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Goatsrue (*Galega officinalis*) Seed Biology, Control, and Toxicity

Michelle Oldham
Utah State University

GOATSRUE (*Galega officinalis)* SEED BIOLOGY, CONTROL, AND TOXICITY

by

Michelle Oldham

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of

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Approved:

_____	_____
Corey V. Ransom Major Professor	Steven A. Dewey Committee Member
_____	_____
Ralph Whitesides Committee Member	Mike Ralphs Committee Member

_____
Byron R. Burnham
Dean of Graduate Studies

UTAH STATE UNIVERSITY
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ABSTRACT

Goatsrue (*Galega officinalis*) Seed Biology, Control, and Toxicity

by

Michelle Oldham, Master of Science

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Major Professor: Dr. Corey V. Ransom
Department: Plants, Soils, and Climate

Goatsrue is an introduced perennial plant that has proven to have great invasive potential, leading to its classification as a noxious weed in many states and at the federal level. This research focused on seed biology, herbicide control, and toxic dynamics of goatsrue. Physical dormancy of mature goatsrue seed was tested through scarification using sulfuric acid with exposures of up to 60 minutes resulting in 100% germination. Comparison of dormancy for 26-year-old and 6-month-old goatsrue seed indicated aged seeds had reduced dormancy levels compared to newly harvested seeds, but had similar viability. Goatsrue seedling emergence was inversely related to burial depth; emergence was greatest at 0.5 cm soil depth (93%), and no emergence occurred from 12 and 14 cm. Goatsrue seed density ranged from 14,832 seeds m^{-2} to 74,609 seeds m^{-2} in the soil seed bank of five goatsrue-infested areas. Viability and dormancy of seeds recovered from the soil seed bank survey ranged from 91 to 100% and 80 to 93%, respectively. Goatsrue was most sensitive to the ALS inhibitor herbicides chlorsulfuron and imazapyr in greenhouse trials. Field studies showed that plots treated with dicamba, chlorsulfuron,

metsulfuron, aminopyralid, triclopyr, and picloram provided at least 93% control of goatsrue 12 months after treatment at two field sites and increased perennial grass cover at one site. All treatments at one site decreased seedling goatsrue cover 11 months after treatment. The concentration and pools (dry weight x concentration) of the toxin galegine, found in goatsrue, vary over plant tissues and phenological growth stages. Galegine concentration is significantly different among plant tissues; reproductive tissues have the highest levels of galegine (7 mg/g), followed by leaf (4 mg/g), and then stem (1 mg/g) tissues. Galegine pools or the total amount of galegine per stalk was lowest at the vegetative growth stage and increased until reaching a maximum at the immature pod stage, but decreased nearly in half at the mature seed stage. Average galegine concentration also peaked at the immature pod stage and decreased by half at the mature seed stage. Thus, goatsrue is most toxic in its phenological development at the immature pod stage.

(77 pages)

Dedicated to my mom, Kathy,
for instilling in me a love of learning
and a desire to do my best.

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CHAPTER 1

LITERATURE REVIEW

Classification and History

Galega officinalis, commonly known as goatsrue, is a member of the Fabaceae family. It is native to Europe and Western Asia; its distribution spanning from Spain and Great Britain east to Iran and north to Scandinavia (Patterson 1992). In the late 1800's goatsrue seemed to have potential as a quality forage crop in Europe. Due to these reports, goatsrue was introduced in 1891 to Logan, Utah for testing at the Utah Agricultural Experiment Station. According to the station's fourth annual report, plots containing several potential forage crops, including goatsrue, were located on the benches east of the buildings then present on the Utah State University campus. Goatsrue did not perform well in these deficit irrigation test plots, yielding only half as much in biomass and crude proteins as alfalfa. Additionally it was found to have low palatability to cattle and horses; thus its testing as a potential forage was discontinued. There is no other record of goatsrue until 1909 when Charles Piper Smith of the Utah State University Botany Department collected and mounted a goatsrue plant (Tingey 1971). By 1974 goatsrue had spread substantially in Cache Valley and was placed on the Utah Noxious Weed list. In the late 1970's it was estimated to have infested 60 square miles in Cache County, Utah (Evans 1984). In 1980 USDA-APHIS (United States Department of Agriculture - Animal Plant Health Inspection Service) selected goatsrue for an eradication project. This project became a major effort involving, APHIS, Utah Department of Agriculture, Cache County Weed District, local landowners, and Utah

State University. In 1984 it was estimated that 85 percent or more of mature plants were eliminated, and the remaining mature plants were substantially weakened by control measures (Evans 1984). Unfortunately after several more years funding for the project was eliminated and goatsrue again began to spread throughout Cache Valley.

Morphology

Goatsrue is considered a taprooted perennial, grows from 0.6 to 1.5 meters tall, and reproduces by seed. It has oddly-pinnate leaves with 6-12 pairs of leaflets. The plant bears most commonly purple, but also blue to white papilionaceous flowers in terminal and axillary racemes. The seed pods are roughly 2.5 cm long, narrow and round in cross section (Whitson et al. 2000). Seeds are about 2.5 times the size of alfalfa seed, and yellowish in color, and can remain viable in the soil for at least 15 years (Evans et al. 1997). Seed coats have a physical dormancy, and must be scarified to allow water up take and germination (Patterson 1992).

Biology

As goatsrue is in the beginning stages of its invasion in the United States, there has been little research done on its basic biology. For instance, seed depth which allows germination and emergence, the level of dormancy in newly matured seed, as well as soil seed bank quantities, dormancy, and viability. Goatsrue is frequently noted in the literature for its ability to fix atmospheric nitrogen when it is associated with a bacterium in a symbiotic relationship. The bacteria strain, *Rhizobium galegae*, associated with

goatsrue is host specific and does not infect other legumes; and likewise goatsrue will not associate with any other rhizobium (Baimiev et al. 2005). The Galega rhizobium is a fast growing rhizobium with an indeterminate root nodule, which is common among temperate legumes. Due to its indeterminate nature, the nodules continually have bacteria in different stages of differentiation (Lipsanen and Lindstrom 1988).

Distribution and Habitat

Goatsrue is found in 10 states throughout the United States: Utah, Colorado, Washington, Nebraska, Maine, Massachusetts, New York, Pennsylvania, Connecticut, and Washington D.C. It is found on 13 state noxious weed lists and is also classified as a federal noxious weed (USDA 2008). Goatsrue has also become problematic in New Zealand, England, Chile, Ecuador, and Argentina (Gresham and Booth 1991; Oehrens and Gonzalez 1975; Patterson 1992). It is not well adapted to the large diurnal variations in temperature as are found in many of its current infestation areas in the United States, perhaps why its spread has been relatively contained since its introduction. However, the temperatures and higher moisture conditions found in the South or Midwest could be much more favorable to its growth and spread (Patterson 1992).

Goatsrue is most commonly found where there is an ample water supply keeping the soil moist throughout the year. It is often found along waterways, pastures, fencelines, roadways and wet marshy areas in Utah (Evans and Ashcroft 1982). Most seed dispersal is through waterways, but dissemination may also occur through harvesting equipment, animal manures, soil moving operations, and contaminated alfalfa seed (Whitson et al. 2000).

Toxicity

Goatsrue is sometimes reported as a beneficial plant with potential as, a forage crop (Peiretti and Gai 2006), a lactogenic compound in sheep (Gonzales-Andres et al. 2004), as well as an herbal medicine for diabetes. There is a common saying originating from Paracelsus (1493-1541) which states, “the dose makes the poison,” which is certainly true for goatsrue, as it contains the toxic compound galegine which at certain doses can have adverse effects. Gresham and Booth recorded the first reported case of sheep poisoning in the United Kingdom in 1991; and cite other instances of poisoning in France, Romania and New Zealand (Gresham and Booth 1991). Research done at the United States Department of Agriculture – Agriculture Research Service (USDA-ARS), Poisonous Plant Research Laboratory in Logan, UT, confirmed goatsrue’s toxicity due to the alkaloid galegine (Keeler et al. 1986, 1992).

Galegine is believed to lower blood pressure and is a central nervous system paralytic (Keeler et al. 1986). Galegine was found at an average concentration of 0.46% in goatsrue leaves (Keeler et al. 1992). Clinical signs of poisoning in sheep can occur at doses as low as 0.8 g of dried plant per kg body weight per day (Keeler et al. 1986). However, research has shown there is a great amount of individual animal variation in toxicity responses, as a does of 24 g of goatsrue per kg animal body weight has also been shown to induce no pathological lesions (Keeler et al. 1988). This wide spectrum of individual animal variation may be due to differences in metabolic response to galegine (Keeler et al. 1988). There is very little information on how galegine concentration in

goatsrue changes as a plant matures, nor on the presence or levels of galegine in goatsrue seed.

Beneficial Uses

Goatsrue is reported to lower blood sugar levels, as well as prevent diabetes mellitus, and can be found sold as a herbal supplement. However, several studies done on its hypoglycemic activity have not been able to prove its abilities to lower blood sugar (Lemus et al. 1999; Neef et al. 1995; Pundarikakshudu et al. 1994;). Also, Witters (2001) explains the belief that goatsrue could aid in the treatment of diabetes mellitus arose from a mistaken notion of guanidines true mode of action.

A preliminary study claims goatsrue can increase milk production in sheep (Gonzales-Andres et al. 2004). Acknowledging goatsrue toxicity, the objective of this study was to find a dose which would increase milk production without inducing toxicity symptoms. Sheep given daily does of 2.0 g dry matter/kg body weight from the first month after lambing for a duration of 60 days increased total milk yield 16.9%, without any signs of toxicity.

Control

Control of goatsrue will become an increasing problem as it continues to spread and may become a major problem unless it is addressed with effective control methods, including prevention. Mowing, clipping and cutting goatsrue are not effective control methods; as they will only reduce seed production and reduce the plants' vigor (Evans

1984). However, it has been found that 2,4-D and dicamba are effective herbicides for control. Mixtures of 2,4-D at 0.6 kg/ha and dicamba at 0.3 kg/ha applied twice during the growing season for two consecutive years will provide control (Evans 1984). However, control was most effective when mechanical methods, clipping plants when the initial growth reaches 61 cm tall, were combined with herbicide applications, spraying the re-growth at 61 cm (Evans 1984). In cropping systems, crop rotation is an effective means of control as it interrupts the natural life cycle of goatsrue (Evans and Ashcroft 1982). Many new herbicides have entered the market since the original herbicide testing was done on perennial goatsrue, thus, there is an opportunity to find more efficient and effective herbicides for control.

Research Objectives

The objectives of this research are to 1) Understand seed biology by determining the level of physical seed dormancy of recently harvested seed; the effect of long-term dry storage on seed dormancy and viability; the impact of seed burial depth on emergence; and the quantities, viability, and dormancy of seed found in the soil seed bank in areas of high plant density. 2) Determine the most effective herbicidal control for goatsrue through greenhouse and field trials. 3) Determine concentration of galegine in goatsrue plant parts as well as the average concentration and galegine pools over phenological growth stages.

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CHAPTER 2

GOATSRUE (*Galega officinalis)* SEED BIOLOGY[*]

Goatsrue is an introduced perennial plant which has proven to have great invasive potential, leading to its classification as a noxious weed in many states and at the federal level. Very little research has been done on its basic biology. Physical dormancy of mature goatsrue seed was tested through scarification with sulfuric acid for up to 60 minutes, resulting in 100% germination. Comparison of dormancy for 26-year-old and 6-month-old goatsrue seed indicated that aged seeds had reduced dormancy levels compared to newly harvested seeds. Maximum germination was similar among the 6-month old and 26-year-old seed lots suggesting no loss of viability had occurred in seed stored dry for 26 years. Goatsrue seedling emergence was inversely related to burial depth and decreased as burial depth increased. Emergence of seed buried at 0.5 to 3.0 cm soil depth was 93 to 87%, respectively, and no emergence occurred from 12 and 14 cm. In sampling the soil seed bank of five goatsrue infested areas, the largest density of seeds found was 74,609 seeds m^{-2} while the lowest was 14,832 seeds m^{-2}. Dormancy and viability of seeds recovered from the soil seed bank survey ranged from 91 to 100% and 80 to 93%, respectively. Management which reduces the soil seed bank and controls emerging seedlings is as essential as control of mature goatsrue plants in order to avoid seedling establishment and re-invasion of a location.

