. Sarah Ward, Colorado State University, pers. comm.).

Eteobalea serratella is a root-mining moth that has been released to control yellow toadflax in mint production (Volenberg et al. 1999). Volenberg et al. (1999) found that *E. serratella* decreased yellow toadflax root biomass by an average of 20% under controlled conditions. *E. serratella* shortened the flowering period and reduced the seed weights of yellow toadflax, but early feeding on the root crowns increased the number of shoots that subsequently developed (Saner et al. 1994).

There has been some success in decreasing the productivity and reproductive potential of yellow toadflax using biocontrol agents in specific studies; however, long-term and sustainable results have yet to be observed. Although the observed success is promising, there are unintended impacts that should be addressed. *M. janthinus* may attack a California native; *Sairocarpus virga* (Gray), of the Schrophulariaceae (Gassmann 2002 in Sing et al. 2005) and larvae of *C. lunula* can attack desirable ornamentals: *Antirrhinum majus*, *Cymbalaria muralis*, and *Linaria maroccana* (Sing et al. 2005). Also, Saner et al. (1994) found that *E. serratella* increased vegetative development of yellow toadflax and did not decrease its competitive ability. These negative impacts

highlight the importance of proper agent screening. Agents that have negative impacts on desirable species or improve the success of a target pest are certainly not suitable.

Chemical. Herbicides that have been used to control yellow toadflax include 2,4-D (2 ,4-Dichlorophenoxyacetic acid) with dicholorprop, amitrole, chlorthal-dimethyl, chlorsulfuron, dicamba, diflufenzopyr, diquat, fluroxypyr, glyphosate, and picloram (Saner et al. 1995; Baig et al. 1999; Lajeunesse 1999; Lajeunesse et al. 2000). Many herbicide efficacy trials have been conducted in attempt to control yellow toadflax and results to date have been variable, particularly with respect to long-term control (Sebastian and Beck 1989; 1998; 1999; 2001; Lajeunesse 1999).

Fair to good control 1 year after treatment was achieved using high rates of picloram plus fluroxypyr (1.12 kg + 0.89 kg ae ha^{-1}) applied pre-bloom (Sebastian and Beck 1989); however, others have had limited or no success with these herbicides (Ferrell and Whitson 1989; Hansen et al. 1989 in Lajeunesse 1999). Glyphosate applied early bloom at 1, 2, and 4 kg ae ha^{-1} provided 40, 70, and 90% current season control in barley, but resurgence occurred the following spring (Saner et al. 1995). Baig et al. (1999) found that pre-harvest application of glyphosate reduced yellow toadflax by more than 80% in barley, canola, and flax fields in Alberta. Other herbicides including, , 2,4-D, MCPA, 2,4DB, fluroxypyr, MCPB, mecoprop, metsulfuron, and triclopyr have been tested for control of yellow toadflax, but found to be ineffective (Saner et al. 1995).

Among many reported studies, chlorsulfuron and picloram have been the most effective herbicides for controlling yellow toadflax, but at high rates picloram can be injurious to desirable vegetation (Lajeunesse 1999; Sebastian and Beck 2001) and long term control has not been achieved. Research conducted by Colorado State University

Weed Science between 2007 and 2009 found that picloram + diflufenzopyr + dicamba (560 + 56 + 140 g ae ha^{-1}) controlled 97 and 98% of yellow toadflax 1 YAT and 2 YAT respectively (Sebastian and Beck 2009). Picloram alone was used in this study, but only controlled 63 and 53% of yellow toadflax with 560 g ae ha^{-1}1 YAT and 2 YAT respectively. Picloram alone at 1.12 kg ae ha^{-1} controlled 70 and 68% of yellow toadflax 1 YAT and 2 YAT respectively. These results indicate synergism with the tank mix and suggest that picloram rates can be decreased to avoid damage to desirable species. Imazapyr + metsulfuron + 2,4-D (560 + 168 + 560 g ae ha^{-1}) provided 95, 94, and 88% control of yellow toadflax 1, 2, and 3 YAT, respectively (Sebastian and Beck 2006). In the same study, metsulfuron + 2, 4-D (168 + 560 g ae ha^{-1}) controlled only 21, 31, and 33% yellow toadflax control 1, 2, and 3 YAT, respectively.

In 2001 Sebastian and Beck showed that timing of treatment improved long term control of yellow toadflax. Chlorsulfuron (88 g ae ha^{-1}) applied in September (post-bloom growth stage) controlled 50% of yellow toadflax 2 YAT and the same rate of chlorsulfuron applied in August (early-bloom) only controlled 9% of yellow toadflax (Sebastian and Beck 2001).

As demonstrated by previous studies, short-term control of yellow toadflax can be achieved, but until recently long term control had been unsuccessful using herbicides. Difficulty with long-term control may be attributed to the perennial habit of the plant, its expansive root system, and large seed banks (Whitson et al. 2000). It may be necessary to treat infestations every 3 to 4 years up to 12 years to achieve complete eradication of the seed bank and root reserves (Lajeunesse 1999).

Cultural and Physical. With large creeping root systems, mature and large stands of yellow toadflax are not good candidates for control by mechanical means; however, there has been some success on large scales (Morishita 1991; Baig et al. 1994). Hand pulling small, localized infestations can be effective if conducted on a regular basis and over an extended period of time and success is increased in sandy or moist soils where more of the root system can be excavated (Lajeunesse 1999). Lajeunesse (1999) suggests that 5 to 6 years of hand pulling is necessary to deplete root reserves and 10 to 15 years of monitoring will be necessary to remove seedlings that emerge from the seed bank; however, these suggestions are not supported by scientific studies.

Tillage has been successfully implemented to control yellow toadflax in cropping systems (Morishita 1991; Baig et al. 1994). It is recommended that tillage begin on summer fallow in June and cultivations should be conducted every 3 to 4 weeks, then after planting, cultivation should be shallow to avoid spreading root fragments (Darwent 1975). In the fall, tillage should be implemented right after harvest and be conducted every 3 to 4 weeks until top growth is killed by frost (Saner et al. 1995).

Non-intensive tillage is not recommended because yellow toadflax will readily re-sprout from root fragments (Nadeau et al. 1992) and the act of tillage may in fact increase the spread of a population. Tillage practices are also not practical for rangeland systems. In cropping systems it can increase erosion and can be responsible for dispersing yellow toadflax when equipment is used on multiple fields (Saner et al. 1995).

Grasses have been found to compete well with yellow toadflax (Carder 1963; Nadeau et al. 1991). Responsible grazing and reducing disturbance in grass stands will keep them healthy and competitive with yellow toadflax. Disturbed sites can be seeded

with vigorous, hardy, and well adapted grasses (Lajeunesse et al. 1993). Established grass communities may also inhibit the establishment of new seedlings (Lajeunesse 1999).

Fertilization with potassium at 336 or 672 kg ha^{-1} for 6 years resulted in a decrease in density from 32 plants m^{-2} in control plots to 11 or 9 plants m^{-2}. However, fertilization with nitrogen at 336 kg ha^{-1} caused an increase in density from 8 plants m^{-2} in the control to 44 plants m^{-2} (Allured et al. 1974 in Saner et al. 1995).

Burning yellow toadflax is not a recommended control measure (Lajeunesse et al. 1993). Fire can destroy shoots, but underground buds and root systems that can reach 1 m in depth will likely be unharmed. Fire may actually stimulate populations by releasing nutrients and removing susceptible competition.

Integrated Management. Yellow toadflax has proven to be a challenging plant to manage. This is likely driven by its plastic nature, extensive creeping root system, perennial habit, and a high degree of genetic variation (Zilke 1954; Saner et al. 1995; Lajeunesse 1999; Ward et al. 2008). Successful management will implement a combination of two or more management techniques and management should be focused on preventing vegetative spread and reducing seed production (Lajeunesse 1999).

CONCLUSION

Yellow toadflax has a long history with humans where it has been appreciated and despised. It is a well adapted plant with a highly plastic nature, capable of inhabiting a wide range of ecosystems. It has many characteristics that lead to successful establishment, dispersal, and persistence. Cropping systems, rangelands, and natural areas are threatened by this plant that has worldwide distribution. It is capable of

decreasing crop yields, competing with desirable forage, and disrupting the balance of natural ecosystems. In most areas of the United States, yellow toadflax is a naturalized plant that can be admired for its showy flowers and populations rarely provoke management. However, in the Intermountain West it is a serious problem. Its range is expanding in Colorado, Idaho, Montana, and Wyoming. Attempts to control this plant have revealed inconsistent results. A number of biological, chemical, and cultural control methods have been implemented, but results from these methods have not provided consistent recommendations for successful long term control.

Managers of yellow toadflax face challenges of genetic variation and environmental variation. It is suspected that multiple introductions to North America have occurred. As an obligate outcrossing species, yellow toadflax exhibits a high degree of genetic variation within and among sites and each generation is a new mixing of genes capable of expressing desirable traits for fitness. There is a wide spectrum of environments throughout Colorado and the state is host to USDA Plant Hardiness Zones 3 to 7. In the east, there are grass prairies and large cropping systems. In the central part of the state there are rugged mountains that reach elevations over 4,000 m. Farther west in Colorado, there is desert rangeland that receives very little precipitation. Yellow toadflax has invaded ecosystems throughout this spectrum, but tends to be most problematic in the mountainous regions.

Weed researchers and weed managers have experienced variable responses to control practices for more than 20 years in Colorado. The source of variation is likely driven by genetics and environment; however, the source of this variation has yet to be determined. Colorado's wide spectrum of invaded ecosystems provides a great

opportunity to learn about geographically separated populations and determine whether managers are facing genetic challenges or environmental challenges. With a better understanding of the source driving the variable response to control, researchers and managers can modify their management practices to overcome the previously observed variation. More recent studies conducted by CSU Weed Science show promise with respect to long term control; however, there is still site to site variation that has yet to be quantified.

The objectives of this research are to better understand the source that is responsible for site to site variation and to determine whether this variation can be overcome with better management practices. Based on studies conducted by CSU Weed Science, chlorsulfuron and imazapyr have provided promise for long term control of yellow toadflax and control has been more effective with fall treatments when compared to summer treatments. This knowledge serves as the basis for conducting identical field studies at five sites in Colorado with chlorsulfuron and imazapyr. The response of geographically separated field sites under identical experiments was supported by a common garden study and an ALS enzyme bioassay. Results from these studies have provided a better understanding of the source of variation. In addition to the field trials and the common garden, an observational study of root bud phenology was conducted. The combined results from these studies indicate that environment is influencing response and spatial variation can be overcome with better management practices.

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Chapter 2: Temporal Changes in Yellow Toadflax Root Bud Phenology

ABSTRACT

Yellow toadflax is an aggressive perennial forb that threatens cropping systems, natural areas, and rangelands in North America. It is an obligate outcrossing species that reproduces by seed, but more importantly by vegetative means. Adventitious shoot buds that develop on the roots are key reproductive structures and allow this species to survive in temperate climates. An observational study was conducted between January 2009 and December 2010 where the developmental patterns of yellow toadflax root buds were monitored over time. The study showed that the majority of buds develop on the "root crown," but buds will also develop to a lesser extent on lateral roots. Root bud development exhibits a pattern related to other plant growth stages. During the dormant growth stage (fall and winter months), bud counts are highest. Bud dormancy break occurred when soil temperatures were above 4 C for 3 to 4 weeks. Following bud break, bud counts decreased until majority of the population was in full bloom to post bloom growth stage. Following the full bloom growth stage, bud counts increased. Tracking root bud phenology has led to a better understanding of root bud development patterns throughout the season and how other growth stages; i.e. bud break, flowering, seed set, and senescence are related to this pattern. When comparing this observational study to

herbicide efficacy trials, it appears that making herbicide applications when bud numbers are increasing might be a key component for achieving maximum success. Additional biological characteristics were also recorded in this observational study.

