THE ECOLOGY AND INTEGRATED MANAGEMENT

OF TALL BUTTERCUP (*RANUNCULUS ACRIS* L.)

by

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ABSTRACT

Tall buttercup (*Ranunculus acris* L.) is a perennial invasive forb found in pastures and irrigated meadows. It has been problematic in New Zealand where it excludes important forage species. The impact of tall buttercup is of interest in North America, especially in Montana where it has invaded over 8300 hectares. Minimal published research exists in the region regarding its impacts and methods of control. My research objectives were to 1) determine the associations of tall buttercup with forage, species richness, and plant diversity, 2) test integrated management strategies to control tall buttercup, and 3) assess the importance of soil moisture on seedling emergence and growth. Objective 1 was carried out in flood and sub-irrigated hayfield meadows over two years near Twin Bridges, Montana. Three transects were established along a gradient of increasing tall buttercup cover at two sites. In general, tall buttercup was found to have minimal associations. It was not associated with species richness, and was positively correlated to plant diversity. It was negatively associated with perennial grasses at one site. Objective 2 was conducted in flood and sub-irrigated hayfield meadows over two years near Twin Bridges, Montana. Treatments were applied in a split-plot design with four replications at two sites. Herbicide treatments occurred at the whole-plot level; non-sprayed, aminocyclopyrachlor + chlorsulfuron, aminopyralid and dicamba. Split-plots consisted of mowing and fertilization. All herbicides provided up to two years of tall buttercup control and mowing and fertilization controlled tall buttercup at one site. Forage production increased following aminopyralid and dicamba treatments; however, aminocyclopyrachlor + chlorsulfuron reduced perennial grasses. Objective 3 was explored in the greenhouse testing tall buttercup seedling emergence and growth along a soil moisture gradient. Seeds were planted in one of three different moisture treatments including 25, 50 and 100% field capacity. The 50% and 100% treatments had the highest seedling density, while the 50% treatment had the highest seedling biomass and height. Integrated management should be utilized for tall buttercup control, and altering irrigation practices may provide control by reducing seedling emergence and growth. Future research is warranted to understand tall buttercup invasion potential across habitat types.

CHAPTER ONE

PROJECT BACKGROUND AND OBJECTIVES

Introduction

Tall buttercup (*Ranunculus acris* L.) is a perennial, invasive forb that occurs in moist habitats including pastures, river flats, grasslands and in irrigated and sub-irrigated meadows (Lamoureaux and Bourdôt 2007). Tall buttercup is native to central and northern Europe (Coles 1971) where it is a weed of old pasture and hay meadows (Harper and Sagar 1953). It was first recorded in North America in the early 1900's and has since been found in all but eight states and one Canadian Province (USDA PLANTS Database). The impact of tall buttercup on forage production and plant diversity has been of interest in North America, especially in Montana where it has now invaded over 8300 hectares (Montana Noxious Weed Summit Advisory Council 2008). Tall buttercup has the potential to reduce forage productivity, posing risks to the livelihoods of landowners and the state's economy. In addition, tall buttercup invasion may result in the loss of plant community diversity and species richness. The negative impacts of tall buttercup have been documented in other regions of the world, including New Zealand where it has been an economically significant species in the dairy industry because it has excluded palatable pasture plants (Lamoureaux and Bourdôt 2007). Currently there is minimal published research on tall buttercup in both North America and the state of Montana. In Montana tall buttercup has been assumed to reduce forage production and plant diversity,

but research is necessary to quantify these assumptions.

Tall buttercup taxonomy, general ecology and demography have been studied, but only in regions of Europe and New Zealand. The species has the potential to be an economically significant species in other regions where it invades, including Montana. Therefore, further research on its ecology, impacts, and integrated management strategies is essential for improved control and to prevent the species from becoming established in areas that are currently free of tall buttercup.