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[*] Oldham and Ransom. Weed Science, Weed Science Society of America, Allen Press Publishing.

Galega officinalis L., commonly known as goatsrue, was introduced in 1891 for testing as a potential forage at the Utah Agricultural Experiment Station located in Logan, Utah (Tingey 1971). It was later discovered that goatsrue had low palatability to cattle and horses and contains the toxic alkaloid compound galegine (Keeler et al. 1986, 1988, 1992; Puyt et al. 1981). Eighty-six years after its introduction, goatsrue had become a significant weed problem and infested approximately 60 square miles in Cache County, Utah (Evans 1984). Beginning in 1981 and continuing through the late 1990's it became the subject of an unsuccessful United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA – APHIS) eradication project. Goatsrue currently occurs in 10 states in the U.S., is included on 13 state noxious weed lists, and is classified as a Federal Noxious Weed (USDA 2008). It has also become problematic in New Zealand, England, Chile, Ecuador, and Argentina (Patterson 1992; Gresham and Booth 1991; Oehrens and Gonzalez 1975). Goatsrue is not well adapted to large diurnal temperature fluctuations found in many areas of current infestations in the United States, which may partially explain its limited rate of spread (Patterson 1992).

Goatsrue is a member of the Fabaceae family, and is native to Europe and Western Asia (Patterson 1992). It is a taprooted perennial which forms vigorous crowns, grows 0.6 to 1.5 meters tall, and reproduces by seed. It can be found growing in and infesting cropland, pastures, wetlands, and waterways in Utah (Evans et al. 1997). The seed pods of goatsrue are roughly 2.5 cm long, narrow, and round in cross section (Whitson et al. 2000). Seed pods can contain from one to nine seeds and an individual plant may have upwards of 15,000 pods (Evans and Ashcroft 1982). Seeds are roughly 2.5 times larger than alfalfa (*Medicago sativa* L.) (Evans and Ashcroft 1982), have a

mustard yellow color, and are believed to be capable of remaining viable in the soil for at least 15 years (Evans et al. 1997). As is common in legumes, goatsrue seeds have physical dormancy induced by water impermeable layers of palisade cells in the seed coat (Finch-Savage and Leubner-Metzger 2006). In papilionoid legumes the lens (or strophiole) is a point of structural weakness in the seed coat due to its elongated palisade cells. These cells are under considerable stress and can split apart and create fissures allowing water entry to break dormancy. This process occurs in both scarified seeds and in those which gain permeability naturally (Murray 1984).

There has been little research on goatsrue's basic biology due to its relatively isolated infestations and confined spread within the United States. The objectives of this research were to determine (1) the level of physical seed dormancy of recently harvested seed, (2) the effect of long term dry storage on seed dormancy and viability, (3) the impact of seed burial depth on emergence, and (4) the quantities, dormancy, and viability, of seed found in the soil seed bank in areas of high plant density.

Materials and Methods

Evaluation of Physical Seed Dormancy. Trials were conducted to determine the level of physical dormancy of newly harvested goatsrue seed with sulfuric acid scarification. Mature seeds were hand harvested and bulked from approximately 10 plants at each of two locations in Cache County, Utah in the fall of 2006. Pods were hand threshed and seed was separated by blowing away the chaff. Seed was stored in plastic containers at room temperature (21-27 C). The experimental design was a

Randomized Complete Block with four replications. Due to limited seed resources each replicate was composed of 10 seeds and the trial was repeated. Forty seeds were placed in each of six glass beakers with 30 ml of undiluted sulfuric acid (H_2SO_4). The seeds in each beaker were exposed to sulfuric acid for; 0, 10, 20, 30, 40, 50, or 60 minutes. Then, the sulfuric acid was poured off and seeds were rinsed by swirling with 30 ml distilled water for 10 seconds, repeated three times. Seeds were then placed in petri dishes[1] lined with a filter paper[2] and moistened with 5 ml of distilled water. Petri dishes were sealed with parafilm[3] and placed inside a germination chamber at 23 C in the dark. Germination was recorded when at least a 2 mm length of radicle emerged from the seed coat and was monitored daily for 7 days. Seeds were incidentally exposed to light each day during germination evaluations.

Effect of Seed Age on Physical Dormancy. To elucidate the effect of seed age on physical seed coat dormancy, germination after scarification was studied on 26-year-old and 6-month-old goatsrue seed. Old and new seed lots were collected from different locations in Cache County, Utah, in fall of 1982 and 2007, respectively. The 26-year-old seed was stored in mesh bags in the dark, in a laboratory with relatively constant air temperatures between 21 – 27 C for approximately 21 years. Seed was then moved to a metal shed where it was exposed to ambient air temperatures for at least the last 5 years of storage. Ambient air temperatures for Logan Utah, range from average low to high temperatures of -9 to 31 C. The new, 2007 seed lot was collected from approximately 10 plants at each of two locations. Pods were hand threshed and seed was separated by blowing the chaff from the seed. Seed was stored in plastic containers in a lab cupboard in the dark at room temperature (21 - 27 C) for 6 months. The same sulfuric acid

scarification treatments used in the experiments to evaluate physical seed dormancy were used to conduct these tests. The experimental design was a Randomized Complete Block with four replications. Each replicate was composed of 10 seeds and the trial was repeated. Germination was recorded when at least a 2 mm length of radicle emerged from the seed coat and was monitored daily for 7 days. Seeds were incidentally exposed to light each day during germination evaluations.

Impact of Depth on Seed Emergence. To evaluate the impact of seed burial depth on goatsrue seedling emergence, seeds were planted in pots at depths of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, and 14.0 cm. Trials were conducted as a Randomized Complete Block Design with 6 replications of 10 seeds and the trial was repeated. Seed from the 2007 seed lot collection was used and was stored in plastic containers at room temperature (21-27 C) prior to burial in black pots 17.5 cm tall and 16 cm in diameter. Seeds were buried in a Kidman fine sandy loam field soil with 7.5 pH and 0.72% organic matter. Seeds were scarified for 60 minutes in sulfuric acid to remove their physical dormancy prior to burial at appropriate depths. The pots were placed in a greenhouse with average day and night temperatures of 25 and 20 C, with approximately 16 hours natural and supplemental lighting. The soil was kept moist by watering with overhead sprinklers for 5 minutes twice daily. Seedling emergence was monitored daily for 30 days by counting the number of seedlings in each pot.

Soil Seed Bank Sampling. In order to determine the amount of goatsrue seed in the soil seed bank, five areas of high goatsrue incidence were sampled in November, 2007, in Cache County, Utah. Site one was an infestation along a canal in a dry field lot,

with a clay loam soil texture and 7.4 pH (Table 2-1.). Site two was an infestation near a spring in a weedy field lot with a clay loam soil texture and 7.6 pH. Sites three and four were infestations in non-irrigated, long-term pastures, with loam soil textures and pH values of 7.3 and 7.4, respectively. Site five was also in a non-irrigated, long-term pasture, alongside a small ditch with running water; the soil was a clay loam texture with a pH of 7.5. A 30 m transect was established at each site with seven, 1 m^2 plots placed at 5 m intervals. The number of goatsrue stems within each 1 m^2 plot were counted and recorded to estimate the population density at each site. Subsequent to counting, each 1 m^2 plot was split into four quadrants and a soil core (10 cm deep and 10 cm in diameter) was taken from each section using a golf course cup hole cutter. Thus, four samples were taken in each 1 m^2 plot for a total of 28 samples per site. Soil cores were stored in sealed plastic bags and placed in cold storage at 4 C until seeds could be extracted with a sieve system and counted. Soil samples were washed with running water through a stacked series of three sieves, sizes, 3.35 mm, 2 mm, and 850 µm; the smallest significantly smaller than goatsrue seed enabling accurate recovery of seed from the soil. Extracted seeds were stored in plastic containers in dry conditions between 21 and 27 C until germination tests were conducted.

A germination test was conducted to determine the viability of seeds extracted from the soil collected from each site. A bulk sample of seeds for each site was created by selecting seeds collected from each of the 28 soil samples. A randomized Complete Block Design with four replications of 25 seeds each was established and tested for germination. Seeds were scarified with sulfuric acid for 60 minutes to relieve dormancy and were triple rinsed with distilled water as previously described. Seeds were then

placed in petri dishes lined with moist filter paper and sealed with parafilm. Seeds were considered to have germinated when at least 2 mm of the radicle emerged form the seed coat. Germination was monitored daily for 7 days and the trial was repeated. Dark conditions were not maintained during germination counts as exposure to light did not affect seed germination. Seeds which did not germinate were considered non-viable.

The percentage non-dormant seed in the soil seed bank was estimated with two sets of data. First, the average number of seeds per location which germinated while in cold storage. Secondly, since germination conditions were not optimal at 4 C cold storage temperatures, a germination test of extracted seed was needed to determine the remaining percentage of non-dormant seed. By combining the results from these two data sets an estimate of the percent non-dormant seed in the soil seed bank at the time of sampling was found. Previously explained design and methods for the viability tests were used to conduct these germination tests. The tests were carried out several months after seed extraction from the soil samples.

To determine soil characteristics at each surveyed site one soil sample was taken in each 1 m^2 plot, with a 1.9 cm diameter soil probe to a depth of 30.5 cm. Samples from each site were combined, mixed and submitted to the Utah State University Analytical Laboratories for analysis.

Statistical Analysis. There were no iteration by treatment interactions in any of the scarification, dormancy, viability, or depth of emergence studies, allowing combination of data across runs for analysis. Data were checked for normality and homogeneity of variances prior to analysis. All studies were subjected to an analysis of variance. Data from the comparison of 26-year-old and 6-month-old seed were analyzed

with two-way t-tests to determine significance between seed lots at each different scarification time. Data from the experiments to overcome physical seed dormancy (2006 seed lot scarification) were fit to a 4-parameter logistic curve:

$$y = y_0 + \frac{a}{1 + (x / x_0)^b} \qquad [1]$$

where *y* is the percent goatsrue germination, *yo* is germination without scarification, *x* is duration of scarification, *a* is the maximum germination, *xo* is the scarification duration causing 50% germination, and *b* is the slope at *xo.* Data from the depth of emergence experiment were best described by a quadratic function (Zhou et al. 2005):

$$y = bx^2 + ax + y0 \qquad [2]$$

ANOVA and t-tests were performed using GLM in NCSS[4]. Non-linear regression was preformed using SigmaPlot[5].

Results and Discussion

Evaluation of Physical Seed Dormancy. Scarification of goatsrue seeds in undiluted sulfuric acid (H_2SO_4) effectively overcame physical dormancy of the seed coat. The sulfuric acid altered the impermeable seed coat, most likely at the lens, making it permeable and thus allowing imbibiton and germination. Without scarification, seed germination was 8%, which increased with increasing durations of scarification to 100% at 60 minutes (Figure 2-1). These results are consistent with previous work on other

legume species where sulfuric acid scarification was effective in removing seed coat dormancy (Mackay et al. 1995; Parera and Ruiz 2003; Teketay 1995). It has also been found to increase germination percentages in other plant families with impermeable seed coats, such as Malvaceae and Convolvulaceae (Chauhan et al. 2006; Todd et al. 2002). This highly effective scarification method may be useful for future research where goatsrue plants are required.