INTRODUCTION

Yellow toadflax (*Linaria vulgaris* Mill.) is an exotic perennial forb that was introduced from Eurasia into the United States during the 1600's by a Welsh Quaker (Mitich 1993). Today, this plant has a worldwide distribution where it threatens cropping systems, natural areas, and rangelands in its non-native range (Reed and Hughes 1970; Saner et al. 1995). Yellow toadflax is naturalized throughout the United States (USDA-NRCS 2010). It is most common throughout the northeastern states, but more localized in other parts of the country (Lajeunesse 1999). Its range is expanding in the Intermountain West, particularly Colorado, Idaho, Montana, and Wyoming (Markin 2002; Wrage 2009; Beck 2010); and has become a serious problem in this region. In the eastern states and throughout most of the Midwest, yellow toadflax is common along roadsides, ditches, railroads, and other abandoned areas (Reed and Hughes 1970; Lajeunesse 1999), but these populations rarely justify management. In the Intermountain West, populations are expanding and hundreds of thousands of dollars are spent annually managing this plant (Hal Pearce, USDA Forest Service Pers. Comm.).

Yellow toadflax is a clonal species that reproduces by seed and vegetatively from adventitious buds that develop on an extensive creeping root system (Nadeau and King 1991; Nadeau et al. 1991; 1992). It is an obligate outcrossing species and seed production can be as high as 30,000 seeds per plant; however, seed viability in many populations is less than 50% and typically around 10% (Nadeau and King 1991; Nadeau

et al. 1991; Saner et al. 1995). Adventitious shoot buds (hereinafter referred to as root buds) develop on crowns and roots systems and are important for perennial species with clonal growth habit (Coupland and Alex 1955; Eliasson 1961; Nadeau and King 1991; Nadeau et al. 1992; Lenssen et al. 2004). Root buds are responsible for dormancy-imposed inhibition of new shoot growth and is one of the key characteristics leading to persistence of perennial weeds (Coupland et al. 1955). Little is known about the molecular mechanisms that control dormancy and growth of root buds of perennial plants (Horvath et al. 2002). However, some investigations of root bud development have been conducted on leafy spurge (*Euphorbia esula* L.); another introduced weedy species that has a growth habit similar to that of yellow toadflax (Nissen and Foley 1987; Hovath 1999; Horvath et al. 2002). These studies revealed that correlative inhibition of underground buds is maintained by at least two signals: one produced in the shoot apices and the other in mature leaves (Horvath 1999). The signal from shoot apices derives from young tissues and is likely auxin (Horvath 1998). The second signal is derived from mature leaves, requires photosynthesis for production and transport, and can be overcome by exogenous application of gibberellic acid (GA) (Horvath 1999). The second signal is likely a sugar. As little as 30 mM sucrose or glucose can inhibit underground adventitious bud growth through a mechanism that is not reversible by exogenous GA applications (Chao et al. 2006).

Root buds are important adaptations that permit species like yellow toadflax to survive dynamic environmental conditions including temperate fluctuations, fire, flooding, and intense grazing (Nadeau and King 1991; Nadeau et al. 1992; Lenssen et al. 2004). Root buds are important for vegetative reproduction and environmental

persistence (Coupland and Alex 1955; Eliasson 1961; Raju et al. 1964; McIntyre 1972; 1979; Budd 1973). Morphological studies showed that yellow toadflax produces long roots and short roots, and most of the reproductive buds develop from the short roots (Charlton 1966). The rate of bud formation is highly variable, can be stimulated by exposure to light (Charlton 1966; Saner et al. 1995), but somewhat inhibited by soil disturbance (Bakshi and Coupland 1960). Root bud initiation does not occur until some secondary growth has occurred in the parent root (Charlton 1965). Plants respond to stimuli in their environment that results in physiological changes. Observing and quantifying developmental patterns of root buds over time should provide a better understanding of external factors that yellow toadflax plants respond to and their subsequent relationships to bud development.

The objective of this observational study was to track the root bud phenology of yellow toadflax populations over time and determine whether a better understanding of the phenology might improve management practices. Genetic differences and environmental differences may be responsible for the previously observed variable response to control measures. Determining and quantifying genetic differences is impractical for land managers; however, understanding environmental factors is more attainable and more practical. A better understanding of root bud phenology and how this phenology develops in relationship to observable plant characteristics and environmental factors may provide land managers with practical methods to improve management.

MATERIALS AND METHODS

Study Sites. Two sites were selected in Colorado to track root bud development of yellow toadflax populations over time. The first site, Milliken, is on private property in Weld County (40°19'12.55"N, 104°48'43.50"W) 2.5 miles south and east of the town of Milliken along the South Platte River. This property is used for recreational purposes and land managers do not target yellow toadflax for control. Yellow toadflax was the dominant species at this site and there was low species richness. Three species coexisted in the sampling area: common ragweed (*Ambrosia artemisiifolia* L.), wild licorice (*Glycyrrhiza lepidota* Pursh), and field bindweed (*Convovulus arvensis* L.). These species made up less than 10% of the foliar cover and yellow toadflax was 75 to 90% foliar cover in the sampled area. Other species in the surrounding area include poison hemlock (*Conium maculatum* L.), common teasel (*Dipsacus fullonum* L.), and willow (*Salix* spp.).

The second site, Greenland, is in Douglas County (39°14’91” N, 104°86’07”W) 4 miles north of the town of Monument on open space land managed by Douglas County. There was greater species richness at this site including other weedy species. Other species in the sampling area included bluegrass (*Poa* spp.), diffuse knapweed (*Centaurea diffusa Lam.*), musk thistle (*Carduus nutans L.*), Colorado rush (*Juncus confusus* Coville), Louisiana sage (*Artemisia ludoviciana Nutt.*), western yarrow (*Achillea millefolium L.*), tufed hairgrass (*Deschampsia cespitosa (L.) P. Beauv.*), needle grass (*Stipa* spp.), wheatgrass (*Agropyron* spp.), cinquefoil (*Potentilla* spp.), common mullein (*Verbascum thapsus L.*), and white heath aster (*Symphyotrichum ericoides L.*). Yellow

toadflax made up 40 to 60% foliar cover in the sampled area. Additional site characteristics for both locations are displayed in Table 2.1.

Data Collection and Analysis. Beginning in January of 2009 each site was visited twice monthly. During each visit, soil temperature was recorded at a depth of 8 cm (Table 2.2) and 15 randomly selected root segments, 10 to 15 cm in length were excavated and brought back to the Colorado State University Weed Research Lab. Soil was washed from the root segments and all root buds were counted, measured, and recorded. Root segment harvests occurred from January 2009 into December 2010. In addition to root bud phenology, the phenology of bud break (buds emerging from the soil and transitioning into shoots), flowering, seed set, and senescence were also monitored. A time point was recorded when 90% or more of harvested root segments exhibited these growth stages (Figure 2.1). Precipitation data and average air temperature were also collected from weather stations near the harvest sites (Table 2.2). For each sampling period, root bud numbers were counted and measured. The average number of buds per 15 cm root segment was calculated for each collection period and that number was graphed with soil temperature on the same scale (Figure 2.1). Each site was graphed separately by year. Average root bud numbers were determined and standard errors were calculated for each month during the study (Table 2.3).

RESULTS AND DISCUSSION

Each site had similar root bud development patterns with respect to seasons and soil temperatures (Table 2.3). Root bud development patterns were also related to other phenological patterns: bud break, flowering, seed set, and senescence. Root buds were present on the root crowns and lateral roots of yellow toadflax throughout the year, but

the number and length of buds fluctuated. Some buds may transition into shoots as early as February in Colorado, when soil temperatures are greater than 0 C; however, very few buds emerged during these colder periods. Bud counts declined following bud break and reached their lowest counts during full bloom growth stage. Following full bloom, bud counts increased and peaked following seed set, and then maintained more consistent levels during the dormant season.

Bud Break. In 2009 at the Milliken site, very few buds had transitioned into shoots in early February when soil temperatures were between 0 and 4 C (Figure 2.1). When soil temperatures were greater than 4 C for a duration of 3 to 4 weeks, >90% of harvested root segments had a least one root bud that had transitioned into a shoot. At the end of March >90% of harvested root segments were exhibiting bud break. At the Greenland site in 2009, the soil did not warm up as quickly and >90% of harvested root segments contained at least one transitioned root bud by the middle of April. At the Milliken site in the middle of March 2010, when soil temperatures exceeded 4 C for a period of 3 to 4 weeks, >90% of harvested root segments had at least one root bud that had transitioned into a shoot. This pattern occurred 1 to 2 weeks later at the Greenland site in 2010 where the soil did not warm up as quickly. As the majority of plants in these populations transitioned from the bud growth stage to the vegetative growth stage, average root buds numbers decreased.

Flowering. In early June of 2009, the Milliken population began to flower and by the end of June >90% of harvested root segments had at least one flowering shoot. In 2009, flowering did not begin until July at the Greenland site and by late July >90% of harvested root crowns exhibited flowering shoots. In 2010, the Milliken population

began to flower at the end of June and >90% of harvested root segments had flowering shoots in late July. The Greenland population in 2010 did not reach peak flower until late August. As the majority of plants in these populations transitioned to the flowering growth stage, average bud numbers continued to decrease.

Seed Set and Senescence. By late July 2009, seed pods were developing at the Milliken population and in early August >90% of harvested root segments exhibited shoots setting seed. Seed set continued into September when the plants began to senesce. At the Greenland site in 2009, >90% of harvested root segments exhibited shoots that were setting seed in late August and by mid September >90% of harvested root segments had shoots that were starting to senesce. In 2010 at the Milliken site, seed set began in the middle of July and reached its peak in early September. By late September, >90% of harvested shoots were beginning to senesce. At the Greenland site in 2010, >90% of harvested root segments exhibited shoots setting seed in the middle of September and by the middle of October, >90% of harvested root segments from the Greenland population had shoots that were senescing. The common trend observed at each site and for each year was that average bud numbers reached their lowest counts when plants were transitioning from flowering into seed set. Following this transition, bud numbers began to increase. The Milliken site exhibited a less dramatic pattern than the Greenland site. After bud numbers reach their lowest counts at Milliken, they continued to increase for about 16 weeks. The Greenland site showed a more dramatic increase in bud numbers when they peaked 4 to 5 weeks following their lowest counts.

Tracking the phenology of root bud development over time has provided a better understanding of how root buds develop with respect to other aspects of yellow toadflax

phenology. This study revealed that yellow toadflax has root buds present throughout the year, but the numbers of buds fluctuate over time. The pattern of root bud development was similar between 2009 and 2010. Root bud counts were highest from late fall to early spring and reached their lowest counts during late bloom and seed set. The relative number of root buds can be predicted based on the growth stage of a population.

Flowers and developing seeds are demanding sinks and require large amounts of assimilate (Kigel and Galili 1995). Root buds also serve as resource sinks (Davis and Haissig 1994; Gesch et al. 2007). Root buds of leafy spurge (*Euphorbia esula* L.) serve as sinks for photosynthate in late summer and autumn (Gesch et al. 2007) and it is likely that root buds of yellow toadflax also serve as sinks during this time period. Russian knapweed (*Acroptilon repens* (L.) DC.) is another perennial weed species that develops root buds and root carbohydrate reserves of this species peak after the growing season and are lowest when plants bloom (Jacobs and Denny 2006). Canada thistle (*Cirsium arvense* (L.) Scop.), an aggressive creeping perennial weed, develops root buds that are more abundant during fall and winter (McAllister and Haderlie 1985). It is likely that bud numbers reach their lowest counts during late bloom and seed set growth stage because plants are partitioning resources to the developing seeds and not to the root system. When seeds are ripening, it is likely that yellow toadflax transitions to another growth stage, i.e., root bud initiation, and partitions more assimilate to the root system.

Previous research has shown that fall application of herbicides is a good time to apply to control perennial species (Donald 1992; Lym and Zollinger 2000). During the fall, plants are in a period of senescence and theoretically, applied herbicides should have a better chance of being translocated into the root system. Post bloom may well be the

best time to make herbicide applications; yellow toadflax plants are moving assimilate to the root systems, but have not yet progressed far enough into senescence where foliar applied herbicide absorption and translocation would be affected. In (2001), Sebastian and Beck showed that specifically timed treatments improved long term yellow toadflax control. Chlorsulfuron (88 g ae ha^{-1}) applied in September (post flower) controlled 50% of yellow toadflax 2 YAT and the same rate of chlorsulfuron applied in August (early flower) provided only 9% control of yellow toadflax (Sebastian and Beck 2001) at the same time interval. These results show the benefit of making applications at a more advanced growth stage.