The overarching goals for this project were to determine whether tall buttercup is associated with a decrease in forage production, species richness, and plant diversity in flood and sub-irrigated hayfield meadows where it typically occurs in Montana, and to develop integrated management strategies to control the species. This chapter reviews the literature pertinent to this project. The existing literature on tall buttercup biology, ecology, economic and ecologic impacts, and methods of control that have been tested in other regions (e.g. New Zealand) will be reviewed. Due to the lack of data on tall buttercup in North America, the successful use of integrated management in plant communities with other invasive forbs will be discussed along with its potential to control tall buttercup infestations. Moreover, because this project seeks to determine tall buttercup's impact on species richness and plant diversity, literature from research studying these effects by other invasive plants is examined. Finally, project justifications and objectives will be outlined.

Literature Review

Distribution and Habitat

The native range of tall buttercup spans central and northern Europe, the southern United Kingdom (U.K.), and Scandinavia. It is typically found within wet temperate, continental and subarctic climates (Bourdôt et al. 2013). The plant is found in habitats with ample water supply either from precipitation or high water table. It can tolerate low-oxygen conditions created by flooding for up to 30 days (Harper 1957, He et al. 1999), including damp meadows and pasturelands (Bourdôt et al. 2013). In its native range it is typically a member of grazed or mown grassland communities, and it tends to increase following overgrazing or cropping for hay (Harper 1957).

Tall buttercup has spread from its native range to other countries in the Northern Hemisphere including Morocco, Ethiopia, Newfoundland, and most of the upper continental regions of the United States and southern provinces of Canada (Bourdôt et al. 2013). In the Southern Hemisphere tall buttercup is found in South Africa, Tasmania, Australia and New Zealand (Webb et al. 1988). Its naturalized distribution occurs over several climactic zones including subtropical regions in the south and subarctic and temperate regions in the north (Bourdôt et al. 2013). Additionally, tall buttercup has been recorded within dry-land areas of the United States in New Mexico as well as Mediterranean regions in Spain (Bourdôt et al. 2013). Specifically in Montana, tall buttercup is found predominately in moist fields, meadows, pastures, and in flood irrigated and sub-irrigated meadows. It has been collected from elevations as high as 2,500 meters (Jacobs et al. 2010). In Montana, the species has been listed as a Facultative

Wetland species, meaning 67 to 99 percent of tall buttercup occurrences are in wetlands (Lesica and Husby 2006).

Irrigation and Occurrence of Tall Buttercup**.** Irrigation has a large influence on the persistence of tall buttercup in many locations where it is invasive; in fact, suitable habitat for the species declines in the absence of irrigation in areas that do not receive rainfall in adequate amounts (Bourdôt et al. 2013). In Montana tall buttercup appears to be the most prevalent in flood and sub-irrigated hayfield meadows (Jacobs et al. 2010). A survey of landowners in Madison County, Montana, in June 2012 indicated that landowners also have noticed a decrease in the prevalence of the species following a reduction in irrigation. One landowner stated; "We have had tall buttercup for years. We have converted from flood to sprinkler [irrigation]. The reduction of irrigation in wetter areas allows the other plants to out compete the buttercup. Where we still flood irrigate, there is more buttercup" (Appendix A).

Bourdôt et al. (2013) hypothesized that tall buttercup has expanded into more arid regions like Montana due to irrigation. To quantify this hypothesis, they developed a CLIMAX model to project potential global distributions of tall buttercup under current and future climate scenarios both in the presence and absence of irrigation. Current global occurrences of tall buttercup suggest that it favors modified environments including irrigated or high-rainfall, heavily grazed pasturelands, river banks and roadsides. CLIMEX models predicted that under the current climate and in the absence of irrigation, 34 million km^2 of land is suitable for tall buttercup. The majority of suitable land is confined to the Northern Hemisphere including Asia (12 million km^2), North America (9