Effect of Seed Age on Physical Dormancy and Viability. A high percentage of 26-year-old and 6-month-old goatsrue seed subjected to acid scarification germinated. However, the two seed lots differed in scarification requirements ($P = 0.0001$). Maximum germination for old seed (99%) was reached at 10 minutes scarification, while newly harvested seed required 50 minutes for maximum germination (89%) (Figure 2-2). Old seed had greater germination than new seed for scarification times of 20, 30, and 40 minutes. However, germination was similar between new and old seed at 50 and 60 minutes of acid scarification. It is likely that 60 minutes of scarification caused damage to old seed as germination dropped to 89%. Without scarification old seed had significantly higher germination than new seed, at 61 and 23%, respectively. These results indicate that viability of 26-year-old goatsrue seed was similar to 6-month-old seed though percent dormancy was significantly less. The level of physical dormancy in 26-year-old seed may also be lower, as a much shorter scarification time was needed to reach maximum germination.

The results of long term seed viability studies have discovered many species which are capable of germination after extremely long periods of burial in the soil. One of the most notable studies established by Duvel discovered that after 38 years, 36 of 107

weed species still had viable seed (Monaco et al. 2002). Another important study by Beal determined 9 of 20 weed species still had viable seed after 40 years of burial (Monaco et al. 2002); including several species with physical seed coat dormancy (Baskin and Baskin 2006). Lewis (1973) also determined that seed of grass and crop species do not have long persistence or viability in the soil whereas legumes and weeds are classified as persistent, and remain viable for much longer periods. Legumes were also found to have similar levels of persistence in dry storage and in soil. However, many factors have been found to influence the longevity of seed in the soil including dormancy characteristics of the seed, environmental conditions, and biological interactions (Radosevish et al. 1997). Thus, though this research indicates goatsrue seed is capable of remaining viable in dry storage for at least 26 years, field studies are also needed to determine whether persistence under field conditions is similar to dry storage.

Seed Depth of Emergence. Goatsrue seedling emergence was inversely correlated with burial depth. Emergence decreased as depth increased. Seeds buried at 0.5 cm soil depth had the highest emergence at 93% (Figure 2-3). At 1, 2 and 3cm depths, emergence ranged from 90 to 87%. Emergence decreased to 56% at 8 cm and then rapidly dropped to 21% at 10 cm. No emergence occurred at 12 and 14 cm seed burial depths. Seeds placed on the soil surface had 87% germination, however, only 15% became established (data not shown in figure). These results demonstrate that soil disturbance which moves goatsrue seed to near the soil surface may be necessary for seed establishment. However, burial of goatsrue seed to depths of 12 cm or greater can prevent seedling emergence. Burying goatsrue seed with tillage could be an effective method for reducing seeding emergence in some situations. However, tillage is not

possible in many areas where goatsrue is found (i.e., wetlands, canals, etc.). Also, the persistence of goatsrue seed would enable it to germinate if subsequent tillage brought seeds back to the soil surface. The effect of seed depth on goatsrue seedling emergence may vary with different soil textures and moisture levels.

This research is similar to seedling emergence patterns found in many different species, where increasing seed burial depth results in decreasing seedling emergence (Chauhan et al. 2006; Chauhan and Johnson 2008; Benvenuti and Micchia 2001; Zhou et al. 2005). Similar to goatsrue, giant sensitive plant (*Mimosa invisa* Mart. ex Colla) a perennial legume species, and little mallow (*Malva parviflora* L.) have lower seedling emergence on the soil surface than seeds buried shallowly under the soil (Chauhan et al. 2006; Chauhan and Johnson 2008). However, some species, such as curly dock (*Rumex crispus* L.), perennial sowthistle (*Sonchus arvensis* L.), and field pennycress (*Thlaspi arvense* L.) have higher emergence on the soil surface than at shallow depths when the soil is at full water capacity (Boyd and Acker 2003). Goatsrue is different from many weed species in its ability to emerge at depths where others are inhibited or have very low emergence. Hairy nightshade seedling emergence is totally inhibited at seed burial depths between 7 and 8 cm (Zhou et al. 2005). A study of 20 weed species by Benvenuti and Micchia (2001) found seedling emergence was inhibited in most species at depths considerably less than 10 cm. Giant sensitive plant and little mallow have virtually no emergence at 8 cm seed burial (Chauhan et al. 2006; Chauhan and Johnson 2008); whereas in this study 56% of goatsrue seedlings emerged from 8 cm.

Soil Seed Bank Sampling. Goatsrue population density**,** as estimated by stem numbers were similar across sites one, three, four and five; site two was similar to site

four but had a higher density than sites one, three, and five (Table 2-2). Seed densities ranged from 74,609 seeds m^{-2} at site four to 14,832 seeds m^{-2} at site two. No correlation was found between stem densities and seed bank quantities. Though site two had the greatest number of stems m^{-2}, it had the lowest number of seeds. This may be due to the small diameter and length of the stems present at site two (observationally noted), which though greater in number may not have had the same seed production capabilities as larger stems present at other locations. Viability of goatsrue seed was high at all sites, ranging from 91 to 100% (Table 2-2). The greatest quantity of goatsrue seed found (74,609 seeds m^{-2}) in this study is higher than total seed bank numbers of weed and native plant seed found in a multitude of studies covering a variety of environments, ranging from arable fields to secondary forest (Marshall and Arnold 1994; McLaughlin and Bowers 2007; Radosevich et al. 1997; Tipping 2008; Williams and Harvey 2002).

The length of time goatsrue plants exist in a location may partially explain differences in seed quantities among different sites, which was not accounted for in this research. The previously discussed germination tests of 26-year-old goatsrue seed demonstrates its longevity and potential to persist in the soil seed bank. Thus, some sampled locations may have been infested for longer periods of time than other sites, resulting in a larger accumulation of seeds in the soil. Other plant measures such as plant biomass and height, not collected in this study, may have correlated with observed differences between seed densities. Soil factors such as levels of organic matter or nutrients, and other environmental factors like temperature or competition have an interaction with plant growth and development. Consequently, these factors may affect goatsrue's seed production and the amount of seeds found in the soil seed bank. More

research is needed to determine if correlations exist between infestation duration, plant biomass, soil properties, and goatsrue seed bank quantities. Though seed numbers differed across sites, even the lowest amount of seeds found was a substantial number, capable of reinvading a site cleared of mature plants.

The amount of non-dormant seed, as estimated by the percentage of goatsrue seed which germinated in the soil samples while in cold storage and in post extraction germination tests ranged from 7 to 20%. Thus, seed dormancy ranged from 93 to 80 % across the sampled goatsrue infested locations at the time of sampling (Table 2-2). These differences in dormancy levels could be due to environmental factors such as, soil moisture and texture found at individual sites. These factors can have an effect on the percentage of dormant seeds a plant produces (Quinlivan 1971) and on the break down of dormancy in seeds in the soil seed bank. Unpublished data (Oldham and Ransom) have shown that storage of goatsrue seeds for 140 days in cool (4 C) or warm (17 C) temperatures in either moist or dry conditions resulted in no detectable change in dormancy levels. Seeds in moist soil exposed to 1 to 5, 24 hour cycles of freeze (-6 C) and thaw (7 C) temperatures transitioned every 12 hours, also showed no change in seed dormancy. Seed dormancy release may not have been achieved in these studies due to the relatively short duration of the experiments. Goatsrue seed banks persist much longer than the length of these studies; thus, temperature and moisture may still be contributing factors to dormancy release which simply act slowly. More research is needed to determine if temperature and moisture over long periods of time contribute to the break down of goatsrue seed physical dormancy.

Understanding goatsrue seed biology has important implications to management plans as was shown in the USDA-APHIS eradication project in Cache County, Utah. During the project, goatsrue soil seed bank capabilities were not understood and thus became an obstacle to eradication (Evans et al. 1997). Our data is further proof that goatsrue forms large, highly viable, persistent seed banks which must be dealt with in order to achieve successful management of goatsrue infestations.

Past research has shown that the soil fumigant sodium methyldithiocarbamate does not sufficiently decrease goatsrue seed viability for its use as an effective management tool (Evans and Peiterson 1987). Though placement of goatsrue seed at 12 cm or deeper in the soil will prevent emergence, the tillage operations required to do this may not be feasible in all situations. Mature plants should be controlled mechanically or chemically to prevent further additions to the seed bank. However, continued research is needed to determine effective methods for goatsrue seed bank control. Research into herbicidal control of emerging seedlings, as well as seed predation or other methods to decrease the viability of the seed bank may be valuable areas of exploration. Effective methods to control goatsrue seed bank reserves will be vital to creating successful goatsrue management plans, in order to prevent seedling establishment and re-invasion of a location.

Sources of Materials

[1] Falcon 90 mm Petri dishes 1029, Becton Dickinson and Company, Franklin Lakes, NJ. USA 07417-1886.

[2] Whatman No. 3 Filter Paper, 90 mm circles, Whatman Inc., 200 Park Ave Suite 210 Florham Park NJ. 07932 USA.

[3] Parafilm, Pechiney Plastic Packaging, Menasha, WI 54952.

[4] Number Cruncher Statistical Software, NCSS, 329 North 1000 East, Kaysville, UT 84037.

[5] Sigma Plot 9.0, Sgma Plot 2004 for Windows, Version 9.0.1., SYSTAT Software, Inc., 501 Conal Blvd, Suite C. Point Richmond, CA 94804-2028.

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Table 2-1. Soil test results from goatsrue soil seed bank sampling sites in Cache Valley, Utah.

Soil properties	Unit[a]	Site 1	Site 2	Site 3	Site 4	Site 5
Texture	--	Clay loam	Clay loam	Loam	Loam	Clay Loam
pH	--	7.4	7.6	7.3	7.4	7.5
Salinity- EC_e	dS m^{-1}	0.7	0.81	0.8	1.1	0.7
Organic Matter	%	3.3	5.4	5.7	5.1	3.4

[a]dS m^{-1} = desi Siemens

Table 2-2. Goatsrue stem density, seed density, and viability, at five locations in Cache Valley, Utah.[a]

Site	Stem density		Seed density		Viability[b]		Dormancy[c]
	---------- no. m^{-2} ----------				---------- % ----------		
1	60	b	25,651	cd	91	b	80
2	85	a	14,832	d	99	a	93
3	55	b	65,447	ab	100	a	90
4	62	ab	74,609	a	94	b	87
5	53	b	45,955	bc	99	a	93

[a] Values in each column with different letters are significantly different at p = 0.05.

[b] Viability was determined using a bulk sample of seed recovered from the soil seed bank survey.

[c] Dormancy was determined by combining the percentages of seed which did not germinate in the soil sample prior to seed extraction and the percentage of seed from a bulked sample which did not germinate in a germination test.