The above ground growth stage of yellow toadflax is easily recognized and has a relationship to root bud development. When comparing each site over years, there were time differences when flowering occurred, but post bloom was consistent with initiation of increased root bud development. Land managers can use this information to target treatments to a particular time period to achieve greater success. Timing applications based on observable yellow toadflax phenology can improve control. Additionally, economic and ecological costs can be minimized by making fewer applications.

Additional Biological Characteristics

While conducting this study, additional observations of yellow toadflax were made with respect to seed dispersal. The literature does not reference the dispersal mechanisms: "hitch-hiking" and "catapulting." Hitchhiking seeds use animals and humans as dispersal agents. Yellow toadflax seeds were observed clinging to shoes, clothing, and skin; particularly the hairy areas of the forearms (Nicholas Krick, Pers. Obs.). It is conceivable that this same mechanism could occur easily on the undersides of

large mammals including livestock and wildlife, but no studies have been conducted to support this hypothesis. "Catapulting" refers to a dispersal mechanism that also involves physical interaction with a plant. The seed capsules dehisce in an upward direction and dry seeds are held loosely in the open capsules. When walking through a patch of yellow toadflax, some stalks bend over with contact, and when that pressure is released, the stalks spring back into an upright position, launching seed in a 'catapult-like' fashion.

Table 2.1: Site Characteristics for two root bud harvest sites in Colorado.

	----------paste----------				----------ppm----------							----------%----------				
Site	pH	EC	Lime Estimate	% OM	NO_3-N	P	K	Zn	Fe	Mn	Cu	Sand	Silt	Clay	Texture	Ecoregion[a]
Greenland	6.2	0.2	Medium	7.0	2.5	5.6	238	3.4	49.4	8.6	2.2	63	25	12	Sandy Loam	Pine Oak Woodlands
Milliken	8.0	3.1	Low	1.9	5.7	35.5	171	15.8	47.6	6.7	13.5	69	18	13	Sandy Loam	Flat to Rolling Plains

[a] Ecoregions defined by Chapman et al. (2006).

Table 2.2: Environmental data for two sites in Colorado where root buds of yellow toadflax were harvested.

	Milliken						Greenland					
	2009			2010			2009			2010		
	Soil Temp[a]	Air Temp[b]	Precip[c]	Soil Temp	Air Temp	Precip	Soil Temp	Air Temp	Precip	Soil Temp	Air Temp	Precip
January	2	-1	0.0	0	-4	0.2	-1	0	1.3	0	-1	0.2
February	3	2	0.0	0	-2	0.5	0	2	0.7	0	-4	2.5
March	4	4	0.2	3	5	1.1	5	4	1.8	2	3	5.0
April	8	7	6.0	8	9	8.4	5	6	8.1	8	7	8.1
May	14	15	2.9	12	13	5.8	13	12	5.0	12	11	41.2
June	20	18	5.1	20	21	5.7	16	15	8.1	19	18	4.1
July	23	21	7.5	20	23	0.8	18	18	14.7	23	19	9.2
August	22	21	2.9	22	23	2.5	18	18	2.8	23	19	4.5
September	18	17	1.8	25	18	0.1	17	13	4.8	19	17	0.1
October	9	6	2.1	16	12	1.0	7	4	5.1	11	10	1.8
November	3	3	0.5	3	2	1.4	3	4	1.5	3	3	1.4
December	0	-7	0.2	1	0	0.0	-2	-5	1.3	1	1	0.3

[a] Average soil temperature (C) for each month recorded at a depth of 8 cm.

[b] Average air temperature (C) collected from nearest weather station.

[c] Average monthly precipitation (cm) collected from nearest weather station.

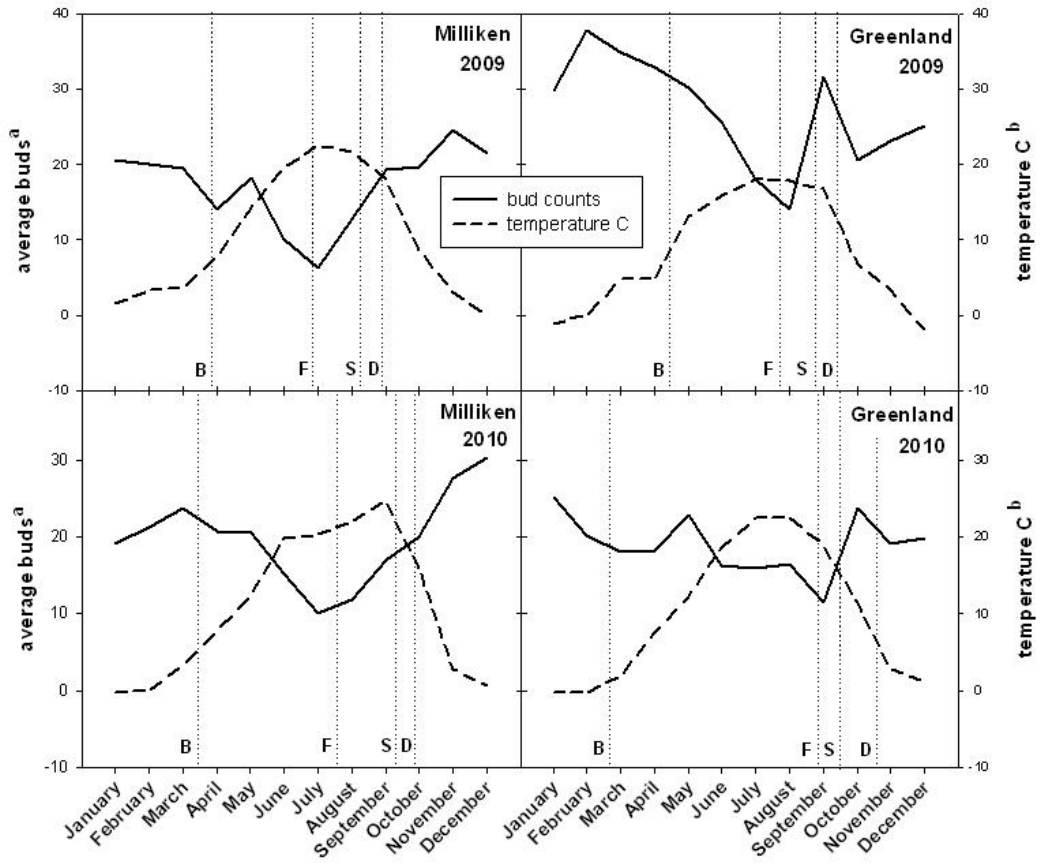

Figure 2.1: Average number of root buds per 15 cm root segment of yellow toadflax and soil temperature at Milliken and Greenland harvested in 2009 and 2010.

[a]Average number of root buds per 15 cm root segment represented by solid line.

[b]Average soil temperature in C at time of harvest represented by dashed line.

Vertical dotted lines are represented by the majority of the population exhibiting: **B**: bud break; **F**: flowering, **S**: seed set; **D**: senescence.

Table 2.3: Average number of root buds per 15 cm root segment of yellow toadflax with standard errors for each month for 2 years.

	Milliken				Greenland			
	2009		2010		2009		2010	
	Root Buds[a]	Standard Error[b]	Root Buds[a]	Standard Error[b]	Root Buds[a]	Standard Error[b]	Root Buds[a]	Standard Error[b]
January	21	1.63	19	1.68	30	1.95	25	1.58
February	20	1.49	21	1.84	38	2.01	20	1.49
March	20	1.9	24	2.33	35	1.89	18	1.83
April	14	1.33	21	1.27	33	2.31	18	1.49
May	18	1.64	21	1.85	30	2.1	23	2.58
June	10	1.02	15	1.69	26	2.57	16	1.68
July	6	0.82	10	0.99	18	1.78	16	1.47
August	13	1.58	12	0.93	14	1.7	17	1.06
September	19	1.76	17	1.39	32	2.42	12	1.62
October	20	1.39	20	2.09	21	1.73	24	2.24
November	25	2.14	28	2.48	23	1.77	19	1.41
December	22	1.59	30	2.31	25	1.86	20	3.48

[a] Average number of root buds per 15 cm root segment of yellow toadflax for each month.

[b] Standard error calculated from the average number of root buds for each month.

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Chapter 3: Geographically Separated Yellow Toadflax Populations Response to ALS Inhibiting Herbicides.

ABSTRACT

Yellow toadflax is an introduced creeping perennial forb that is problematic in the Intermountain West. Unexplainable site to site variation in control efficacy has been observed over more than 20 years of field research and consistent recommendations for control of yellow toadflax are not available. Yellow toadflax expresses significant genetic variation and can inhabit a wide range of ecosystems. The influence that site characteristics and genetic variation have on control has yet to be identified. Identical herbicide efficacy trials were conducted at five discrete yellow toadflax populations using four rates of two herbicides. Plants were harvested from these sites for evaluation in a common garden study and an ALS enzyme bioassay. One year after treatment (YAT) chlorsulfuron applied at 94 g ae ha^{-1} resulted 76% or higher yellow toadflax control at all sites; while, 380 g ae ha^{-1} of imazapyr was necessary to provide consistent control($\geq$73%) at four of five sites. Evaluations 2 YAT showed that yellow toadflax recovered at two sites. It appears that recovery was largely driven by length of growing season and the growth stage when herbicide treatments were applied. Lower elevation sites and sites with a higher percentage of flowering shoots at the time of application were more difficult to control. The common garden study and ALS enzyme bioassay revealed that

these populations were susceptible to these herbicides on a whole plant level and on a mechanistic level, confirming that herbicide resistance is not responsible for spatial variation in control. Managers can improve their success by increasing herbicide rate and treating infestations at their most susceptible growth stage. Land managers that use what we have learned will improve control, apply less herbicide, minimize treatment costs and minimize environmental impacts.

INTRODUCTION

Yellow toadflax (*Linaria vulgaris* Mill.) is an exotic perennial forb that was introduced from Eurasia into the United States during the 1600's by a Welsh Quaker (Mitich 1993). Today, this plant has a worldwide distribution where it threatens cropping systems, natural areas, and rangelands in its non-native range (Reed and Hughes 1970; Saner et al. 1995). Yellow toadflax is naturalized throughout the United States (USDA-NRCS 2010). It is most common throughout the northeastern states, but more localized in other parts of the country (Lajeunesse 1999). Its range is expanding in the Intermountain West, particularly Colorado, Idaho, Montana, South Dakota, and Wyoming (Markin 2002; Wrage 2009; Beck 2010); and has become a serious problem in this region. In the eastern states and throughout most of the Midwest, yellow toadflax is common along roadsides, ditches, railroads, and other abandoned areas (Reed and Hughes 1970; Lajeunesse 1999), but these populations rarely justify management. In the Intermountain West (roughly, the region between the Rocky Mountains and the Sierra NevadaMountains), populations are expanding with annual management costs exceeding $300,000 (Hal Pearce, USDA-Forest Service Pers. Comm.).

Yellow toadflax is a clonal species that reproduces by seed and vegetatively from adventitious shoot buds that develop on an extensive creeping root system (Nadeau and King 1991; Nadeau et al. 1991; 1992). It is an obligate outcrossing species and seed production can be as high as 30,000 seeds per plant; however, seed viability in many populations is less than 50% and often around 10% (Nadeau and King 1991; Nadeau et al. 1991; Saner et al. 1995). Vegetative reproduction is an important mode of propagation at high latitudes and for long-term persistence (Nadeau et al. 1991; Nadeau et al. 1992). Yellow toadflax meets many of the ideal weed characteristics proposed by Baker (1974): the plant is able to quickly colonize open sites (large seed production, potential long distance dispersal, variable germination, early vegetative reproduction), adapt its growth form to the site conditions (rapidly spreading roots, shoots branch, all branches flower), and persists (deep roots, perennial habit) (Saner et al. 1995). Because of these factors, yellow toadflax can be a serious problem on rangelands where it has a reputation for being difficult to control.