million km^2), and Europe (8 million km^2), in addition to the entire land area of New Zealand. However, when irrigation was included in the current climate model, the area suitable for tall buttercup increased 30 percent to 45 million km$^{2.}$ These expansions in suitable habitat were found mainly in central Asia and central and northern regions of North America. When the model was used to examine future climate scenarios, little effect on potential distribution was found. Bourdôt and others (2013) speculated that this is likely due to the wide tolerance of tall buttercup to varying air temperatures. However, in contrast to climate change, irrigation had a large influence on the potential distribution of tall buttercup both under current and future climate scenarios. This is attributed to the extreme sensitivity of tall buttercup to drought stress (Lamoureaux and Bourdôt 2007). When irrigation was included with future climate change scenarios in the model, suitable habitat for tall buttercup expanded into central Asia and northern portions of North America, in addition to the southern coastal regions of Australia, high altitude regions in Africa, and central and northern Chile and Patagonia (Bourdôt et al. 2013).

Overall, the influence of irrigation on the invasion potential of tall buttercup could have considerable economic consequences. Both climate change and increases in global human population are likely to diminish current productive agricultural lands and increase the demand for products from these reduced agricultural areas. This demand may encourage conversion of marginal land to intensive agriculture, including irrigation, thus increasing habitat for tall buttercup into areas that were not otherwise climactically suitable. Invasion into new habitats due to increased irrigation will likely come with high

economic costs to control the species and decreased land productivity (Bourdôt et al. 2013).

Identification

Tall buttercup is a true perennial growing from a stout, abruptly ending root stock. It is rhizomatous and also spreads by seed. The plant has basal leaves that grow directly from the rhizome. The basal leaves are deeply divided into three to five palmate lobes (Figure 1.1). The flower stems are freely branching and erect, reaching 30 to 100 cm in length, and there may be one to several flower stems per plant. Stems have soft hairs that are sparse to many and appressed. Stem leaves are similar to basal leaves but are smaller and lack petioles. Because the plant is so tall and has leaves on its flowering stems, it is able to compete with tall perennial grasses (Harper 1957).

Waxy, bright yellow tall buttercup flowers have radial symmetry with typically five, but as many as eight, glossy petals. It has five reflexed floral sepals about 4 to 6 mm in length with long spreading hairs. There are 30 to 70 stamens with 15 to 40 pistils. Flower pedicels can reach up to 12 cm in length (Figure 1.1). The fruit of tall buttercup is a small, dry-walled achene with a single seed (Figure 1.2). Achenes are 2 to 3 mm long and compressed with a prominent keel and hooked beak at the tip (Jacobs et al. 2010). The hooked beak on the achene is often used to distinguish tall buttercup from sharpleaf buttercup (*R. acriformis*), which is native to North America and similar in appearance. The hooked beak on tall buttercup is only 0.3 to 0.6 mm in length while on the native sharpleaf buttercup it is often greater than one mm in length (Dorn 1984).

Tall buttercup is a genetically variable species with a number of intra-specific

taxa within the *acris* species. The most widespread and common of these is *R. acris* subspecies *acris* (often referred to as tall buttercup, giant buttercup, or meadow buttercup). This subspecies varies in its stem and petiole hair characteristics, size of the achenes and petals, and the degree of leaf dissection (Coles 1971). *Ranunculus acris* subspecies *acris* is most likely the subspecies found throughout Montana (Jacobs et al. 2010), and it is also the only subspecies that occurs in Britain (Coles 1971) and New Zealand (Webb et al. 1988).

Life History

Tall buttercup is a polycarpic perennial herb that produces a short rhizome. In New Zealand, dense clones of tall buttercup greater than one meter in diameter are common in dairy pastures (Lamoureaux and Bourdôt 2007). In the late winter and early spring, 40-50 new leaves form on the apex of the rhizomes. In Europe daughter shoots have been found to form in the fall after flowering (Sarukhan and Harper 1973), and in New Zealand daughter shoots form throughout the summer and fall (Lamoureaux and Bourdôt 2007). The rhizomes of tall buttercup may persist for two or more years in peaty soils, while in aerated soils they often decay quickly and disappear before flowering (Harper 1957). Lamoureaux and Bourdôt (2007) suggested that the rhizomes of tall buttercup have little impact on the spread of tall buttercup populations in pastures. However, regenerative shoots will form following defoliation or fungal attack (Green et al. 1998), and trampling by stock may also promote asexual reproduction (Diemer and Schmid 2001). These processes of regeneration are likely important mechanisms of the

persistence and growth of tall buttercup in New Zealand (Lamoureaux and Bourdôt 2007).