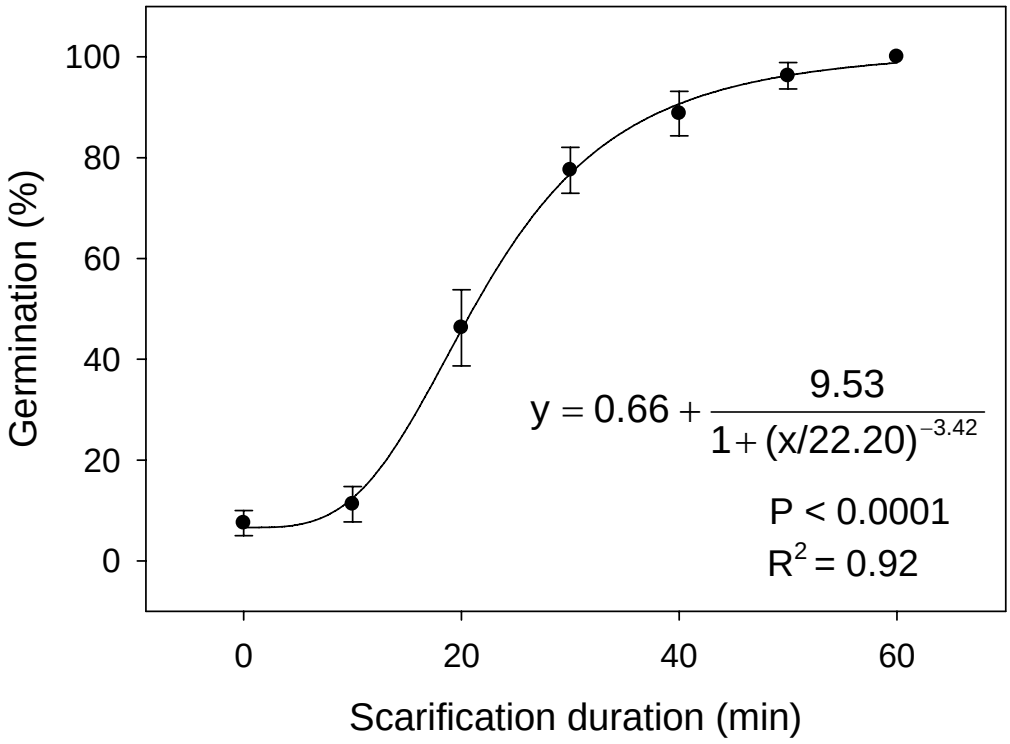

Figure 2-1. Goatsrue seed germination in response to increasing durations of scarification in undiluted sulfuric acid. Symbols represent means (n= 8) of data combined over replicates and combined over two experiments; whiskers represent the standard error of the mean

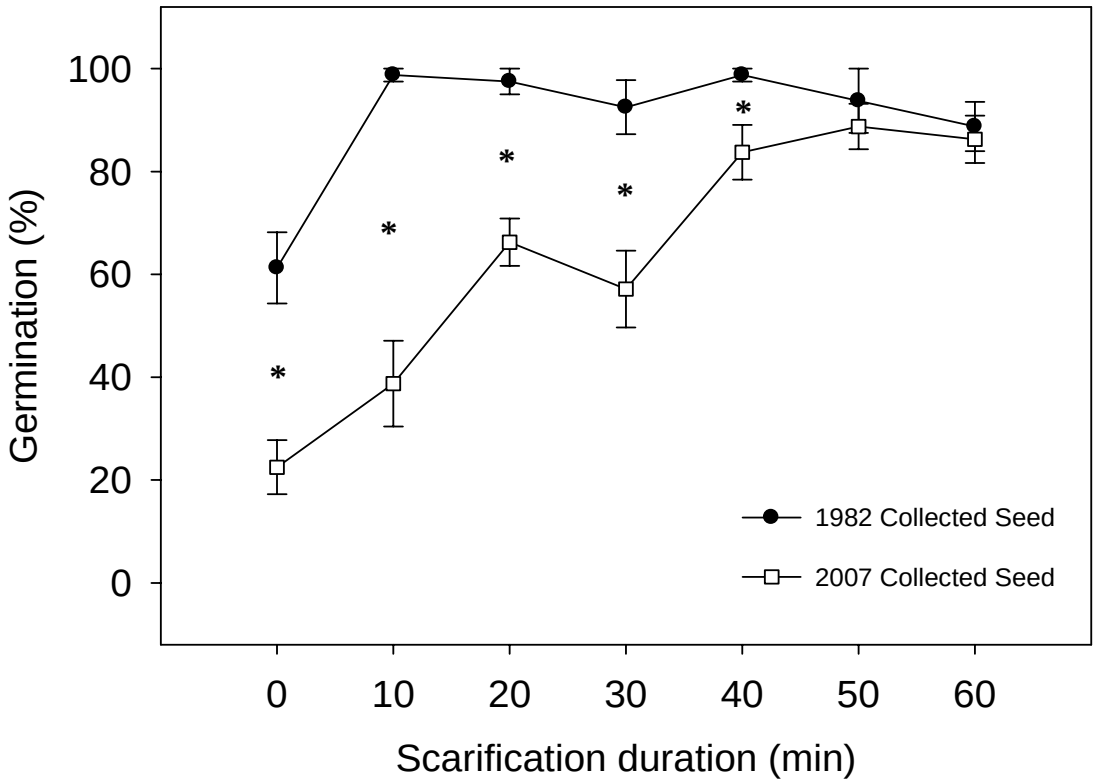

Figure 2-2. Germination response of 26-year-old and 6-month-old goatsrue seed to increasing durations in undiluted sulfuric acid. Symbols represent means (n= 8) of data combined over replicates and combined over two experiments; whiskers represent the standard error of the mean. Values with an asterisk between new and old seed lots and within scarification duration are significantly different at ($P < 0.05$) according to a paired t-test.

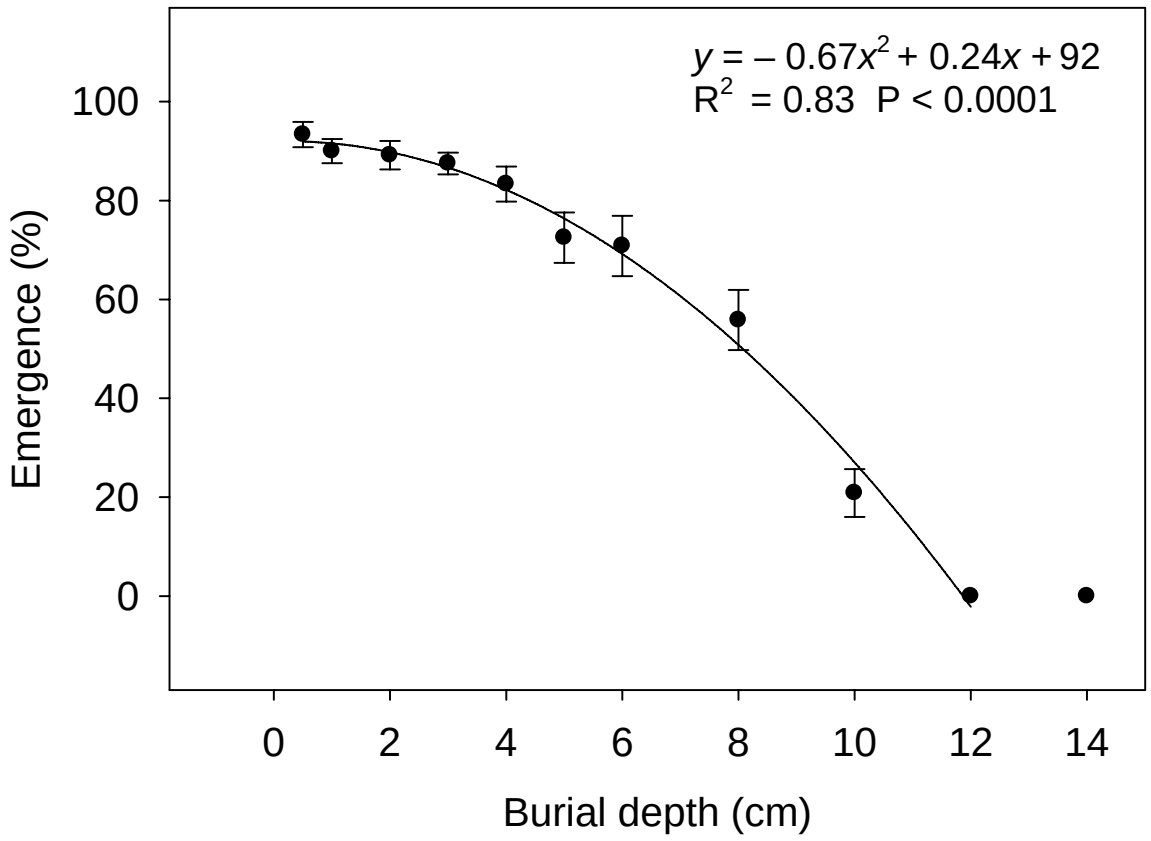

Figure 2-3. Percent goatsrue emergence from seeds buried in pots at increasing depths in a fine sandy loam soil in the greenhouse. Symbols represent means (n= 12) of data combined over replicates and combined over two experiments; whiskers represent the standard error of the mean.

CHAPTER 3

HERBICIDES FOR CONTROL OF GOATSRUE (*Galega officinalis*)

Goatsrue response to eight herbicide treatments was evaluated in greenhouse and field trials. Herbicides tested on goatsrue grown from seed in the greenhouse included; 2,4-D amine, dicamba, chlorsulfuron, picloram, imazapyr, imazamox, aminopyralid, and triclopyr. Each herbicide was applied at doses of 0.125X, 0.25X, 0.5X, 1.0X, and 2.0X, where X is equal to a typical field use rate. Goatsrue was most sensitive to the ALS inhibitors chlorsulfuron and imazapyr, with I_{50} values of 0.07X (3.7g ai ha^{-1}) and 0.16X (90 g ai ha^{-1}) respectively. Goatsrue did not respond to increasing 2,4-D and imazamox rates. The herbicides evaluated in the greenhouse were also tested at two field sites, except imazamox which was replaced by metsulfuron. Field studies gave some varying results, but overall showed that all of the tested herbicides, excluding 2,4-D and imazapyr, had greater than 93% control of goatsrue at both sites. Treatments of chlorsulfuron, dicamba, metsulfuron, aminopyralid, triclopyr, and picloram were effective at controlling established perennial goatsrue as well as increasing perennial grass cover at one site. All treatments at one site decreased seedling goatsrue cover, while only aminopyralid and picloram decreased seedling cover at another site 11 months after treatment.

Galega officinalis L., or goatsrue, was originally introduced to the U.S. in 1891 at the Utah Agricultural Experiment Station in Logan, Utah, for testing as a potential forage (Tingey 1971). During initial testing, cattle and horses showed low palatability towards goatsrue, foreshadowing the later discovery of galegine, a toxic alkaloid compound contained in goatsrue (Keeler et al. 1986, 1988, 1992). This compound is believed to cause death in sheep and other livestock which consume goatsrue (Puyt et al. 1981). By the 1980's, 86 years after its introduction, goatsrue had spread to infest approximately 60 square miles in Cache County, Utah (Evans 1984). Consequently, goatsrue became the target of a United States Department of Agriculture – Animal Plant Health Inspection Service (USDA – APHIS) eradication project beginning in 1981 and continuing through the late 1990s. The project ended without reaching eradication, primarily due to goatsrue soil seed bank reserves (Evans et al. 1997). Currently, the USDA Plants Profile identifies 13 state noxious weed lists which include goatsrue, 10 states where it occurs, and notes its classification as a Federal Noxious Weed (USDA 2008). Goatsrue has also become problematic in New Zealand, England, Chile, Ecuador, and Argentina (Gresham and Booth 1991; Oehrens and Gonzalez 1975; Patterson 1992;).

Goatsrue is a member of the Fabaceae family and is native to Europe and Western Asia (Patterson 1992). In Utah it grows in many environments including cropland, pastures, wetlands, and waterways (Evans et al. 1997). Goatsrue is a taprooted perennial which forms vigorous crowns, grows 0.6 to 1.5 meters tall, and reproduces by seed. It is not well adapted to large diurnal fluctuations in temperature found in many of its current locations in the U.S., which may partially explain its limited rate of spread (Patterson 1992).

Mowing, clipping, and cutting goatsrue are not very effective control methods as they will only reduce seed production and plant vigor, and may not be possible in all situations (Evans 1984). However, past research shows that 2,4-D and dicamba are effective herbicides for control. Mixtures of 2,4-D at 0.6 kg/ha and dicamba at 0.3 kg/ha applied twice during the growing season for two consecutive years will provide control (Evans 1984). However, control was most effective when mechanical methods were combined with herbicide applications, by clipping plants when the initial growth reaches 61 cm tall, followed by spraying the re-growth at 61 cm (Evans 1984). In cropping systems, crop rotation is an effective means of control as it interrupts the natural life cycle of goatsrue (Evans and Ashcroft 1982).