Yellow toadflax's growth habit and obligate outcrossing nature result in various phenotypes and genotypes. In the Intermountain West, managers encounter genetic differences among yellow toadflax populations and a wide spectrum of environmental variation where this plant is found. Ward et al. (2008) sampled 220 individuals from 11 yellow toadflax populations in Colorado, Wyoming, Montana, and North Dakota. Using inter simple sequence repeat's (ISSR's), the authors found 30.6% genetic variation among populations and 69.4% within populations (Ward et al. 2008). Of the 220 plants sampled, 216 had unique genotypes (Ward et al. 2008). These results quantify the degree of genetic variation in the studied populations and suggest multiple introductions;

however, further investigation is needed to determine how these differences may influence response to control measures.

The state of Colorado exists in the Intermountain West and is host to many unique environments. Colorado has 6 level III ecoregions which are further divided into 35 level IV ecoregions (Chapman et al. 2006). Large cropping systems and perennial grasslands can be found in eastern Colorado. In the central part of the state there are rugged mountains that reach elevations greater than 4,000 m and on the western border there is desert. Yellow toadflax has successfully invaded plant communities across this range of ecosystems in Colorado, but it is generally most problematic in the mountainous regions.

More than 20 years of field research on yellow toadflax conducted by Colorado State University Weed Scientists and land managers in Colorado have provided no consistent control strategies for yellow toadflax (Sebastian and Beck 1989; 1998; 1999; 2001; 2006). In other locations in North America, biological, chemical, and cultural techniques have been used to manage this plant and results have also been inconsistent (Saner et al. 1995). One hypothesis is that variable response to control is driven by genetic differences and environmental differences, but the source of the variation has yet to be determined.

Based on studies conducted by CSU Weed Scientists, chlorsulfuron, imazapyr, and picloram have provided good yellow toadflax control 1 YAT and control has been more effective with fall treatments when compared to summer treatments (Sebastian and Beck 2001; 2006; Daniel et al. 2010). The objectives of this study were to determine whether genetic or environmental factors impact yellow toadflax's response to control measures. Understanding the source of the variation, the secondary objective was to

determine whether the spatial variation (variable response to control measures among geographically separated populations) could be overcome by changing management practices.

MATERIALS AND METHODS

Field Experiments.

Study Sites. Five study sites were selected in Colorado to conduct identical field experiments (Figure 3.1). These geographically separated sites are on publically managed rangeland or open space. One site (Camp Hale; 39°42'57"N, 106°30'93"W) is in Eagle County and another site (White River; 40° 0'31.06"N 107°25'53.83"W) is in Rio Blanco County. Both of these sites are on the White River National Forest, a multi-use, federally managed land. Another site (Hot Sulphur; 40°03'85"N, 106°17'74"W) is in Grand County and the fourth site (Wildcat Canyon; 37°24'47"N, 107°93'78"W) is in La Plata County . These latter two sites are on state land managed by the Colorado Division of Wildlife. The fifth site (Greenland) is in Douglas County (39°14'91" N, 104°86'07"W) on open space managed by the County. Each site is unique and can be differentiated from the other sites based on a number of characteristics depicted in Table 3.1.

Treatments and Experimental Design. At each location, identical studies were conducted from September 2008 through September 2010. Experiments at each site included two herbicides at four rates and were designed as a randomized complete blocks with four replications. Plots were 3 m x 9.1 m (10 ft. x 30 ft.). Treatments were: chlorsulfuron (Telar[1]) at 0, 40, 64, and 94 g ae ha^{-1}(0, 0.75, 1.25, and 1.75 oz product $ac^{-1)}$ and imazapyr (Arsenal/Habitat[2]) at 0, 127, 253, and 380 g ae ha^{-1}(0, 0.5, 1.0, and 1.5 pt

product ac^{-1}). Crop oil concentrate at 1% v/v was applied with each herbicide treatment. Treatments were applied on each site during a 10-day period in September 2008, when populations were in the bloom to post bloom growth stage (Table 3.2), using a $C0_2$ pressurized backpack sprayer that delivered 187 L ha^{-1} (20 gal ac^{-1}) at 276 kPa (40 psi) through Tee-Jet™ 11002 flat-fan nozzles[3].

Data Collection. Data were collected at three times during the study period: before herbicide treatments (baseline), 1 year after treatment (1 YAT), and 2 years after treatment (2 YAT). For baseline data collection, two subplots (0.25 m^2 quadrats) within each plot were sampled for percent yellow toadflax canopy cover, yellow toadflax density, yellow toadflax flowering shoots density, and individual percent foliar canopy cover for all additional species. The subplots were positioned at two predetermined locations within each plot and measurements were averaged over the two subplots to represent the whole plot. For 1 YAT data collection, the same subplots were sampled for the same variables measured during baseline collection. Data collection 2 YAT included the same measurements taken 1 YAT with an additional sampling of biomass. Above ground biomass was harvested from two 0.25 m^2 quadrats in each plot then pooled to represent each whole plot. Precipitation was collected from nearby weather stations and soils were analyzed by CSU's Soil Analysis Lab (Table 3.1).

Common Garden Experiment. Plant materials were collected from the five field sites in Colorado (Figure 3.1), which were used for the herbicide field experiments. Before initiating the field herbicide applications, plants were randomly selected from outside the plot area, excavated, taken back to the university, potted, and placed in a greenhouse. Plants were hand watered to field capacity every other day and fertilized with Miracle-

Gro All Purpose Plant Food[4] every 3 weeks. Eighteen weeks after being potted in the greenhouse, five plants from each site were selected as genets. The five genets from each site were chosen based on overall appearance and a large number of shoots. Clones were propagated from each genet via stem cuttings.

Twelve stem cuttings, 8 to 12 cm in length, were taken from each of the 25 genets, dipped in Hormex[5] hormone solution (Napthaleneacetic Acid and Indolebutyric Acid; 0.240% and 0.013%) for several seconds then placed in moist potting soil. The cuttings were placed under a mist irrigation system for 4 weeks where they developed roots after 3 weeks. All clones were grown in the greenhouse for 12 weeks. When outdoor conditions were favorable, the clones were moved to a shade box for a period of 7 days. After this period, six clones from each genet were selected for the study. The selected plants were potted in 23 L pots filled with Miracle-Gro Potting Soil[6]. Potted plants were moved to CSU's Agricultural Research, Development and Education Center (ARDEC) and placed inside wooden boxes sunk in the ground to a depth of 50 cm. The top of the wooden boxes and the top of the pots were level with the soil surface. Pots were buried to approximate temperature and moisture conditions that would be encountered under field conditions. Six sunken boxes contained all 150 plants in a completely randomized design, with three replications for each genet. Drip irrigation was supplied to each plant using 1.9 L hr^{-1} (0.5 gal hr^{-1}) emitters. Irrigation was calibrated to deliver 2 to 8 L (0.5 to 2.1 gal) to each plant per day. Water quantity was adjusted to field capacity throughout the growing season to avoid soil desiccation and soil saturation.

Six weeks after potting, plants were re-randomized in the boxes and Miracle-Gro All Purpose Plant Food[4] was applied to all plants. Six weeks following re-randomization

and fertilizer application, three potted clones from each genet were removed from the sunken boxes and treated with 40 g ae ha^{-1} chlorsulfuron plus 1% v/v crop oil concentrate using a $C0_2$ pressurized backpack sprayer, that delivered 187 L ha^{-1} at 276 kPa through Tee-Jet™ 11002 flat-fan nozzles[3]. Treated plants were then returned to the boxes. Thirty days after treatment, all plants were harvested. Shoots and roots were separated, dried in an oven at 30 C to a constant weight and then dry weights were measured. Throughout the growing season, mature seed pods were collected from plants and saved to be included with end of experiment biomass. For each plant, root and shoot biomass were pooled to represent total biomass for each plant and plants were combined by site. This experiment was conducted in the summer of 2009 and repeated in the summer of 2010 using new clones from the same genets.

In Vivo Acetolactate Synthase (ALS) Enzyme Bioassay. Plants subjected to this assay were the same plants used in the common garden study. The assay was conducted in 96 well microtiter plates where actively growing leaf tissue (4 mm^{-2}) was subjected to three solutions: blank (alanine (50 mM) with ampicilin (50 µg ml^{-1})), standard (alanine (50 mM), ampicillin (50 µg ml^{-1}), and HOE 704 (1.5 µM)), and knockout (alanine (50 mM), ampicilin (50 µg ml^{-1}), HOE 704 (1.5 µM), and chlorsulfuron or imazapyr. Each plant was subjected to eight concentrations of chlorsulfuron (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0.078 µM) and imazapyr (200, 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µM) with the knockout solution. Tissue from each population was incubated in 150 µL of each solution at 25 C for 24 h then frozen for 10 h. Plates were then thawed and 37.5 µL HCL (1.25 M) was added to all wells and incubated at 60 C for 30 min. The reaction was then incubated for 15 minutes with creatine and α-napthol (0.17 and 1.7% in 2N NaOH),

then absorbance (acetoin, representative of ALS activity) was measured spectrophotometrically at 535 nm (Westerfeld 1945) using a plate reader (Biotek).

Statistical Analysis.

Field Experiments. Data from the field experiments were analyzed with the Statistical Analysis Systems (SAS Version 9.2) PROC MIXED procedure. Before statistical analysis, data for density were square root transformed and data for biomass were log transformed to satisfy the assumptions of ANOVA. The transformations improved homoscedasticity and normality of distribution. The primary interest of this study was to evaluate the effects of individual herbicides; therefore, chlorsulfuron and imazapyr were analyzed separately. Likewise, data collected 1 YAT were analyzed separate from data collected 2 YAT. Means were compared using Fisher's Protected LSD at $\alpha = 0.05$.

Site Variation. To better understand site variation and the influence of environmental factors, regression analysis was conducted for the percent of yellow toadflax controlled. GR_{50} values, herbicide dose that reduces growth by 50% (Zawierucha and Penner 2001), for biomass (collected 2 YAT) were calculated for chlorsulfuron at each site, and then subjected to a correlation matrix (PROC CORR) with several site characteristics (Table 3.1). This analysis was also done with imazapyr and revealed similar results; however, yellow toadflax biomass was not reduced by 50% at Wildcat Canyon with imazapyr which resulted in an unrealistic GR_{50} value. Imazapyr GR_{50} values are discussed in the appendix.

Non-Target Impacts. To examine the impacts on non-target species, the change in percent foliar cover for each treatment was averaged over sites and then compared back to baseline data. Percent foliar cover of non-target species by treatment was subjected to

ANOVA using PROC GLM and means were compared using Fisher's Protected LSD $\alpha = 0.05$.

Common Garden Experiment. Levene's test for homogeneity revealed that data from 2009 and 2010 could be combined. Each clone served as a replication for each genet. Common garden data were analyzed using the PROC MIXED procedure where clone was nested within genet and means were compared using Fisher's Protected LSD at $\alpha = 0.05$.

In Vivo ALS Enzyme Bioassay. Quantities (determined spectrophotometrically with a plate reader) of the knockout solutions were compared to the blank and standard solutions. Quantities of the knockout solutions were subtracted from quantities of the standard solutions and negative values indicated enzyme inhibition.

RESULTS

Field Experiments. Herbicide effectiveness was determined by yellow toadflax density (1 YAT and 2 YAT) (Table 3.3) and biomass (2 YAT) (Table 3.5). Analysis revealed main effects of site, herbicide, rate, and year were significant. Analysis also revealed a site by rate interaction where the rate effect was dependent upon site for chlorsulfuron 1 and 2 YAT and for imazapyr 2 YAT.

Chlorsulfuron Treatments—1YAT. Chlorsulfuron treatments decreased the density of yellow toadflax at all sites and all rates compared to untreated plots (Table 3.3); however, site variation was most dramatic at the lowest rate (40 g ae ha^{-1}). Acceptable control of a creeping perennial was established at 75% by leafy spurge researchers so the same criteria were used to evaluate yellow toadflax control (Beck et al. 1993). Acceptable control of yellow toadflax was achieved at Camp Hale and Hot Sulphur using the lowest

rate (40 g ae ha^{-1}), which decreased yellow toadflax density from 75 and 137 (untreated control) to 17 (77% control) and 24 (83%) shoots 0.25 m^{-2} respectively. Equivalent control was achieved at the other three sites; however, the highest rate (94 g ae ha^{-1}) was required. With the highest rate (94 g ae ha^{-1}), yellow toadflax stem density was decreased at Greenland, Wildcat Canyon, and White River from 95, 156, and 72 to 20 (79%), 18 (88%), and 17 (76%) shoots 0.25 m^{-2} respectively. Variable response to control was observed among sites, but site variation was overcome by increasing herbicide rate.