Figure 1.1. Root, stem, leaves, inflorescence and achene of tall buttercup (Britton and Brown 1913, available from USDA NRCS PLANTS Database).

Figure 1.2. Achenes of tall buttercup (USDA NRCS PLANTS Database).

The development of flower buds begins in the late summer the year prior to flowering (Totland and Eide 1999). Flowering is promoted by low winter temperatures (Bocher 1945), and Harper and Sagar (1953) found that the duration of flowering in Europe was extended following high soil moisture conditions. The majority of studies from Europe indicate that flowering will last about two months from late spring through mid-summer. However, in Norway flowering can continue into early fall (Lamoureaux and Bourdôt 2007). In New Zealand peak flowering is in summer, but some flowering may occur all year (Popay et al. 1989). In invaded regions in Montana tall buttercup flowering begins in late May and peaks in mid-June (Strevey, Personal Observation 2012). Seed set continues from late summer onward, and germination can occur during late autumn or late spring (Harper and Sagar 1953). In Russia germination only occurs in spring, however, in New Zealand germination has been the greatest in autumn (Lamoureaux and Bourdôt 2007). After flowering, both flower stalks and leaves die and are replaced by an overwintering rosette (Grime et al. 1988). The seeds of tall buttercup do not have an obvious dispersal mechanism and often fall close to the parent plant (Harper 1957), but they can be carried long distances via water currents, infested hay, fur, hooves or through the guts of grazing animals (Dore and Raymond 1942, Tuckett 1961, Bourdôt 1983). Fully matured seeds of tall buttercup can pass through the digestive tract of cows unharmed and remain viable in their feces (Sarukhan 1974). Dore and Raymond (1942) calculated that one cow may disperse approximately 9,400 tall buttercup seeds ha^{-1} during a 165-day grazing season.

Seedbank densities in tall buttercup infested areas vary greatly depending on the

severity of the infestation. In sparse populations in Quebec, the seedbank contained only three seeds m^{-2} (Dore and Raymond 1942). However in the U.K and New Zealand, seed densities ranged from 300 to 2,600 m^{-2} in dense infestations (Champness and Morris 1948, Tuckett 1961). The proportion of seeds of tall buttercup in grassland seedbanks in the U.K and Wales is similar to the proportion of tall buttercup plants present in the grassland (Champness and Morris 1948), suggesting that seeds do not accumulate in the soil (Lamoureaux and Bourdôt 2007). This observation is supported by experiments that have found tall buttercup seeds survive for less than two years in the soil, particularly if the seeds are located in the top 2 cm of soil (Bourdôt 1983, James and Rahman 1999, Williams 1983). Seed losses are likely due mainly to germination (Sarukhan 1974, Bourdôt 1983). In the dairy pastures of New Zealand, the majority of tall buttercup seeds tend to be in the top two cm of soil (Tuckett 1961). However, if seeds are buried deeply, they may survive much longer. James and Rahman (1999) found that 20 percent of tall buttercup seeds buried below four cm were still viable after 16 years.