A rust fungus, *Uromyces galegae* has been investigated and used as a biological control agent for infestations of goatsrue in Chile (Oehrens and Gonzalez 1975). Tests were conducted in Zurich Switzerland with a rust strain from Toulouse, France and plants originating from Chilean goatsrue seed. These tests showed goatsrue had a high susceptibility to the *Uromyces galegae* which reduced seed production, while other legume species were unaffected. After this discovery the fungus was distributed across Chile. The south central zone of the country had the best results due to a climate which promoted distribution and growth of the rust fungus. More research is needed to determine if *Uromyces galegae* could be an effective biological control for goatsrue management in the United States and other countries.

The objective of this research was to evaluate the effectiveness of six herbicides not yet tested for goatsrue control, as well as 2,4-D and dicamba which were primarily used in previous control efforts.

Materials and Methods

Greenhouse Herbicide Trials. 2,4-D amine, dicamba, chlorsulfuron, picloram, imazapyr, imazamox, aminopyralid, and triclopyr were tested for goatsrue sensitivity in greenhouse conditions. Each herbicide treatment was applied at five different dose treatments of 0.125X, 0.25X, 0.5X, 1.0X, and 2.0X, where X is equal to the typical field use rate. Rates of each herbicide are listed in Table 3-1. Plants were grown from seed collected from approximately 10 plants at each of two locations in Cache County, Utah in the fall of 2006. Pods were hand threshed and seed was separated by blowing the chaff from the seed. Seed was stored in plastic containers at room temperature (23 C) until planting. Plants were grown in a 44% peat, 56% vermiculite, by volume medium in cone-tainers[1]. Seeds were scarified with sulfuric acid (H_2SO_4) for 60 minutes prior to planting. The trials were conducted as a Randomized Complete Block design; with six replications each comprised of a single plant. Plants were sprayed after 4 to 6 weeks of growth when they were between 13 and 30 inches in height. Treatments were applied in a laboratory booth sprayer with an 8002 nozzle, at 207 Kpa and 187 L/ha. During the experiment the greenhouse was maintained at day and night temperatures of 25 and 20 C, with approximately 16 hours natural and supplemental lighting. Plants were also measured for height; and above ground biomass was harvested, dried, and weighed.

Herbicide Field Trials. Field trials were conducted at sites heavily infested with goatsrue in Smithfield and Amalga, in Cache County, Utah. The Smithfield site was a field lot with the perennial grass species; meadow foxtail (*Alopecurus pratensis* L.), Reed

Canarygrass (*Phalaris arundinacea* L.), Quackgrass (*Agropyron repens* L. Gould), and Smooth Brome (*Bromus inermis* Leyss). The Amalga site was a long term pasture with Reed Canarygrass (*Phalaris arundinacea* L.) and a sedge (*Carex spp*. L.). The herbicides evaluated in the greenhouse were also tested in the field, excluding imazamox which was replaced by metsulfuron methyl. The 1x rates used in the greenhouse trials were used, except for treatments of 2,4-D, dicamba, and triclopyr where field rates were increased due to poor response in the greenhouse (Table 3-2).

Plots were 3 m wide by 9 m long at Smithfield and 3 m wide by 6 m long at Amalga, and arranged in a completely randomized block design with four replications. Herbicide applications were made with a CO_2-pressurized backpack sprayer calibrated to deliver 187 L/ha at 207 Kpa, when plants were in full flower (July 3 at Smithfield and July 18 at Amalga, 2007). Treatments were evaluated 12 months after spraying with a visual rating system where 0 was no damage and 100 was plant death. A line point method was used to determine vegetation cover (DiTomaso et al. 2006). Vegetation was recorded every 30.5 cm along two parallel 6 m transects. Vegetation cover was determined at 11 and 12 months after treatment (MAT). Vegetation sampling was conducted at 11 MAT because goatsrue seedlings had recently emerged and were easily differentiated from perennial goatsrue regrowth. Sampling was also conducted 12 MAT in order to describe vegetative cover of perennial goatsrue and perennial grasses at a more mature stage.

Statistical Analysis. Dry biomass data from the greenhouse trials were checked for normality and homogeneity of variances prior to Analysis of Variance (ANOVA)[2]. There were no run-by-treatment interactions allowing combination of greenhouse

biomass data across runs for analysis. Dry biomass data from the greenhouse trials were fit to the logistic dose response curve[3]:

$$y = (D - C)/[1 + (x/I_{50})^b] \qquad [1]$$

where *y* is the percentage biomass reduction, x is the herbicide dose, C is the percentage biomass at high doses, D is the percent biomass in the non-treated control, I_{50} is the dose causing 50% biomass reduction, and *b* is the slope at the I_{50} dose (Seefelt et al. 1995) I_{50} values, the relative rate of each herbicide required to reduce biomass by 50%, were derived from the dose response curves (Table 3-3).

Data from field trials were not combined due to location by treatment interactions. Data were checked for normality and homogeneity of variances prior to analysis. Visual ratings and vegetative cover from each field site were subjected to an analysis of variance and a Fischer's protected LSD means separation. Some plots had severe infestation of Canada thistle (*Cirsium arvense* L.) or field bindweed (*Convolvulus arvensis* L.) which became dominant after goatsrue was controlled by the treatments. Thus, plots with greater than 60% perennial weed cover (other than goatsrue) were excluded in the analysis for perennial grass, perennial goatsrue, and goatsrue seedling cover at both field sites.

Results and Discussion

Greenhouse Trials. Goatsrue was most sensitive to the ALS inhibitors chlorsulfuron and imazapyr, with I_{50} values of 0.07X (3.7g ai ha^{-1}) and 0.16X (90 g ai ha^{-}

^{1}), respectively. The synthetic auxin picloram was also quite effective with a 0.27X (153 g ae ha-^{1}) I_{50} value (Table 3-3, Figure 3-1). Goatsrue was also moderately sensitive to aminopyralid and dicamba, indicated by I_{50} values of 0.55X (48 g ae ha-^{1}) and 0.71X (402 g ae ha^{-1}) , respectively. Triclopyr had less effect on goatsrue indicated by the highest I_{50} value at 0.91X (1150 g ae ha^{-1}). Goatsrue did not respond to increasing 2,4-D and imazamox rates, and thus never reached an I_{50} value.

Field Trials. Metsulfuron, chlorsulfuron, dicamba, and picloram gave 100% control of established goatsrue plants at both sites 12 MAT (Table 3-2). Triclopyr, aminopyralid, and imazapyr, resulted in 100% control at the Amalga site (P = 0.0001) and 97, 93, 84% control at the Smithfield site (P = 0.0001), respectively. Goatsrue control with 2,4-D was variable providing 98% control at the Amalga site, but only 9% at Smithfield. Variability between sites may be partially attributed to site differences; the Amalga site was in a pasture on a moist north east facing slope with predominantly reed canarygrass; while the Smithfield site appeared to be drier and had a variety of perennial grasses.

Line intercept vegetation sampling results were well correlated with our visual control results at Smithfield (R^2 = 0.95) (Figure 3-2). Correlations were not necessary for the Amalga site as control was 100% for all treatments except, 2,4-D at 98% control. The line intercept vegetation sampling from Smithfield showed that all treatments excluding 2,4-D and imazapyr, increased perennial grass cover compared to the control plots (P = 0.0001). 2,4-D was ineffective in controlling goatsrue, thus perennial grasses did not increase as goatsrue was still dominant. Although imazapyr had 84% control of perennial goatsrue it also injured perennial grasses; reducing their regrowth and

competitiveness leading to an increase of other weed species. At Amalga, only treatments of dicamba and metsulfuron led to a significant increase of perennial grass cover (P = 0.0390) compared to the untreated. All treatments at Amalga may not have led to a significant increase in grass cover as the site had a strong perennial grass cover prior to treatment. Goatsrue removal at Amalga would be less likely to significantly increase the cover of grass present, as opposed to the removal of an almost totally goatsrue dominated canopy with little perennial grass cover at Smithfield. Imazapyr followed the same trend at Amalga as occurred at Smithfield; good control of goatsrue along with injury to perennial grasses, resulting in the lowest percentage of grass cover (20%) at Amalga.

Eleven MAT all treatments showed a significant decrease in goatsrue seedling cover at Smithfield (P = 0.0001) and picloram and aminopyralid showed a significant decrease at Amalga (P = 0.0001) (Table 3-4). This decrease in seedling cover may be due to herbicide inhibition of seed production the previous year, residual herbicide control, and competition from perennial grasses. Eleven MAT, imazapyr treatments at Amalga resulted in a significant increase in seedling goatsrue cover, due to herbicide injury of perennial grasses. Due to confounding from an increasing perennial goatsrue canopy seedling goatsrue cover 12 MAT is not shown.

In both field and greenhouse trials goatsrue showed high sensitivity to chlorsulfuron and picloram. Imazapyr was effective against goatsrue in the greenhouse with the second lowest I_{50} value and resulted in 100% control at Amalga but only 84% at Smithfield. Though dicamba and triclopyr showed poor response in the greenhouse, the increased rates used in the field resulted in at least 97% control. Aminopyralid, to which

goatsrue showed only marginal sensitivity in the greenhouse, resulted in at least 93% control at both field sites. Goatsrue showed almost no response to 2,4-D in the greenhouse and at the Smithfield field site, however at Amalga it resulted in nearly 100% control. The reasons for this large difference in 2,4-D performance is unknown, but control of goatsrue with 2,4-D is expected to be poor in most conditions. Differences seen between the greenhouse and field trials may be due to many factors which differ between the two environments; including, temperature, moisture, nutrient levels, and competition. A greenhouse environment may favor goatsrue recovery from herbicide damage with its optimal temperature, moisture, and nutrient levels as opposed to the dry and hot field conditions found in the summer after herbicide application. Goatsrue plants in the field were also exposed to harsh winter conditions, as well as competition from perennial grasses which may have further weakened the plants' vigor and ability to recover from herbicide treatment.

Annual goatsrue seedling emergence from soil seed bank reserves became a major inhibitor to eradication success in the USDA-APHIS goatsrue eradication project. Thus, the use of a herbicide which is effective against perennial goatsrue and has residual activity against seedlings may be a benefit to future control efforts. The majority of herbicides tested in this research should provide excellent control of perennial goatsrue with a single application as opposed to the double application, or mechanical followed by chemical treatment found necessary for effective treatment in the past. However, depending on the situation and environment (proximity to water and desirable species present) some of the tested herbicides may be more beneficial than others. Though this research indicates some of the tested herbicides may provide 1 year residual control of

goatsrue seedlings, more research is needed to determine long-term residual control. Determining herbicide residual control of annual goatsrue seedlings will be critical to determining the most effective management plans; in order to prevent seedling establishment and re-invasion of a location cleared of mature goatsrue plants.

Sources of Materials

[1] Cone-tainers, Stuewe & Sons Inc., 2290 SE Kiger Island Drive Corvallis, Oregon 97333-9425.
[2] Number Cruncher Statistical Software, NCSS, 329 North 1000 East, Kaysville, UT 84037.
[3] Sigma Plot 9.0, Sigma Plot 2004 for Windows, Version 9.0.1, SYSTAT Software Inc., 501 Conal Blvd, Suite C. Point Richmond, CA 94804-2028.

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keywordquery=galega+officinalis&mode=sciname. Accessed: September 2, 2008.

Table 3-1. Herbicide rates and corresponding proportions of the field use rate used in goatsrue dose response greenhouse trials.

	Proportion of field use rate[a]				
Herbicide	0.125X	0.25X	0.5X	1X	2X
	----------g ai/ha or g ae/ha----------				
2,4-D amine	140	280	560	1121	2242
Dicamba	71	141	283	566	1132
Chlorsulfuron	7	13	26	53	105.4
Imazapyr	71	141	283	566	1132
Imazamox	71	141	283	566	1132
Aminopyralid	11	22	44	88	176
Triclopyr	158	316	632	1264	2529
Picloram	71	141	283	566	1132

[a]Rates of chlorsulfuron, imazapyr, and imazamox are presented in g ai/ha.