Imazapyr Treatments—1YAT. Analysis of stem density revealed that main effects of site and rate were significant. Yellow toadflax at Camp Hale, Greenland, Hot Sulphur, and White River was controlled more effectively than yellow toadflax at Wildcat Canyon (data not tabled). Yellow toadflax stem density decreased from 89, 73, 99, and 47 to 7 (92%), 27 (63%), 10 (90%), and 11 (77%) shoots 0.25 m^{-2} at Camp Hale, Greenland, Hot Sulphur, and White River respectively. Yellow toadflax density at Wildcat Canyon decreased from 141 to 84 (40%) shoots 0.25 m^{-2}. Yellow toadflax control at Greenland and Wildcat Canyon would trigger re-treatment. The rate effect revealed that the highest rate (380 g ae ha^{-1}) decreased yellow toadflax density from 90 to 17 (81%) shoots 0.25 m^{-2} and this was more effective than the lowest rate (127 g ae ha^{-1}) which decreased density of yellow toadflax from 90 to 4 (59%) shoots 0.25 m^{-2}.

Chlorsulfuron Treatments—2YAT. Site variation was still overcome using the highest rate at four of five sites (Table 3.3). With the lowest rate (40 g ae ha^{-1}) yellow toadflax at Greenland and Wildcat Canyon exhibited recovery, wherestem density decreased from 46 and 84 to 35 (24%) and 46 (45%) shoots 0.25 m^{-2}, respectively. Most treatments at Camp

Hale and Hot Sulphur controlled yellow toadflax best among sites, even at the mid-rate (64 g ae ha^{-1}) where yellow toadflax stem densities were decreased from 28 and 78 to 4 (86%) and 3 (96 %) shoots 0.25 m^{-2} respectively (Table 3.3). At the highest rate (94 g ae ha^{-1}), yellow toadflax stem density was decreased from 28, 46, 78, 84 and 41 to 2 (93%), 16 (65%), 1 (99%), 12 (86%), and 9 (78%) at Camp Hale, Greenland, Hot Sulphur, Wildcat Canyon, and White River respectively. Acceptable control of yellow toadflax was achieved at four sites at the two highest rates. With the mid-rate (64 g ae ha^{-1}), yellow toadflax at all sites, except Greenland, showed reductions in biomass 2 YAT, and at the highest rate (94 g ae ha^{-1}), most site to site variation was overcome. Biomass at Greenland and Wildcat Canyon was decreased from 41 and 138 to 13(68%) and 37 (73%) g 0.25 m^{-2} from the highest rate (94 g ae ha^{-1}); however, the decrease at Greenland was no different from the control plot, and the lack of control at both sites would trigger re-treatment (Table 3.5).

Imazapyr Treatments—2 YAT. Analysis revealed a site by rate interaction and the highest imazapyr rate (380 g ae ha^{-1}) overcame site-to-site variation with the exception of Wildcat Canyon (Table 3.4). The lowest rate (127 g ae ha^{-1}) decreased yellow toadflax stem densities at Camp Hale, Hot Sulphur, and White River. At Greenland and Wildcat Canyon, the lowest rate (127 g ae ha^{-1}) was no different than the untreated. The mid-rate (253 g ae ha^{-1}) at Greenland controlled yellow toadflax better than the untreated, but at the Wildcat Canyon site, no rate effect was apparent 2 YAT. The highest rate (380 g ae ha^{-1}) decreased yellow toadflax stem densities from 42, 65, and 33 to 0 (100%), 2 (97%), and 2 (94%) shoots 0.25 m^{-2} at Camp Hale, Hot Sulphur, and White River, respectively. At the sites that expressed recovery (Greenland and Wildcat Canyon), yellow toadflax

stem densities decreased from 39 and 73 to 17 (56%) and 48 (34%) shoots 0.25 m^{-2}. Excellent control of yellow toadflax was achieved at Camp Hale, Hot Sulphur, and White River where biomass was reduced from 20, 75, and 81 to 0 (100%), 6 (92%), and 1 (99%) g 0.25 m^{-2} respectively (Table 3.5). Yellow toadflax biomass at Greenland and Wildcat Canyon were no longer influenced by rate 2 YAT and even with the highest rate (380 g ae ha^{-1}) biomass was only reduced 55% 4%.

Site Variation. Elevation had a negative relationship with GR_{50} values for biomass with chlorsulfuron (Table 3.6). As site elevation increased, the amount of herbicide required to reduce yellow toadflax biomass by 50% decreased. The lower elevation sites: Greenland and Wildcat Canyon required 80 and 58 g ae ha^{-1} chlorsulfuron, respectively, to decrease yellow toadflax biomass by 50%; whereas, Camp Hale, Hot Sulphur, and White River sites required 6, 26, and 38 g ae ha^{-1}, chlorsulfuron, respectively for 50% reduction in yellow toadflax biomass (Figure 3.2).

Relationships were determined between GR_{50} values for biomass and proportion of population flowering at the time of application in the same manner they were done for elevation (Figure 3.4). The proportion of shoots flowering at the time of application had a positive relationship with GR_{50} values for biomass. At the time of application, yellow toadflax at Camp Hale, Greenland, Hot Sulphur, Wildcat Canyon, and White River displayed 18, 45, 28, 60, and 24% flowering of their respective populations (Table 3.6). More herbicide was required to control yellow toadflax at sites that had a higher percentage of flowering shoots at the time of application, which were Greenland (45%) and Wildcat Canyon (60%). Sites that had a smaller proportion of plants flowering at the time of application, i.e. plants were transitioning from flower to seed set and senescence

growth stages, (Camp Hale 18%, Hot Sulphur 28%, and White River 24%) required less herbicide to control yellow toadflax.

Non-Target Impacts. Averaging over sites, chlorsulfuron treatments 1 YAT and 2 YAT increased the foliar cover of non-target species when compared to imazapyr treatments (Figure 3.4). Chlorsulfuron applied at 40, 64, and 94 g ae ha^{-1} increased non-target species foliar cover by 17, 14, and 19% respectively 1 YAT. Imazapyr applied at 127, 253, and 380 g ae ha^{-1} reduced the foliar cover of non-target species by 2, 10, and 13% respectively. Imazapyr applied at 253 and 380 g ae ha^{-1} reduced the foliar cover of non-target species compared to the untreated control plots 1 YAT. At the same rates 2 YAT chlorsulfuron increased non-target species foliar cover by 22, 28, and 27%; whereas imazapyr at the same rates changed the foliar percent cover of non-target species by 11, 9, and -1%, respectively. The foliar cover of non-target species was lower than the untreated with 380 g ae ha^{-1}imazapyr.

Common Garden and In Vivo ALS Enzyme Bioassay. Main effects of site (population from which germplasm was harvested) and treatment (treated or untreated) were significant at $\alpha = 0.05$. Plants taken from different populations grew differently under uniform conditions (Table 3.7). Most notably, plants from the Hot Sulphur site were more robust than plants from the other populations and produced 40% more biomass than the other sites. Even though there were site differences, treatment was significant and all treated plants were effectively controlled with 40 g ae ha^{-1} chlorsulfuron. At harvest (30 DAT) no re-growth or recovery was observed among any of the treated plants and differences largely appear to be a function of plant size at the time of application. The ALS enzyme bioassay supports the results from the common garden, revealing no

herbicide resistance. Enzyme from all populations was inhibited by chlorsulfuron (10 µM to 78 mM) and imazapyr (200 µM to 2 µM) on a mechanistic level.

DISCUSSION

In many agronomic settings, managers face genetic differences that relate to herbicide resistance (Heap 2011). Herbicide resistance develops after repeated application events that select for plants capable of surviving herbicide applications (Devine and Shimabukuro 1994). Resistance or tolerance to herbicides has yet to be observed among yellow toadflax populations; however, with obligate outcrossing and high seed production, the formation of resistant populations is not unlikely. ALS is a nuclear encoded gene that is transferred via pollen flow and with herbicide selection pressure, populations could quickly become resistant (Funke et al. 1999, Rieger et al. 2002).

ALS-inhibiting herbicides are notorious for their ability to select for resistant populations and there are more weed species resistant to ALS-inhibitors than any other herbicide group (Tranel and Wright 2002). Australian researchers studied *Lolium rigidium* (Gaudin) populations that were never exposed to ALS-inhibiting herbicides and found ALS-herbicide resistant individuals (Preston and Powles 2002). The frequency of resistance to sulfonylurea herbicide varied from 2.2×10^{-5} to 1.2×10^{-4} and the frequency of resistance to imidazolinone herbicide varied from 1×10^{-5} to 5.8×10^{-5} depending on the population (Preston and Powles 2002). These researchers illustrated that resistance to ALS-inhibiting herbicides is present in populations where it has not been selected for, so managers must use caution when making herbicide management decisions.

Germplasm used in the common garden study revealed no resistance to chlorsulfuron; all treated plants were 100% controlled and no re-growth was observed 30 DAT when plants were harvested. The observed differences among sites were largely due to plant size at application, which revealed that these populations expressed different growth potential. The ALS enzyme bioassay supported results from the common garden and revealed that these populations are not resistant to these herbicides; thus, site-to-site variation is not being driven by an herbicide resistance mechanism. Our results support the hypothesis that the long-observed site-to-site variation is being driven by environmental factors. The common garden study and ALS enzyme bioassay revealed genetic differences (expressed by different growth potential in a common environment); however, it is important to note that these genetic differences did not confer resistance to the herbicides used.

It is not uncommon for herbicide effectiveness to decrease or "break" 1 to 2 years after treating weedy perennial herbs (Beck et al. 1993) and acceptable yellow toadflax control likely requires sequential applications made over several years (Beck 2010). Years of field research conducted on yellow toadflax produced variable results and response to control has been unpredictable (Sebastian and Beck 1989; 2001). The results from our new experiments reveal site-to-site variation, but much of the spatial variation was overcome by increasing herbicide rate. Yellow toadflax at Camp Hale and Hot Sulphur was controlled using lower herbicide rates, but the other sites were not. Results showed that increased herbicide rates provided equivalent control among all sites, most apparent 1 YAT. The rates used in this study are representative of recommended field use rates and a dose response was observed from both chlorsulfuron and imazapyr.

Control improved with increasing rate; except for biomass data 2 YAT from imazapyr treatments. Spatial variation 1 YAT was mostly overcome with a higher rate, but 2 YAT yellow toadflax at Greenland and Wildcat Canyon recovered.

Site differences were apparent, primarily the recovery exhibited by yellow toadflax at Greenland and Wildcat Canyon. These differences can be better explained by the relationship of biomass GR_{50} values to elevation and proportion of the population flowering at the time of application. Both these site characteristics were highly correlated with herbicide effectiveness. Relationships reveled that higher elevation sites (Camp Hale, Hot Sulphur, and White River) were more effectively controlled than lower elevation sites (Greenland and Wildcat Canyon). Relationships also revealed that yellow toadflax at sites with a lower percentage of flowering shoots, i.e. a greater proportion of shoots transitioning from flowering to seed set and senescence, at the time of application (Camp Hale, Hot Sulphur, and White River) were more effectively controlled than sites with a higher percentage of flowering shoots at the time of application (Greenland and Wildcat Canyon). These relationships reveal that environment and associated climatic conditions are potential factors influencing control and not genetic differences expressed as resistance.