Seedling survival depends on seed burial depth and on the amount of disturbance the vegetation experiences aboveground. Several seedling emergence studies have shown that many tall buttercup seeds in pasturelands will germinate within the first year (Harper 1957; Williams 1983; James and Rahman 1999). In Sweden vegetation removal caused an increase in tall buttercup seedling recruitment (Kiviniemi and Eriksson 1999). Moreover, in pasture in Canada seedling recruitment was greater on dung pats and mole hills devoid of vegetation when compared to the surrounding pasture (Parish and Turkington 1990). Dung pats are the primary source of disturbance in New Zealand dairy

pastures and smother existing seedlings but later create raised bare ground that is suitable for recruitment. During the winter in the Takaka Valley in New Zealand, pastures are trampled by grazing livestock, creating raised bare ground that provide sites for tall buttercup seedling establishment (Lusk 2009). Tall buttercup seedling recruitment is reduced in well-drained or waterlogged conditions (Harper 1957), suggesting that recruitment is microsite-limited (Lamoureaux and Bourdôt 2007). Additional factors impacting seedling survival include emergence time, seedling density, environmental heterogeneity, field conditions like temperature and competition, and the availability of suitable microsites (Harper 1977).

Several studies have investigated tall buttercup seedling survival (Sarukhan and Harper 1973, Rabotnov and Saurina 1971, Sarukhan 1976). Results indicated low rates of survival; however survival rates can be variable from year to year (Lamoureaux and Bourdôt 2007). For example, one study found that the probability of a seedling surviving to one year was as low as one percent (Sarukhan and Harper 1973), but another study showed survival to be as high as 70 to 97 percent (Rabotnov and Saurina 1971). In contrast to survivability of seedlings, the probability of a new plant surviving to one year from vegetative reproduction via rhizomes may be relatively high. In the only known experiment testing the survival of tall buttercup plants produced through rhizomes, Sarukhan (1976) found that the survival rate in a grassland habitat in Wales was 70 percent. In general, the risk of mortality for tall buttercup is reduced during flowering (Harper and White 1974), and flowering plants have a greater probability of surviving to the following spring when compared to plants that did not flower (Sarukhan 1974). The

life-span of tall buttercup varies between environments. In Russia, populations of tall buttercup were found to survive up to 14 years (Rabotnov and Saurina 1971); however, half of a population of one-year-old plants from a grassland in Wales did not survive beyond four years (Sarukhan and Harper 1973).

Impacts of Tall Buttercup

Ecological Impacts. The recorded ecological and economic impacts of tall buttercup come from New Zealand, where the species invades dairy pastures. In these areas, tall buttercup can to exclude grasses and clovers through competition (Lamoureaux and Bourdôt 2007). During the peak of the growing season tall buttercup can range in cover from 5 to 50 percent (Bourdôt et al. 2003). Furthermore, the species tends to persist in pasturelands, likely due to annual recruitment of new plants from seed where livestock create bare sites conducive to seedling establishment (Lusk et al 2009). Stout rhizomes provide resilience to adverse environmental conditions and higher regenerative capabilities compared to other pasture species (Lamoureaux and Bourdôt 2007). While these ecological impacts have been noted throughout New Zealand, there is little research available that actually quantifies how tall buttercup is impacting the plant community or forage production.

Glycoside Ranunculin. Glycosides are secondary plant products, many of which are active metabolites or allelochemicals that interact with other plants, microorganisms and insects (Rhoads 1979). Many plants in the Ranunculaceae family, including tall buttercup, produce the glycoside ranunculin (Conner 1977), which is a bound form of

protoanemonin found in members of the genera *Helleborus*, *Anemone*, *Clematis*, and most commonly, *Ranunculus* (Bonora et al. 1987). Protoanemonin is an irritant oil derived by autolysis during the breakdown of fresh plant tissue (Bai et al. 1996). When glycoside ranunculin is consumed by grazing animals, enzymatic breakdown occurs and the vesicant, protoanemonin, forms (Harper and Sagar 1953). Protoanemonin can cause blistering of the lips and tongue, irritation of the digestive tract, respiratory failure, ventricular fibrillation, and death in some cases. Protoanemonin is unstable and volatilizes when dried, therefore when tall buttercup is cut and dried in hay, some of its toxic properties are reduced (Cheeke 1998).