Table 3-2. Herbicide rates, visual percent goatsrue control, percent perennial goatsrue cover, and perennial grass cover 12 months after treatments at two field sites in Cache County,Utah.[a]

		Goatsrue control				Goatsrue cover				Perennial grass cover			
Herbicide	Rate[b]	Amalga		Smithfield		Amalga		Smithfield		Amalga		Smithfield	
	g ae/ha	---------- % ----------											
Untreated	---	---	---	---	---	35	a	89	a	32	cd	2	e
2,4-D	2242	98	b	9	c	0	b	80	a	61	abc	9	de
Dicamba	2242	100	a	100	a	0	b	0	b	69	ab	32	cd
Chlorsulfuron	53	100	a	100	a	0	b	0	b	50	a-d	59	ab
Imazapyr	566	100	a	84	b	0	b	3	b	20	d	9	de
Metsulfuron	42	100	a	100	a	0	b	0	b	84	a	78	a
Aminopyralid	88	100	a	93	ab	0	b	3	b	39	bcd	74	a
Triclopyr	1681	100	a	97	a	0	b	0	b	57	abc	48	bc
Picloram	566	100	a	100	a	0	b	0	b	38	bcd	57	abc

[a]Values within columns followed by different letters are significantly different at P = 0.05.
[b]Chlorsulfuron and imazapyr are in g ai/ha. All treatments included NIS at 0.25% v/v.

Table 3-3. Parameter estimates for non-linear regression analysis of goatsrue biomass response to increasing rates of several herbicides.[a]

Herbicide	*D*	*C*	I_{50}	*b*	R^2
	----------%----------		% of field use rate		
2,4-D amine	103.62 (2.29)	89.50 (186.0)	I_{50} > 2X rate	--	--
Dicamba	100.00 (2.01)	6.03 (10.64)	0.71 (0.15)	1.23 (0.18)	1.00
Chlorsulfuron	99.99 (2.84)	13.13 (10.64)	0.07 (0.02)	0.72 (0.37)	1.00
Imazapyr	10.18 (2.97)	26.87 (2.63)	0.16 (0.01)	1.95 (0.36)	1.00
Imazamox	111.75 (8.52)	91.31 (8.55)	I_{50} > 2X rate	--	--
Aminopyralid	97.46 (12.59)	0.00 (81.30)	0.55 (1.18)	0.91 (0.96)	0.93
Triclopyr	92.54 (5.82)	40.22 (20.26)	0.91 (0.37)	2.85 (2.72)	0.93
Picloram	99.08 (10.61)	16.56 (21.48)	0.27 (0.16)	1.27 (0.87)	0.95

[a]C is the percentage biomass at high doses, D is the percent biomass in the non-treated control, I_{50} is the dose causing 50% biomass reduction, and *b* is the slope at the I_{50} dose

Table 3-4. Seedling goatsrue cover 11 months after treatments at two field sites in Cache County, Utah

		Seedling goatsrue cover 11 MAT[a]			
Herbicide	Rate[b]	Amalga		Smithfield	
	g ae/ha	---------- % ----------			
Untreated	---	21	bc	34	a
2,4-D amine	2242	28	bc	8	bcd
Dicamba	2242	36	abc	8	bcd
Chlorsulfuron	53	20	c	0	d
Imazapyr	560	55	a	18	b
Metsulfuron	42	39	ab	3	cd
Aminopyralid	88	4	d	1	d
Triclopyr	1681	36	abc	12	bc
Picloram	560	0	d	0	d

[a]Values within columns followed by different letters are significantly different at P = 0.05.
[b]Chlorsulfuron and imazapyr are in g ai/ha. All treatments included NIS at 0.25% v/v.

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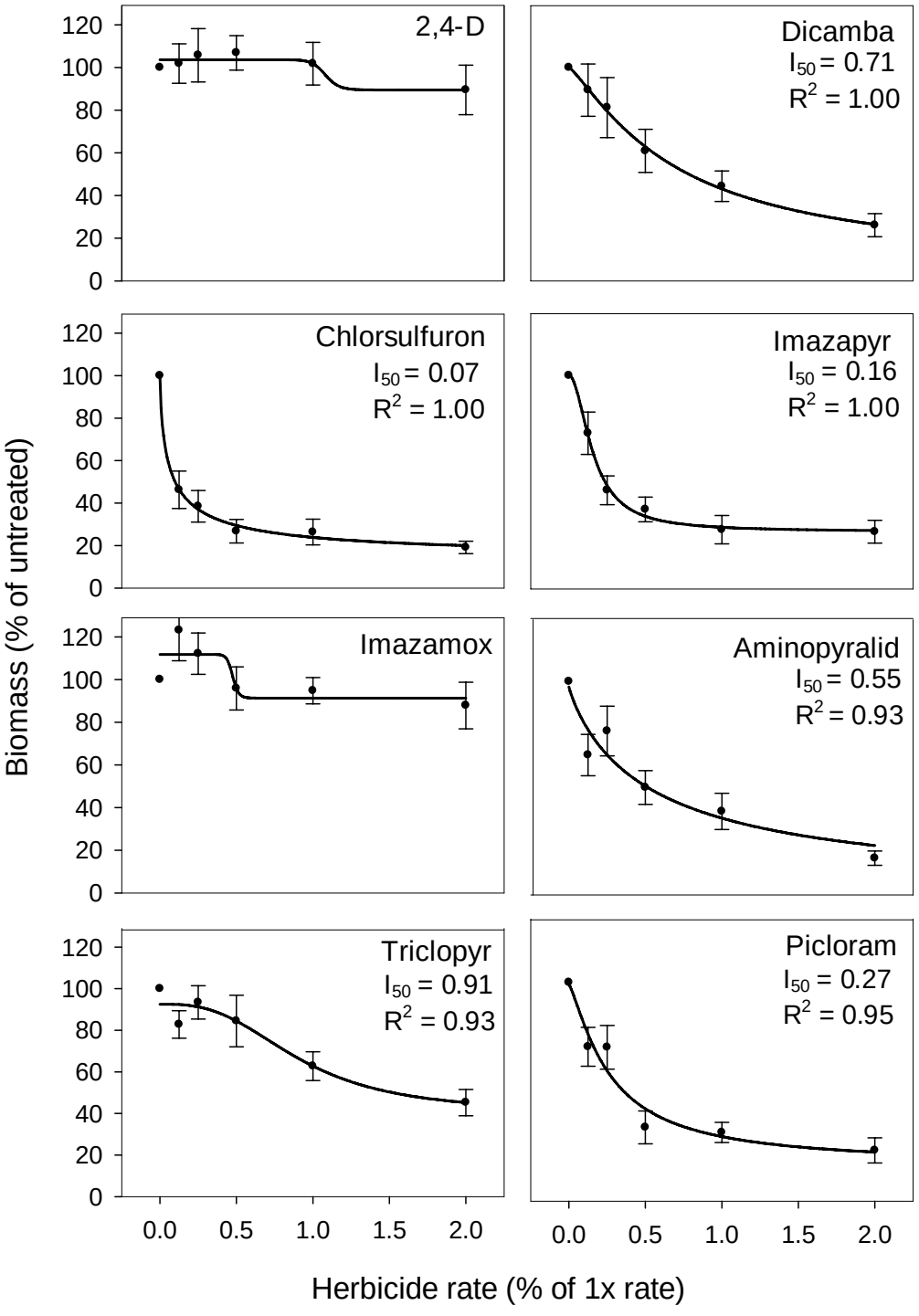

Figure 3-1. Goatsrue biomass response to increasing herbicide rates in greenhouse trials. Regression estimates are shown in Table 3-3. Symbols and whiskers represent the means and standard errors of four replicates of combined runs (n=8).

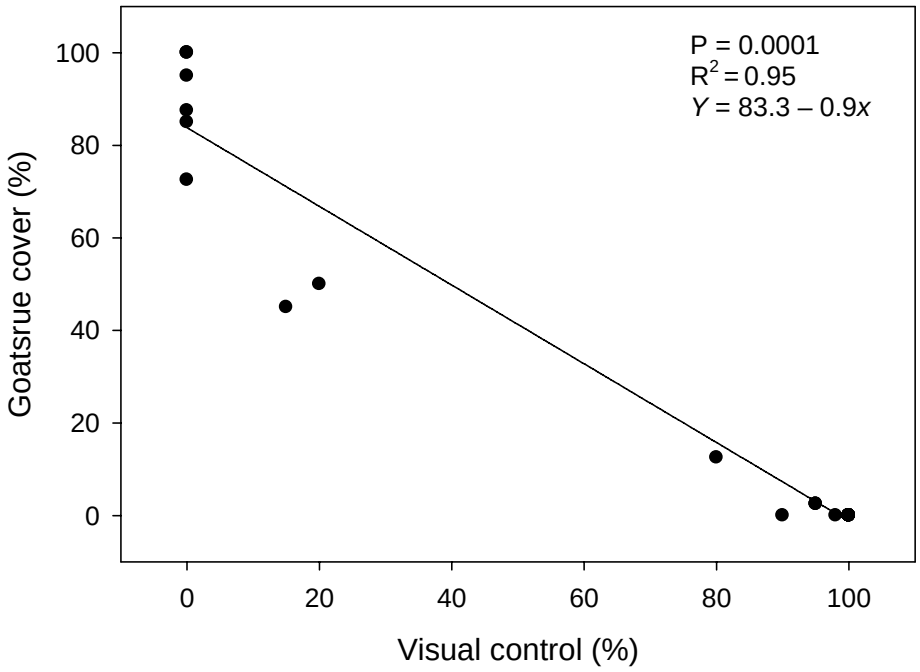

Figure 3-2. Linear regression comparing percent visual estimates of goatsrue control and goatsrue percent cover at the Smithfield site.

CHAPTER 4

GOATSRUE (*Galega officinalis*) TOXICITY

Abstract *Galega officinalis*, commonly known as goatsrue, is a member of the Fabaceae family, native to Europe and Western Asia. It is a toxic plant due to the alkaloid galegine. In order to determine average galegine concentration, concentration in above ground plant parts, and total galegine pools over phenological growth stages, 20 goatsrue plants at four locations were sampled. Plant parts were freeze dried, ground, and analyzed with liquid chromatography/mass spectrometry (LC-MS). Galegine concentration was significantly different in plant tissues; reproductive tissues had the highest levels of galegine (7 mg/g), followed by leaf (4 mg/g) and finally stem (1 mg/g) tissues. Galegine concentration and pools varied over plant tissues and phenological growth stages. Galegine pools (dry weight x concentration) or the total amount of galegine per stalk, were lowest at the vegetative growth stage (2 mg/stalk) and increased until reaching a maximum at the immature pod stage (91 mg/stalk). This maximum decreased nearly in half (48 mg/stalk) by the mature seed stage. Like galegine pools, galegine average concentration also reached a maximum at the immature pod stage (4 mg/g), and decreased nearly in half by the mature seed stage (2 mg/g).

Introduction

Galega officinalis, commonly known as Goatsrue, is a member of the Fabaceae family, native to Europe and Western Asia (Patterson, 1992). It was first introduced to Logan, Utah in 1891 for testing as a potential forage crop. Goatsrue is now known to have low palatability to cattle and horses and contains the toxic alkaloid galegine. It is a taprooted perennial which grows 2 to 5 feet tall and reproduces by seed. Goatsrue currently occurs in 10 states in the U.S., is included on 13 state noxious weed lists, and is classified as a Federal Noxious Weed (USDA, 2008). It has also become problematic in New Zealand, England, Chile, Ecuador, and Argentina (Patterson, 1992; Gresham and Booth, 1991; Oehrens and Gonzalez, 1975). Goatsrue is not well adapted to large diurnal temperature fluctuations found in many areas of current infestations in the United States, which may partially explain its limited rate of spread (Patterson, 1992).