When overlaying the five field sites on USDA Plant Hardiness Zone map (Figure 3.7), it becomes evident that environment, likely climate and length of growing season influenced the recovery of the lower elevation sites. Camp Hale, Hot Sulphur, and White River sites are located in plant hardiness zone 4; Greenland and Wildcat Canyon are located in plant hardiness zone 5. The site locations reveal that the two lower elevation sites experience a longer growing season, which likely promoted recovery from herbicide

injury at least at the Greenland site. It should also be noted that Wildcat Canyon experienced drought conditions throughout the study, particularly during the growing season (Table 3.1). Drought conditions make weed control more difficult (Parker 2005) and the dry conditions at Wildcat Canyon may have been a factor limiting yellow toadflax control at that site. The Greenland site experienced more summer rainfall than the other sites, and this too, may have aided in yellow toadflax recovery at that site. Recovery promoted by a longer growing season with conditions influenced by precipitation seems reasonable; however, when observing the phenology of root bud development (Chapter 2) there is a clear pattern of root bud development that is related to flowering.

The relationship between flowering and root bud development suggests an additional explanation why the higher elevation sites were more effectively controlled. Figure 2.1 in Chapter 2 illustrates that when yellow toadflax populations were in the full bloom growth stage, the number of adventitious root buds present on roots were at their lowest levels. Following the full bloom/seed set growth stage, bud numbers increased and remained at higher levels during dormancy. Leafy spurge (*Euphorbia esula*) root buds serve as sinks for photosynthate in late summer and autumn (Gesch et al. 2007) and it is likely that yellow toadflax root buds are also sinks during this time period. Previous research has shown that applying herbicides in the fall generally results in acceptable control of perennial species (Donald 1992; Lym and Zollinger 2000), probably because herbicides are translocated to the root systems. Based on the evidence provided in Chapter 2, it is likely that the populations growing at higher elevations were more advanced in their growth stage because of shorter growing seasons. These populations at

a more advanced growth stage would have developed more root buds at the time of application. Root buds serve as resource sinks (Davis and Haissig 1994; Gesch et al. 2007) and it is likely that herbicides translocated to these tissues causing mortality and decreasing the number of healthy buds that develop into shoots the following growing season. The lower elevation sites have a longer growing season and it is likely applications occurred when root bud numbers were decreasing or at their lowest counts. Flowers and developing seeds are demanding sinks that often require large amounts of assimilate (Kigel and Galili 1995). It should be expected that herbicides applied during this growth stage would translocate less to the root system of yellow toadflax. Although the growth stage of these populations at the time of application is correlated to the effectiveness of herbicides, both chlorsulfuron and imazapyr have long residual activity and this may be an additional reason for the improved control at higher elevations (Whitcomb 1999).

When comparing chlorsulfuron treatments to imazapyr treatments, differences in recovery were observed (Figure 3.6). Chlorsulfuron is a more selective herbicide that caused less injury to non-target species. Chlorsulfuron treatments 1 YAT and 2 YAT increased the cover of non-target species when compared to imazapyr treatments. Imazapyr treatments were less selective relative to site recovery after treatment and decreased the foliar cover of non-target species when compared to the untreated. Land managers are challenged with removing weeds and maintaining healthy plant communities. Chlorsulfuron and imazapyr are both ALS inhibiting herbicides that can effectively control yellow toadflax populations, but imazapyr is a broad spectrum herbicide (Tu et al. 2001; Anonymous 2007) that will rarely be recommended for

broadcast application because of its tendency to be non-selective especially at rates above 253 g ae ha^{-1} (1 pt product ac^{-1}), which our experiments demonstrated (Figure 3.8). Imazapyr is more injurious to non-target plants and collateral damage can occur with broadcast application. While chlorsulfuron and imazapyr provided similar control of yellow toadflax, with the exception of the Wildcat Canyon site, chlorsulfuron was less injurious to non-target species and promoted site recovery. It can be a challenge to remove an undesirable species while providing conditions that permit a system to recover in absence of the undesirable species and our data reveal the importance of choosing an appropriate herbicide that can selectively remove a weed with minimal collateral damage. It is likely that non-target species were able to recover following the selective removal of yellow toadflax with chlorsulfuron due to a decrease in competition for resources. Imazapyr treatments did not allow non-target species recovery, particularly at the higher rates.

Effective long-term yellow toadflax control has been a challenge for many years. Before this study, site-to-site variation has been unexplainable and consistent recommendations for yellow toadflax control were not available. Our research has provided a better understanding of the influencing factors associated with spatial variation. The length of the growing season, with associated climatic factors, and the yellow toadflax growth stage at the time of application appear to have a strong influence on herbicide efficacy. These factors probably contributed to spatial variation in past research. Despite the wide range of environmental and climatic variation, better yellow toadflax control can be achieved by increasing herbicide rates and this was most evident 1 YAT. Rarely will one application provide acceptable long-term yellow toadflax control

and most managers in Colorado expect to make multiple applications to achieve acceptable control; however, treatments are often considered successful if populations can be reduced to levels below a re-treatment threshold (75% control or better; Beck et al. 1993).

More effective control was achieved at higher elevation sites. Chapter 2 provides good evidence to suggest that higher elevation sites were in a more advanced growth stage, which resulted in better control based on the phenology of root bud development. It is likely that more buds present or developing on a plant resulted in better control because buds are strong sinks (Davis and Haissig 1994; Gesch et al. 2007) and herbicides were likely translocated to those tissues and had a greater negative effect on their root systems. Fall applications, which likely target root systems, have proven to be important for the control of perennial weeds (Donald 1992; Lym and Zollinger 2000).

Managers can utilize what has been learned though these experiments to improve their programs and management success. Management can be improved by simply increasing herbicide rate, but more importantly by targeting populations at their most susceptible growth stage. In the past, land managers have relied on herbicide tank mixes of up to five herbicides to control yellow toadflax. This new research showed that managers can achieve commercially acceptable control using one herbicide across multiple sites. Our research also showed that acceptable control can be achieved for multiple growing seasons, which suggests that managers facing thousands of acres of yellow toadflax can have confidence in the residual effect of their treatment. This makes annual monitoring of sites less important and revisiting treated sites every 2 years might be more appropriate. Although a rate increase of chlorsulfuron is necessary to overcome

site variation, this should result in less overall herbicide use (decrease due to fewer tank mixes and elimination of high rates of ineffective compounds), should reduce costs for managers, and will decrease environmental exposure to herbicides.

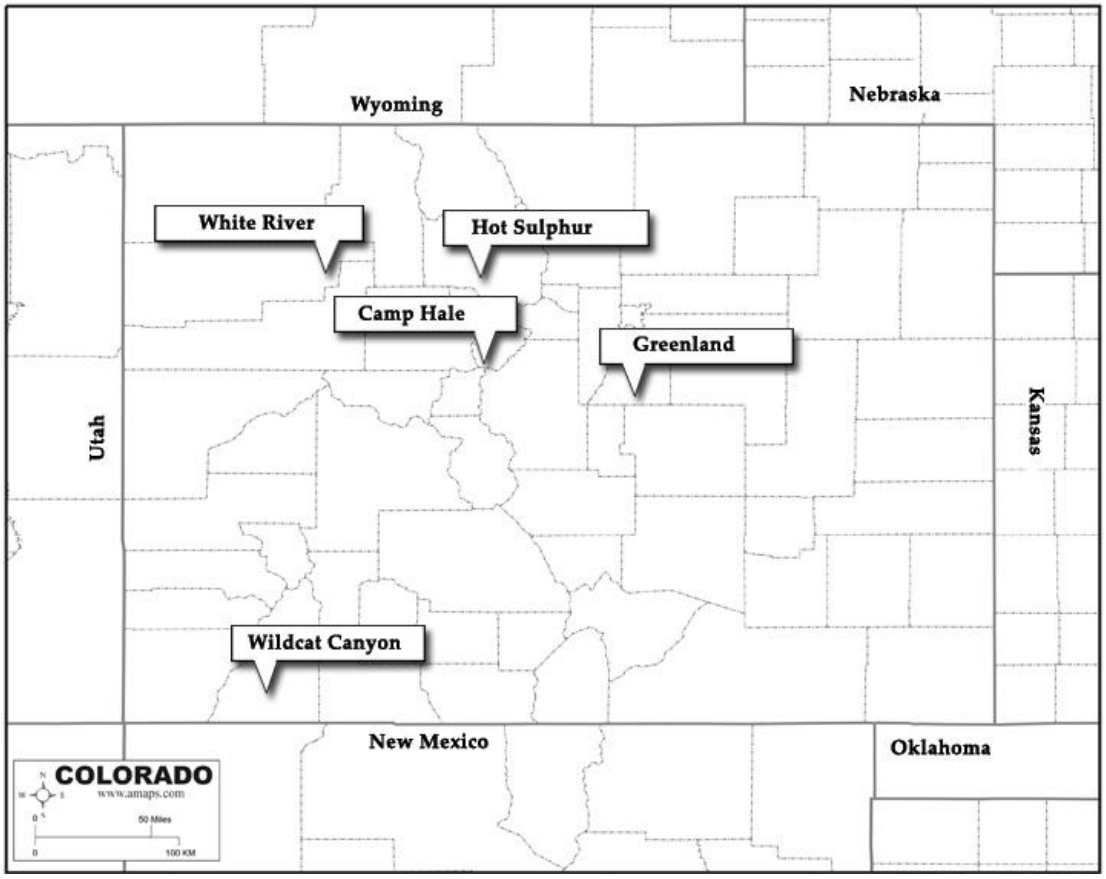

Figure 3.1: Location of yellow toadflax field sites.

Table 3.1: Site characteristics for five yellow toadflax research sites in Colorado.

Site	Camp Hale	Greenland	Hot Sulphur	Wildcat Canyon	White River
Elevation (m)	2837	2186	2323	2142	2481
Aspect	Southwest	Southeast	Northeast	Northwest	Northeast
Species Richness	11	21	11	15	24
Ecoregion[a]	Sedimentary Subalpine Forests	Pine-Oak Woodlands	Sagebrush Park	Semiarid Benchland & Canyonland	Sedimentary Subalpine Forests
Precipitation (cm)					
2007	40.7	28.8	43.9	41.3	35.4
2008	48	32.8	37.8	40.4	104.1
2009	46.2	55.1	34.8	19.1	96.5
2010	31.8	78.2	32.8	27.9	88.9
Total [b]	125.7	160.9	105.2	87.2	289.8
Summer [c]	63.2	131	64.9	38.7	101.9
Soil properties					
pH	6	6.2	7.5	6.5	6
EC	0.2	0.2	0.4	0.5	0.3
N (ppm)	3.1	2.5	9.3	17.6	10.1
P (ppm)	6.8	5.6	9.3	10.6	3.7
K (ppm)	172	238	232	126	337
Lime (ppm)	1	2	3	1	1
Zn (ppm)	98.4	3.4	0.4	1.9	0.8
Fe (ppm)	57.9	49.4	7.9	28	86.3
Mn (ppm)	4.3	8.6	0.7	1.1	3.6
Cu (ppm)	2	2.2	2.5	1.7	1.7
OM (%)	4.9	7	2.5	2.8	8.3
Sand (%)	64	63	40	66	60
Silt (%)	22	25	41	23	32
Clay (%)	14	12	19	11	8
Texture	Sandy Loam	Sandy Loam	Loam	Sandy Loam	Sandy Loam

[a] Ecoregions defined by Chapman et al. (2006).

[b] Total precipitation represent the total amount of precipitation that was recorded throughout the study (2008-2010).

[c] Summer precipitation represents the total amount of precipitation that was recorded April—September for the duration of the study (2008-2010).

Table 3.2: Growth stage of yellow toadflax at the time of application in September 2008.

	Camp Hale	Greenland	Hot Sulphur	Wildcat Canyon	White River
Growth stage	Post bloom /senescence	Early bloom	Late bloom /senescence	Full bloom /early seed set	Late bloom/seed set
% flower	18	45	28	60	24
% post flower	65	8	48	15	35
% vegetative	17	47	24	25	41
Plant height (cm)	2-35	20-50	4-40	25-35	30-75

Table 3.3: Density of yellow toadflax 1 and 2 YAT following chlorsulfuron treatments at five sites in Colorado.