Economic Impacts. Tall buttercup was first recorded in New Zealand in 1872 (Webb et al. 1988) and currently invades dairy pastures in six of 17 dairying regions where the species is considered to have significant economic impacts (Bourdôt and Saville 2010, Bourdôt et al. 2003). Specifically, it occurs in South Auckland, Hawke's Bay and in the Taranaki regions as well as the districts of Wairarapa, Horowhenua and Tasman (Figure 1.3) (Lamoureaux and Bourdôt 2007). The production of the acrid tasting glycoside ranunculin deters grazing cattle, thus reducing the metabolizable energy available in pastures because cattle not only avoid tall buttercup but also nearby palatable forage species (Bourdôt and Saville 2010). Due to reduced available forage, tall buttercup has been reported to cause significant losses in milk production (Bourdôt and Saville 2010). It was estimated that tall buttercup resulted in a revenue loss to dairy farmers of NZ $156 million during the 2001-2002 season across the six infested regions (Bourdôt et al. 2003). Bourdôt and Saville (2010) developed a model to predict national losses to the

New Zealand dairy industry. They incorporated national losses to the dairy industry during the 2001-2002 season and assumed that the extent of tall buttercup infestation had not increased from 2002. The model estimated that tall buttercup costs the dairy industry an average of NZ $118 million annually across the six invaded dairying regions. If tall buttercup were to occur in all 17 of the dairy farming regions, the model predicted national losses of NZ $328 million (Bourdôt and Saville 2010).

Potential Impacts in Montana. In Montana tall buttercup has been particularly troublesome in Madison County, in the southwestern portion of the state. The cost of controlling tall buttercup along roadsides in Madison County over the past 10 years has been about $42,000, and landowners with tall buttercup on their property have spent about $29,000 over the past 10 years to control the species; Madison County weed district personnel have spent about 4,500 hours controlling tall buttercup since it was listed as a noxious weed in 2003 (Edsall, Personal Communication 2013). While current and future impacts of tall buttercup in Montana are unknown, it is clear that its presence has the potential to impart economic losses due to decreases in plant community diversity and productivity in hayfield meadows. Land management practices in these hayfield meadows are likely promoting persistence of the species. Many of these sites are used for spring grazing, are flood or sub-irrigated in the summer, and are hayed in the late summer. Grazing provides a competitive advantage for tall buttercup over more palatable forbs and grasses, and irrigation creates an ideal habitat for the species, as well as a potential dispersal mechanism. Landowners in Madison County have observed that irrigation water carries tall buttercup seeds into their pastures (Appendix A). Moreover,

haying these areas also provides a dispersal mechanism for tall buttercup seeds (Tuckett 1961, Bourdôt 1983) and may encourage the growth of tall buttercup over other competitive pasture species (Harper 1957).

Figure 1.3. Distribution of tall buttercup in New Zealand, shown as the percentages of dairy pasture land infested in each of the main dairying regions (Adapted from Lamoureaux and Bourdôt 2007).

Integrated Management Approaches to Control Tall Buttercup

With the evolution of herbicide-resistant invasive plants, the ability of plant communities to shift in their response to invasive plant control measures, and the rising

complexity of managing both native and agricultural plant communities; developing integrative management approaches to invasive plant management has become an increasing priority (Buhler 2002). Integrated management strategies emphasize a combination of control techniques and consider the cause of invasion rather than just reacting to the invaded plant community (Buhler 2002). Moreover, integrated management approaches can be designed to encourage higher species richness and plant community diversity. These strategies could be advantageous because as plant diversity increases, plant communities may become more resistant to invasion (Sheley et al. 1997, Tilman 1997, Carpinelli 2000).

Specifically in Montana, the successful control and prevention of tall buttercup establishment in areas where it currently does not exist is likely to be achieved through integrated management strategies. The existing research examining potential tall buttercup control methods includes herbicides, mowing, fertilization, biological control, and grazing, however, these studies have only examined control methods individually rather than an integrated approach. Because of this, other studies involving integrated management used to control other invasive forbs will be discussed.