Gresham and Booth (1991) recorded the first reported case of sheep poisoning in the UK in 1991; and cite other instances of poisoning in France, Romania and New Zealand. Research done at the USDA-ARS, Poisonous Plant Research Laboratory in Logan, UT confirmed the presence of galegine in goatsrue as well as its potential to cause death in sheep (Keeler et al., 1986, 1992). Galegine is believed to lower blood pressure and is a central nervous system paralytic (Keeler et al., 1986). It is found at an average concentration of 0.46% in goatsrue leaves (Keeler et al., 1992). Clinical signs of poisoning in sheep can occur at doses as low as 0.8 g of dried plant per kg body weight per day (Keeler et al., 1986). However, research has shown there is a great amount of individual animal variation in toxicity responses, as a does of 24 g of goatsrue per kg animal body weight has also been shown to induce no pathological lesions. This wide

spectrum of individual animal variation may be due to differences in metabolic response to galegine (Keeler et al., 1988).

Little is know about goatsrue and its inherent toxicity due to galegine. Thus, the objectives of this research were to determine (1) concentration of galegine in goatsrue plant parts, and (2) the average concentration and galegine pools over phenological growth stages.

Methods and Materials

Five uniform plants were selected at each of four sites in Cache County, Utah. Two sites were stream/canal side infestations, and two sites were long term pasture sites. One stem was harvested from each plant at preselected phenological stages of development; vegetative (emergence), bud, flower, immature pod, and mature seed pod. Sites were carefully monitored and samples were harvested at the peak of each phenological stage. Sampling began in early May and continued through the beginning of September, 2008. Each stem was separated into plant parts (stem, leaflets, buds, flowers, seed pods) and frozen until subsequent freeze drying. Dry weights were obtained after freeze drying, after which samples were ground through a Wiley mill with a 1-mm screen.

A modified method of Dorling et al. (2004) was used to extract and analyze galegine from goatsrue seed and plant tissues. A 0.1 g sample of dried ground plant tissue from each sample was stirred in 4 mL methylene chloride (CH_2Cl_2) and 5 mL water (containing 50 µg/mL riddelline *N*-oxide) over night. The mixture was separated by centrifuge and a 1.0 mL aliquot of the upper aqueous fraction was added to a 1.5 mL

autosampler vial. Analysis of galegine was accomplished using liquid chromatography/mass spectrometry (LC-MS). For LC-MS experiments, gradient high-performance liquid chromatography (HPLC) conditions were used with a 100 x 2.1 mm column packed with 5 µm biobasic-4 solid phase (Thermo Fisher Scientific); solvent flow at 0.3 mL/min of 20 mM ammonium acetate (A) and methanol (B) starting with an isocratic flow of 5% B for 1 min, followed by a linear gradient to 40% B from 1 – 10 min. The column was returned to the starting solvent composition (5% B) and allowed to equilibrate for 5 min between analyses. Injection volume was 5 µL and flow from the column was connected directly to an atmospheric pressure chemical ionization (APCI) source and LCQ Advantage Max mass spectrometer (Thermo Fisher) operated with source voltage of 4.23kV; a vaporizer temp of 450°C; capillary voltage 23V; capillary temperature 250°C scanning a mass range of 60 – 300 amu. Reconstructed ion chromatograms were used for detection of the target analyte, galegine (m/z = 128), and the internal standard, riddelline *N*-oxide (m/z = 366) and integration of peak areas. Standards were prepared by dilution of 0.107 mL of stock galegine (5 mg/mL galagine sulfate in water with 50 µg/mL riddelline N-oxide) into 0.893 mL of water (50 µg/mL riddelline N-oxide) and serial diluted to give 150, 75, 37.5, 18.75, 9.38 µg/mL galegine standards.

Statistical Analysis. Concentration of galegine was compared among plant parts, growth stages, and site by a mixed model analysis of variance (SAS, 1999). Growth stage, plant parts, and sites were the fixed effects, and individual plants were the random effect. Data did not meet assumptions of normality and homoscedasticity and a log transformation was applied to correct the data. All the main effects had interactions, thus

the model was reduced and each plant part was analyzed separately across stages and sites.

Galegine pools and average concentration were analyzed with a mixed model analysis of variance, with site and growth stage as fixed effects, and plants as the random effect. Data were again corrected with a log transformation in order to meet the assumptions of normality and homoscedasticity. Means were separated by the PDIFF function within the mixed model and sorted by letter differences by PDMIX 800 C. Galegine pools are the total quantity of galegine in each stalk and were calculated by multiplying the galegine concentration by the dry weight of each plant part followed by addition of the parts together. Average galegine concentration was calculated by dividing galegine pools by the total biomass of each stalk.

Results

Galegine concentrations differed significantly among leaf, stem, and floral tissues. When reproductive tissues were present (bud through mature seed stages) they had significantly higher concentrations (7 mg/g) than leaf (4 mg/g) and stem (1 mg/g) tissues ($p = <0.0001$) (Table 4-1). In the reduced model, galegine concentration in stem tissue was significant across stages ($p = < 0.0001$), but not across sites ($p = 0.3008$), with no stage by site interactions (Figure 4-1). Concentrations were highest at the vegetative stage (2 mg/g) and decreased through the growing season reaching the lowest concentration at the mature seed stage (1 mg/g). Reproductive tissue galegine concentration was also significant across stages ($p = < 0.0001$), with no stage by site interactions. Concentrations in reproductive tissues were highest at the bud and

immature pod stages (9 mg/g), and dropped to the lowest concentration at the mature seed stage (5 mg/g). Leaf tissue galegine concentrations were significantly different across stages ($p = < 0.0001$) and sites ($p = 0.0136$), with an interaction that was significant at $p = 0.0514$. Thus, all sites have been graphed across stages to illustrate the interactions. Site two leaf tissue did not decrease in concentration from the immature pod to mature seed stage as did all other sites, as well as all sites within floral and stem tissues. Galegine concentrations in leaf tissue at site two may have remained elevated at the mature seed stage in response to heavy grasshopper feeding at the site. This damage to leaf tissue may have stopped cellular processes thus disabling transport of galegine out of damaged tissues at the mature seed stage.

Average galegine concentration for an entire stem was significantly affected by sites ($p = 0.0133$) and stages ($p = <0.001$). Galegine levels were similar among sites, except site two which was significantly higher than sites four and one; possibly in response to heavy grasshopper feeding. Galegine concentrations were similar between the vegetative and bud stages, decreased at the flowering stage, increased to a maximum at the immature pod stage (4 mg/g), and decreased at the mature seed stage to a level similar to the flowering stage (2 mg/g) (Figure 4-2).

Galegine pools which are influenced by biomass and concentration were significantly different across phenological growth stages ($p= <0.0001$). Pools were lowest in the early vegetative growth stage (1 mg/stem) and increased through the bud and flowering stages, peaking dramatically at the immature pod stage (91 mg/stem) (Figure 4-1). However, after this peak, pools dropped nearly in half at the mature seed

stage (48 mg/stem). Goatsrue biomass was significant across stages ($p = < 0.0001$), and an interaction was present between stage and site ($p = 0.0142$).

Discussion

The results of this research appear to support several theories of plant defense. The resource availability growth rate hypothesis (Coley et al., 1985; Herms and Mattson, 1992) works on an evolutionary time scale to explain the types of defense that have evolved. It states that the optimal level of defense investment varies with growth rate of the plant; as the potential growth rate decreases, the optimal level of defense increases (Coley et al., 1985). Thus in resource rich habitats, fast growing plants with low levels of N-based defenses are most competitive. Goatsrue falls into this category as it grows quickly in resource rich habitats and has the N-based defense compound galegine in relatively low concentrations.

The carbon-nutrient balance theory (Bryant et al., 1983; Herms and Mattson, 1992) suggests the level of investment in secondary metabolites is a balance between photosynthesis and growth, which is sensitive to the carbon / nutrient (C:N) balance of the plant. Synthesis of Nitrogen-based secondary metabolites such as alkaloids are inversely related to the C:N balance, while carbon based compounds are positively correlated. Thus, if growth is slowed by factors which decrease the C:N balance, such as mild water stress or reduced photosynthesis, nitrogen becomes available for use in the production of N- based defense compounds. Goatsrue biomass (an indicator of growth) increased until the flowering stage, and then appeared to remain similar through the immature pod and mature seed stage at three sites. This slow down in growth is

correlated with a dramatic increase in galegine concentration and pools at the immature pod stage. Thus it appears as goatsrue growth slowed (possibly in response to water stress, physiological maturity, or other limiting resources) the extra available nitrogen resources were funneled into galegine production, pushing average concentration and pools to their highest levels. However, previous to these high levels, average galegine concentration decreased at flowering. This decrease in galegine may be due to the nutrient investment required for the formation and maturation of reproductive structures (Alpert et al., 1985; Boege and Marquis, 2005; Herms and Mattson, 1992; Thompson and Stewart, 1981). As alkaloid synthesis competes directly with protein synthesis, periods of intense plant growth may limit alkaloid synthesis due to a lack of substrate or energy (Herms and Mattson, 1992). To help decrease costs to plant growth, some secondary metabolites can be metabolized and made available for primary metabolism (Herms and Mattson, 1992). Thus, it is reasonable to conclude that during a period of increasing plant growth when nutrients are being shifted to reproductive structures (increasing the C:N ratio in the foliage) (Herms and Mattson, 1992), that galegine production ceased and some galegine may have been metabolized to help meet the resource needs of the plant. Galegine pools were not as significantly affected as were average concentration at flowering, remaining similar to the bud stage, as they were buffered by a continued increase in plant biomass.

When examining galegine concentrations in individual plant parts, the optimal defense hypothesis also appears play a role in goatsrue toxicity. This hypothesis states that in order to optimize fitness, plants allocate resources between growth, reproduction, and defense. The optimal level of plant defense must balance metabolic costs of

producing secondary metabolites with the benefits of avoiding tissue loss through herbivory. Defense is costly as it diverts resources away from plant growth, thus it is allocated to tissues that optimize the cost (Herms and Mattson, 1992) and whose protection will most greatly increase the fitness of the plant (McKey, 1974). This research indicates galegine levels were the highest in reproductive tissues, followed by leaf and then stem tissue. Velvet lupine (*Lupinus leucophyllus*) has also been shown to have greater secondary compounds in reproductive tissues (Lee et al., 2007). This unequal distribution of galegine among tissues appears to maximize the cost of defense by protecting tissues which will increase plant fitness the most. Reproductive tissues (buds, flowers, seeds) are defended the most as they are crucial to ensuring another generation of plants. Leaves may be more highly defended than the stems as they are more susceptible to herbivory than the structurally reinforced stem and are more crucial for carbohydrate creation, ensuring enough energy for plant re-growth the following year.

Seed pods were excluded from analysis at the mature seed stage, thus, reproductive tissue at this stage consisted only of mature seeds. The analysis showed that at the mature seed stage, goatsrue seed had a galegine concentration of 5.4 mg/g . As goatsrue often grows along waterways and in wetland areas, there is a potential for ingestion of goatsrue seeds by waterfowl or other birds and consequently a possibility of poisoning. More research is needed to determine the threat goatsrue seed toxicity may pose to wildlife.

The drop in average galegine concentration and pools at the mature seed stage also appears to fit into the optimal defense theory. Goatsrue is a perennial and re-grows from its root system each spring; hence, protection of this tissue (along with seeds) is of

great importance to plant fitness. Thus, the decrease in galegine pools and average concentration in above ground tissues at the mature seed stage may be due to galegine translocation into the root system (McKey, 1974; Ralphs et al., 2000), or possibly galegine metabolization and then translocation into the root system. Another later sampling of goatsrue tissues may have elucidated whether galegine concentration in leaves and stems continue to decrease, eventually removing all galegine from above ground biomass. A small part of the decrease in galegine concentration and pools at the mature seed stage may be due to the exclusion of seed pod material from the analysis.

In summary, goatsrue toxicity due to the alkaloid galegine, varies over plant tissues and phenological growth stages. Galegine concentrations among plant tissues are significantly different and are highest in reproductive tissues, followed by leaf and then stem tissue. Galegine pools increase from their lowest level at the vegetative stage to a maximum at the immature pod stage, followed by a decrease at the mature seed stage. Average galegine concentration also peaks at the immature pod stage and drops nearly in half at the mature seed stage. Thus, goatsrue is most toxic in its phenological development at the immature pod stage.