Yellow toadflax density[a] (shoots 0.25 m^2)																				
	1 YAT										2 YAT									
Rate	Camp Hale		Greenland		Hot Sulphur		Wildcat Canyon		White River		Camp Hale		Greenland		Hot Sulphur		Wildcat Canyon		White River	
g ha^{-1}																				
0	75	bc	95	ab	137	a	156	a	72	bc	28	b-e	46	b	78	a	84	a	41	b
40	17	def	61	bc	24	de	79	bc	39	cd	5	hij	35	bcd	19	d-g	46	bc	17	d-g
64	9	efg	39	cd	2	fg	24	de	21	de	4	hij	24	c-f	3	hij	12	e-h	11	g-j
94	7	efg	20	de	1	g	18	de	17	de	2	j	16	efg	1	ij	12	ghi	9	fgh

[a] Data were analyzed after square root transformation, but means are presented in their original scale. Means followed by the same letters are similar within herbicide and within year. Means were separated with Fisher's Protected LSD $\alpha = 0.05$.

Table 3.4: Density of yellow toadflax 2 YAT following imazapyr treatments at five sites in Colorado.

	Yellow toadflax density[a] 2 YAT (shoots 0.25 m^2)									
Rate	Camp Hale		Greenland		Hot Sulphur		Wildcat Canyon		White River	
0	42	bc	39	bcd	65	ab	73	a	33	cd
127	7	f-i	36	cd	21	de	65	ab	12	efg
253	0	ij	15	ef	8	f-i	52	abc	10	e-h
380	0	j	17	ef	2	hij	48	abc	2	hij

[a] Data were analyzed after square root transformation, but means are presented in their original scale. Means followed by the same letters are similar within herbicide and within year. Means were separated with Fisher's Protected LSD $\alpha = 0.05$

Table 3.5: Biomass of yellow toadflax 2 YAT following herbicide application at five sites in Colorado.

		Field Site Biomass (g 0.25 m^{-2})[a]									
Chlorsulfuron	Rate g ha^{-1}	Camp Hale		Greenland		Hot Sulphur		Wildcat Canyon		White River	
	0	14	e	41	bcd	84	ab	138	a	102	ab
	40	3	fg	35	bcd	29	b-e	113	ab	47	abc
	64	2	fg	35	bcd	2	fg	39	bcd	15	de
	94	1	g	13	de	1	g	37	cde	13	ef
Imazapyr											
	0	20	cde	44	bc	75	ab	138	a	81	ab
	127	8	fgh	42	bc	34	bc	192	a	24	cd
	253	0	i	20	c-f	10	efg	150	a	9	def
	380	0	i	20	c-f	6	ghi	132	a	1	hi

[a] Means were analyzed after log transformation, but are presented in their original scale. Means followed by the same letters within an herbicide are similar, $\alpha = 0.05$, using Fisher's Protected LSD.

Table 3.6: Chlorsulfuron GR_{50} values for biomass with site elevations and the proportion of each population flowering at the time of application. The numbers from this table were used to generate the relationships shown in Figure 3.2 and Figure 3.3.

	Elevation	% Flowering	Equation	GR_{50}
Wildcat Canyon	2142	60	y = 66.221ln(x) - 219.18	58
Greenland	2186	45	y = 58.788ln(x) - 207.81	80
Hot Sulphur	2323	28	y = 42.035ln(x) - 86.951	26
White River	2481	24	y = 45.651ln(x) - 115.89	38
Camp Hale	2837	18	y = 16.019ln(x) + 22.502	6

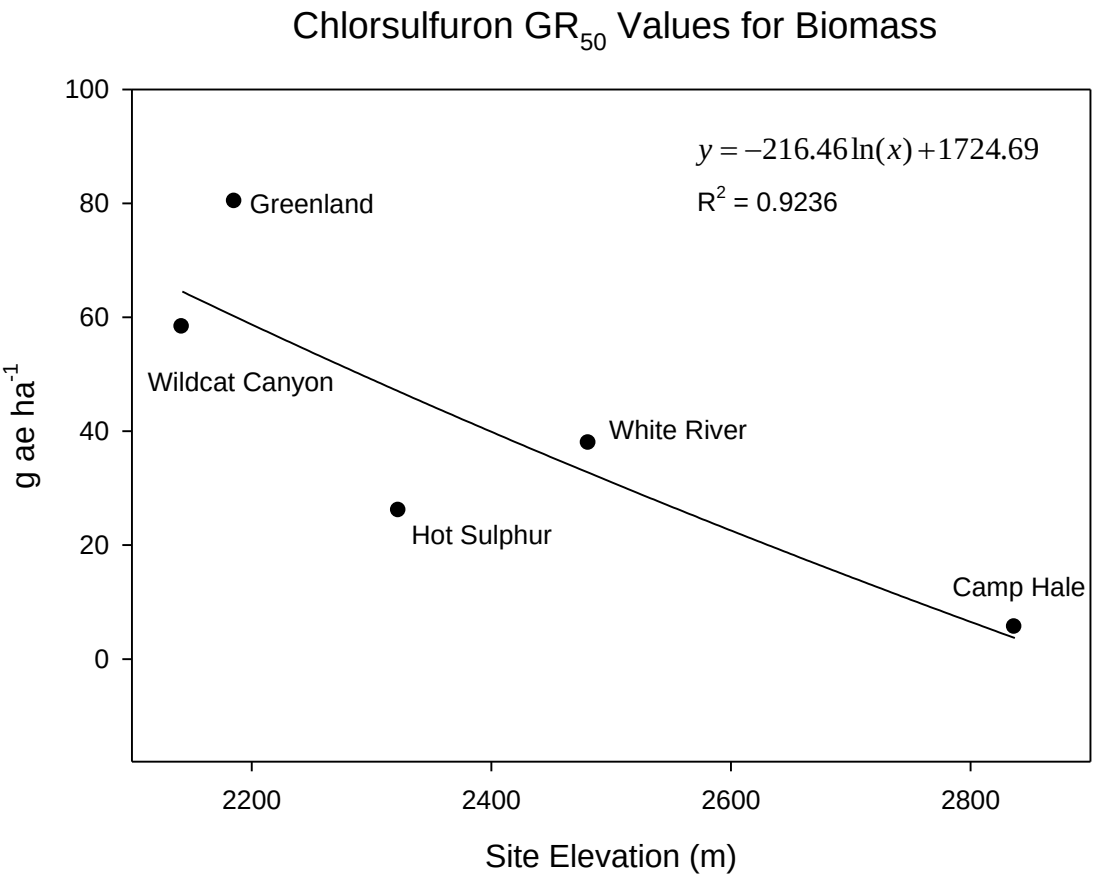

Figure 3.2: Relationship between chlorsulfuron GR^{50} values for yellow toadflax biomass and elevation.

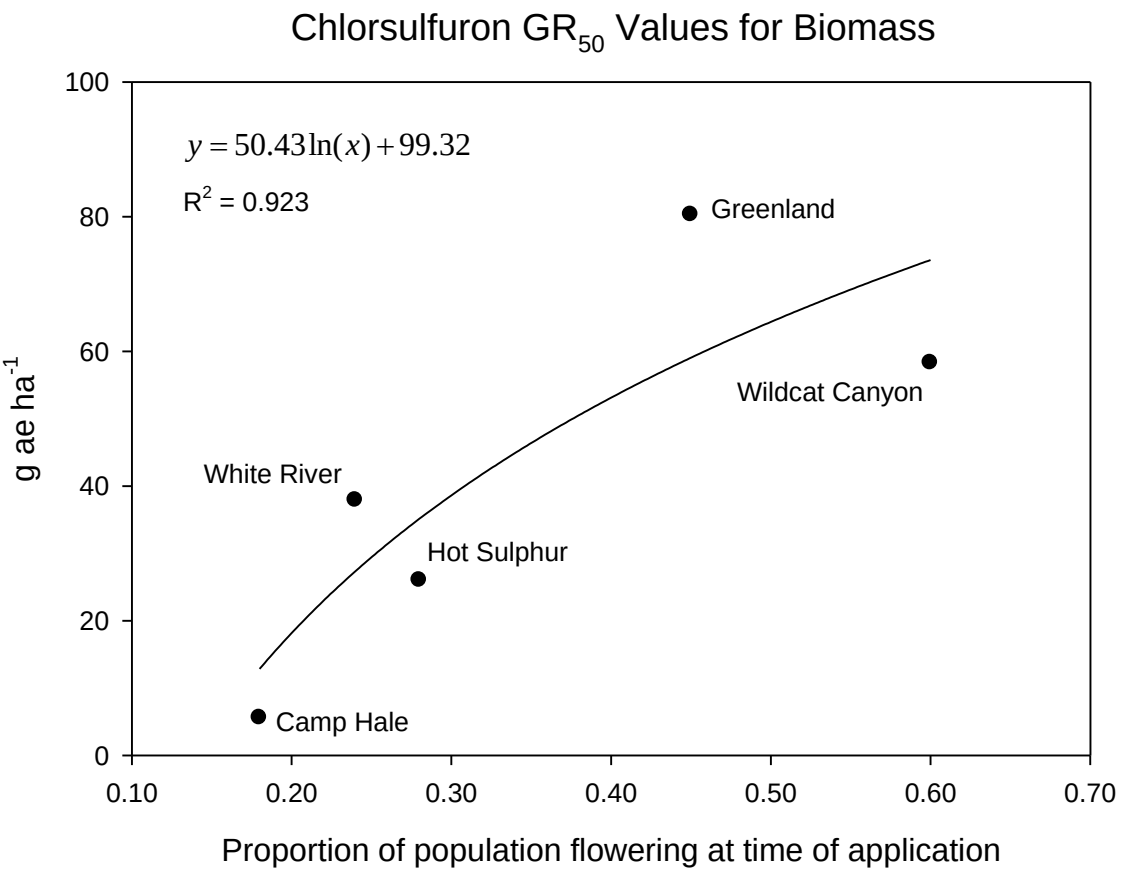

Figure 3.3: Relationship between chlorsulfuron GR^{50} values for biomass and proportion of populations flowering at the time of application.

Table 3.7: Biomass of yellow toadflax subjected to a common garden experiment.

Common Garden Biomass (g pot^{-1})[a]										
	Camp Hale		Greenland		Hot Sulphur		Wildcat Canyon		White River	
Treated	28	bc	24	bc	43	a	32	b	21	c
Untreated	41	bc	31	c	63	a	44	b	38	bc

[a]Means within a row followed by the same letters are similar, α=0.05.

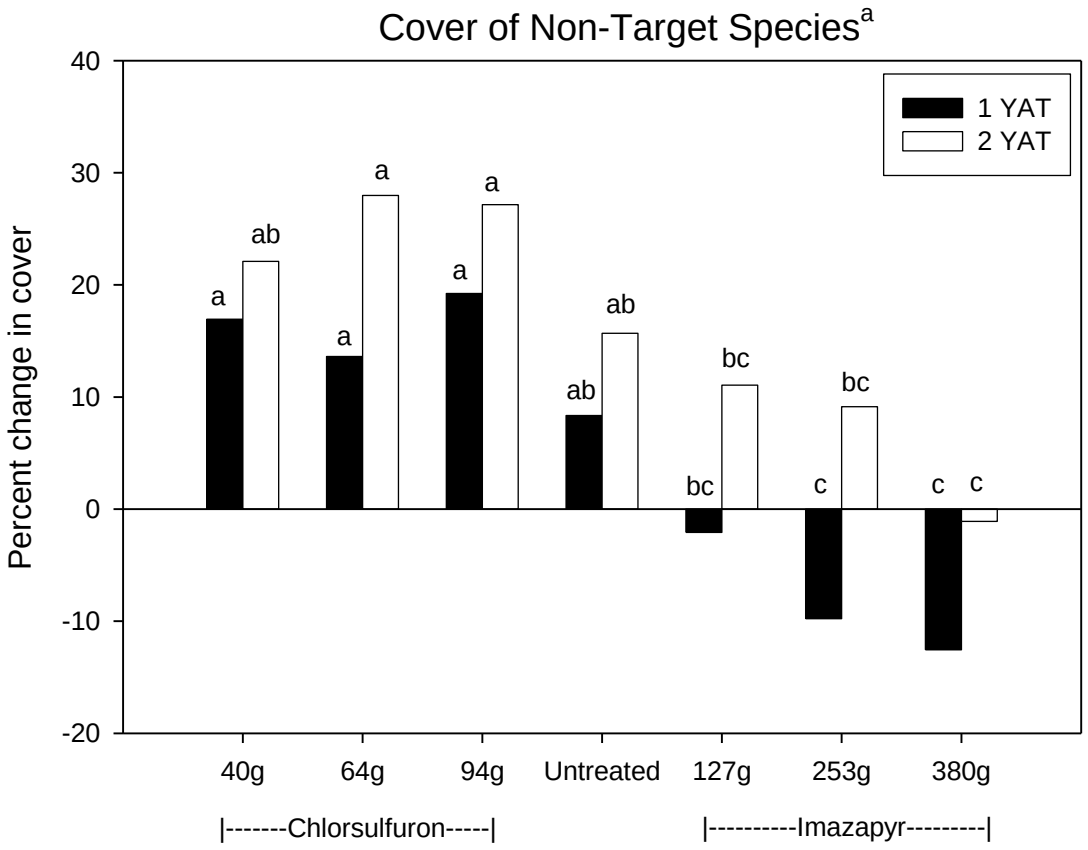

Figure 3.4: Percent change in cover of non-target species 1 YAT and 2 YAT. Values were determined by comparing cover 1 and 2 YAT to baseline data.

[a]Means with different letters are different at $\alpha = 0.05$ with Fisher's Protected LSD.

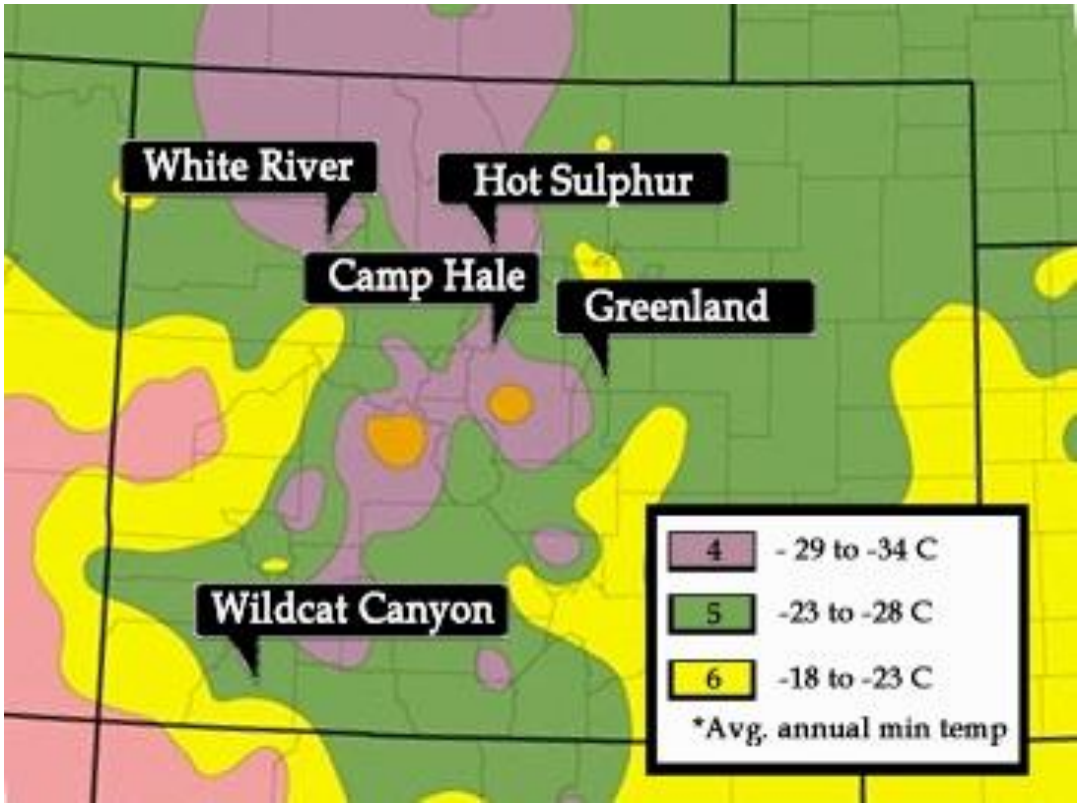

Figure 3.5: Location of Colorado field sites overlaid on the USDA Plant Hardiness Zone map.

SOURCES OF MATERIALS

[1] Chlorsulfuron, Telar® Herbicide, DuPont Crop Protection, Wilmington, DE 19880

[2] Imazapyr, Arsenal/Habitat Herbicide®, BASF Corporation, Agricultural Products, Research Triangle Park, NC. 27709.

[3]11002 flat fan nozzles, TeeJet Technologies, Wheaton, IL 60189

[4]Miracle-Gro Plant Food, Scotts Miracle-Gro Company, Marysville, OH 43041

[5]Hormex® Brooker Chemical Corporation, Chatsworth, CA 91313

[6]Miracle-Gro Potting Soil, Scotts Miracle-Gro Company, Marysville, OH 43041

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APPENDIX

This appendix contains research that was conducted throughout this study, but was not pertinent for journal publication.

Table A.1: Imazapyr GR^{50} values for biomass with site elevations and the proportion of each population at the time of application. The numbers from this table were used to generate the relationships shown in Figures A.1 and A.2.

			Imazapyr	
	Elevation	% Flowering	Equation	GR_{50}
Camp Hale	2837	18	y = 43.467ln(x) - 151.76	104
Greenland	2186	45	y = 49.59ln(x) - 233.4	303
Hot Sulphur	2323	28	y = 32.191ln(x) - 96.094	94
Wildcat Canyon	2142	60	y = 40.504ln(x) - 234.64	1127
White River	2481	24	y = 22.037ln(x) - 32.533	42

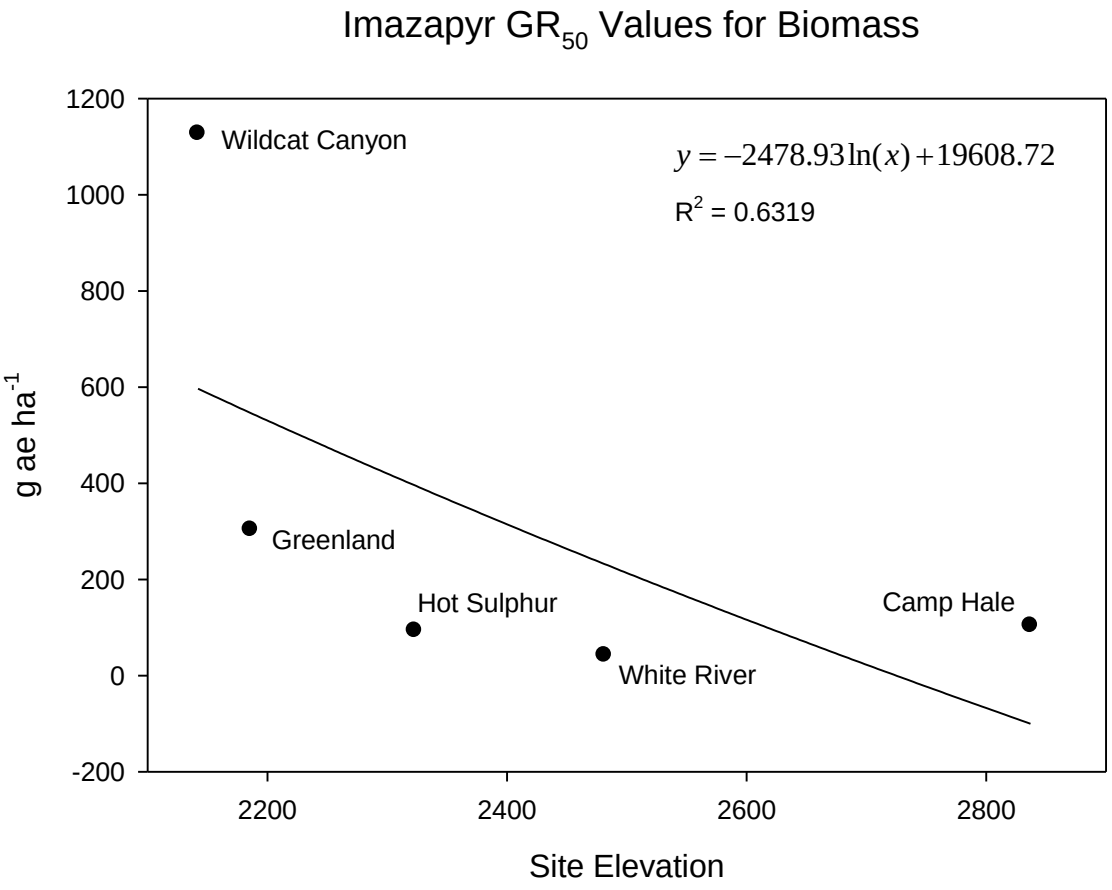

Figure A.1: Relationship between imazapyr GR^{50} values for biomass and elevation.

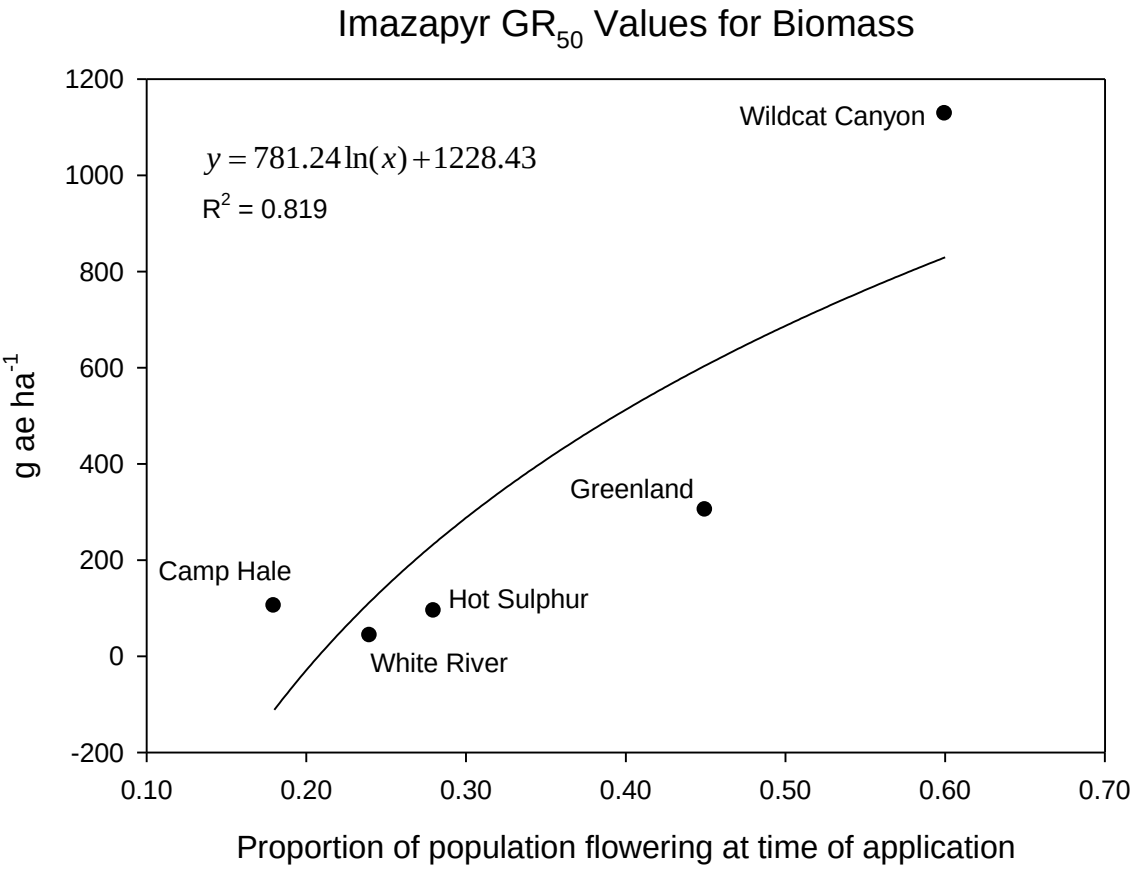

Figure A.2: Relationship between imazapyr GR^{50} values for biomass and proportion of populations flowering at the time of application.