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Table 4-1 Galegine concentrations in stem, reproductive, and leaf tissues over sites.

	Galegine concentration (mg/g)		
Site	Stem	Reproductive	Leaf
1	1.30 a	6.32 b	3.56 bc
2	1.85 a	8.53 a	6.22 a
3	1.34 a	7.92 a	4.43 ab
4	1.27 a	6.64 b	2.80 c
Mean	4.42 B	7.35 A	1.30 C

Values (n=25) within a plant tissue with different lower case letters are significantly different at p = 0.05. Plant tissue means (n= 125) with different capital letters are significantly different at p = 0.05.

Figure 4-1. Galegine concentration over growth stages in goatsrue leaf, reproductive, and stem tissues. In reproductive and stem tissue graphs symbols represent means (n=20) and whiskers represent standard errors; symbols labeled with different letters are significantly different at p = 0.05.

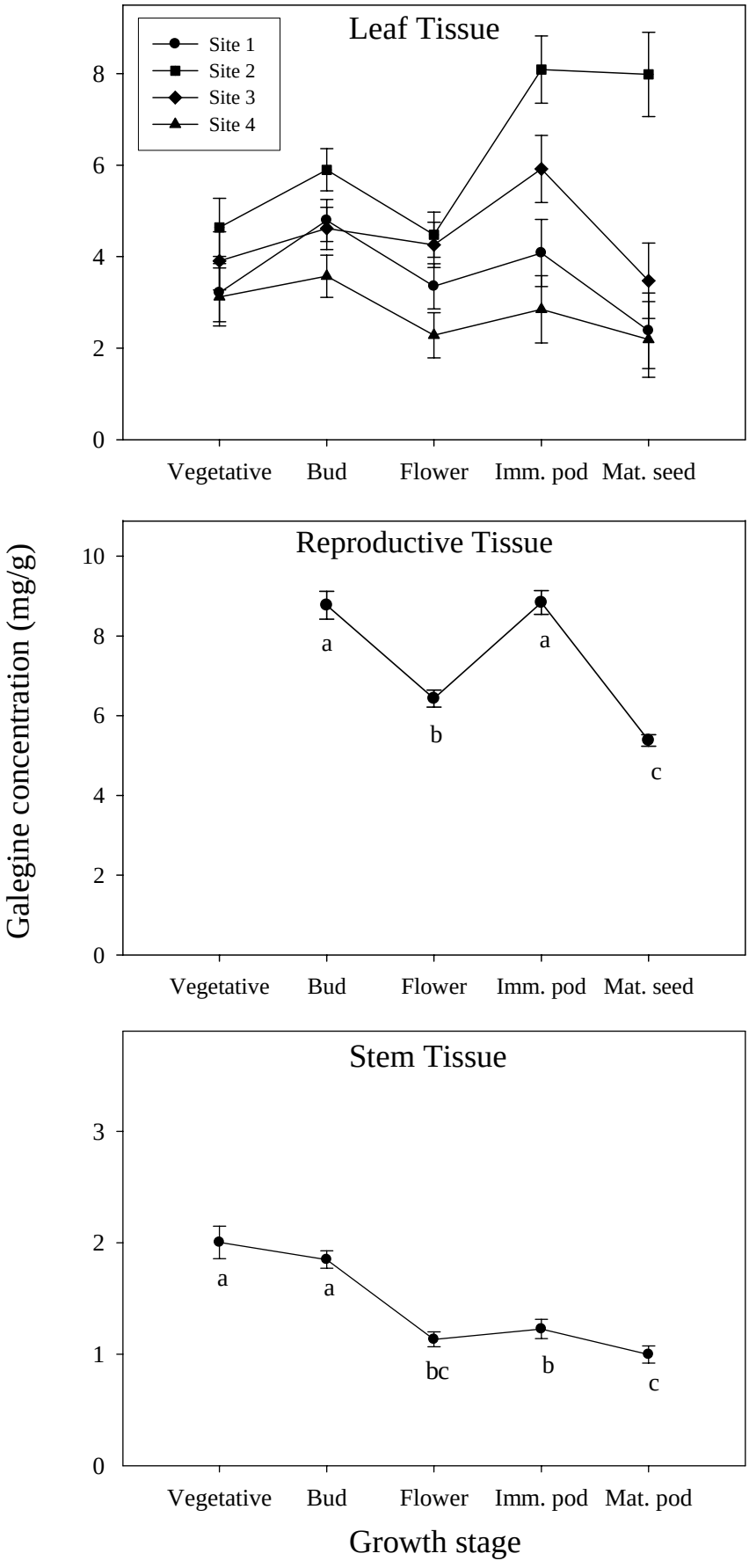
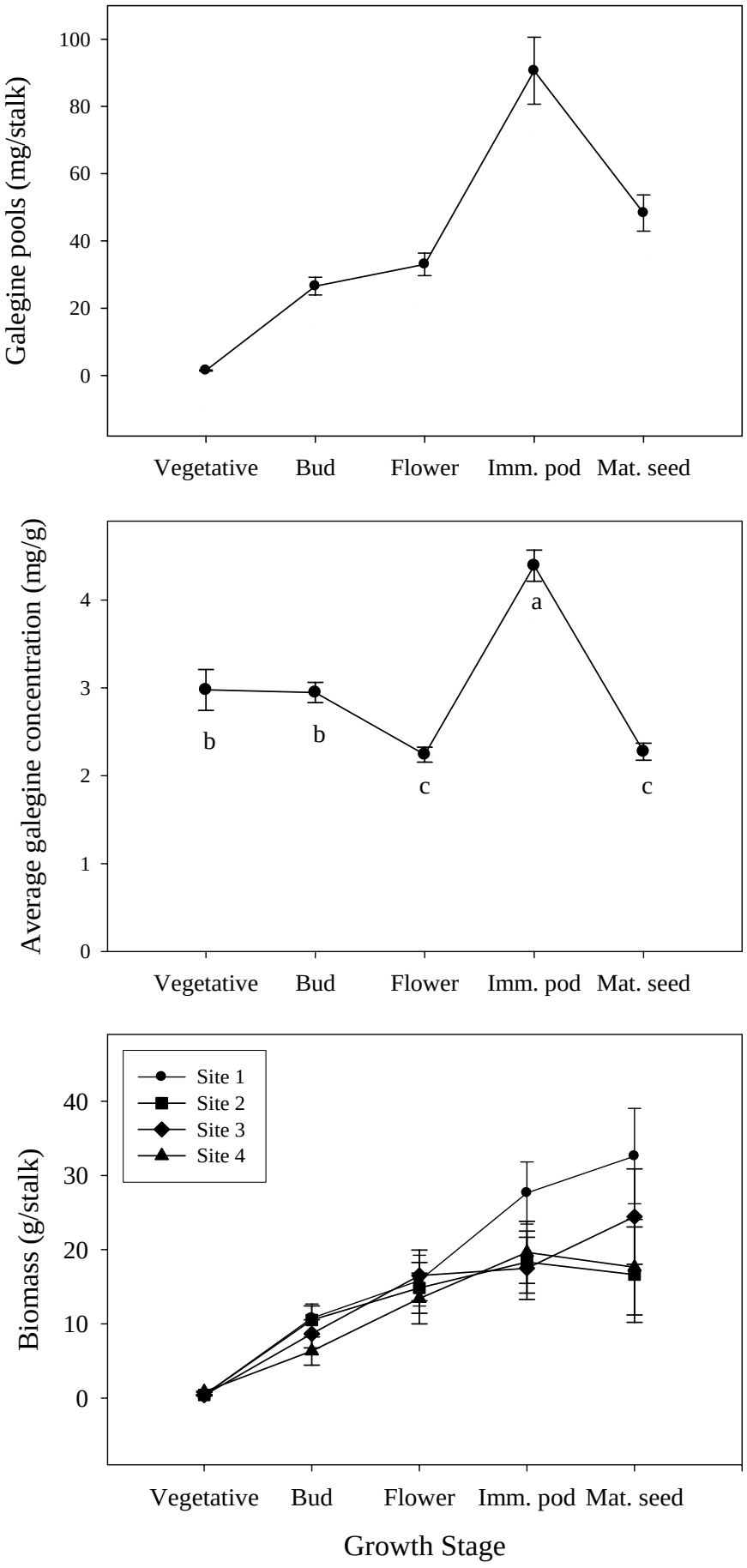


Figure 4-2. Galegine pools, average concentration, and biomass of goatsrue over growth stages. Galegine Pools and Average Concentration graphs symbols represent means (n=20) and whiskers represent standard errors; symbols labeled with different letters are significantly different at p = 0.05.



CHAPTER 5

SUMMARY AND CONCLUSIONS

This research has greatly expanded the current knowledge and understanding of the little studied invasive plant goatsrue. This work furthers understanding of its seed biology, options for control of perennial plants, as well as toxicity concentration and pools through phonological growth stages.

The seed biology studies described in this paper have provided insight into the characteristics of goatsrue seed. Newly matured goatsrue seed was found to be highly physically dormant. Comparison of newly mature seed and 26 year-old-seed showed that while dormancy decreased in old seed, viability remained similar to new seed. Goatsrue seed emerged most readily from shallow burial depths (0.5-3 cm), but remained capable of emergence to 10cm burial depth. A soil seed bank sampling of five goatsrue infested sites showed goatsrue's capability to form a significant seed bank ranging from 74,609 to 14,832 seeds m^{-2}. These findings highlight the importance of preventing additions to the goatsrue soil seed bank, as well as the need for soil seed bank control, as goatsrue infestations are capable of producing very large soil seed bank reserves which may remain physically dormant for long periods while retaining incredibly high viability. Clearly if goatsrue soil seed bank reserves are not taken into consideration in management plans, effective control is not likely as long seed characteristics enable the seed bank to supply seedlings to re-invade locations.

This research also identified many new herbicides which are more effective and efficient for control of perennial goatsrue plants than treatments utilized in the past. Metsulfuron, chlorsulfuron, dicamba, triclopyr, and aminopyralid were all highly

effective treatments, which also resulted in increased perennial grass cover at one location. The above stated herbicides may also be effective in reducing goatsrue seeding cover the spring following treatment, due to seed production inhibition the previous year as well as residual herbicide in the soil. More research is needed to determine long term soil residual control provided by the herbicides effective against perennial goatsrue, as well as alternative methods for controlling the soil seed bank.

This research also showed that goatsrue toxicity, due to the alkaloid galegine, varies over plant tissues and phenological growth stages. Reproductive, leaf, and stem tissues are significantly different, beginning at the bud stage when reproductive tissues are initiated through the mature seed stage, reproductive tissues have the highest levels of galegine, followed by leaf and finally stem tissues. These differences in plant part galegine concentration reflect the optimal plant defense theory, as defenses are differentially allocated to tissues depending on their value to plant fitness. Galegine pools were lowest at the vegetative growth stage and increase until reaching a maximum at the immature pod stage. This maximum decreases nearly in half by the mature seed stage. Like galegine pools, galegine average concentration also reaches a maximum at the immature pod stage and decreases nearly in half by the mature seed stage. As goatsrue growth slows after the flowering stage, the C:N balance likely decreases allowing for an increase in the production of galegine that peaks at the immature pod stage, illustrating the carbon / nitrogen balance theory.

In summary, this research provides greater insight into goatsrue seed biology and highlights the importance of the soil seed bank in goatsrue control efforts. Excellent herbicide control of perennial goatsrue was demonstrated with several herbicides; thus

providing critical goatsrue management information to weed managers. Goatsrue toxicity was also elucidated, giving greater insight into galegine distribution in plant parts and identifying the immature pod stage as the most toxic stage of plant growth.

APPENDIX.
Reprint Permission Letter.

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