Prevention. The surest invasive plant control method is to prevent a species from ever establishing in an area. Invasive plants can be introduced into new areas in many ways including contaminated seed or hay, transport of plant parts and seeds on equipment, livestock, manure and compost, and irrigation and drainage water (Walker 1995). Specifically, tall buttercup has spread via contaminated hay and irrigation water (Tucket 1961 and Jacobs et al. 2010). A landowner in Madison County, Montana, made

the following observation; “We find that water (irrigation) carries it (tall buttercup) into our pastures. If those above us do not participate in management, we end up with their seeds” (Appendix A). Because of the availability and effectiveness of herbicides, less emphasis has been placed on the actual prevention of invasive plants (Buhler 2002). In some cases, prevention is not possible; however, in situations where humans are the primary vectors or have control over the seed source, preventative management can be successful (Walker 1995). Prevention in these instances can be implemented through community action and enforcement of laws and regulations. Noxious weed laws and seed purity laws are a few examples of successful invasive plant prevention programs (Buhler 2002).

Education and awareness programs can also help the public identify invasive plants and report small infestations before they become larger. An example of an education and awareness program with the goal of eradicating an invasive species is the Montana Dyer’s Woad (*Isatis tinctoria*) Cooperative Project (Pokorny and Krueger-Mangold 2007). The program became a formal entity in the state in 1984, and focuses on an educational component at the state, local and site levels. At the state level, dyer’s woad is included in weed education and awareness campaigns including the “Bounty Program” that provides an incentive to notify the project when new infestations are found. At the local level, weed coordinators teach dyer’s woad identification to residents and weed crews, and spread routes of known infestations are surveyed. At the site level, the dyer’s woad field crew visits known infestations every two weeks from May to October. When dyer’s woad is found, control measures are taken to remove or kill the

plant. The project has eradicated the species from several counties, decreased infestation sizes, and has prevented new infestations. Overall, the success of the project can be attributed to early detection and education, repeated treatments, and monitoring (Pokorny and Krueger-Mangold 2007). Programs such as this one are imperative for preventative management efforts to be successful.

Herbicides. Herbicides containing paraquat, diquat, 2,4-D and MCPA (2-methyl-4-chlorophenoxyacetic acid) have reduced tall buttercup in field experiments and promoted more desirable forage species in Czechoslovakia, Russia, Norway and France (Lamoureaux and Bourdôt 2007). In New Zealand phenoxy herbicides like MCPA and MCPB (4-(2-methyl-4-chlorophenoxy)butyric acid) were relied upon heavily by dairy farmers to control tall buttercup starting in the 1950's and 1960's. These herbicides provided control of tall buttercup for a time but are now ineffective because of evolved resistance (Bourdôt et al. 1996). Research proved that MCPA resistance is a heritable trait in tall buttercup, and populations can evolve MCPA-resistance following intense selection pressure (Bourdôt and Hurrell 1991). Winter and spring applications of MCPA were typical for most farmers, which resulted in high selection pressure on the surrounding plant community (Leathwick and Bourdôt 1991). Moreover, MCPA damaged to clovers in dairy pastures (Hurrell and Bourdôt 1993), and researchers concluded that the continued use of MCPA and MCPB would promote tall buttercup due to exclusion of competitive and desirable forb species (Bourdôt and Lamoureaux 2002).

Milestone™ (aminopyralid) and Clarity® (Dicamba) are labeled for tall buttercup control in range and pasture in North America (Jacobs et al. 2010, DowAgroSciences).

Optimum timing for herbicide application is during the leafy phase prior to flower-shoot growth. A herbicide efficacy trial was conducted in a sub-irrigated meadow in Ravalli County, Montana. Milestone™ was applied at two different rates (103 g a.i. ha^{-1} and 172 g a.i. ha^{-1}). There were two different application timings including a summer application in June and a fall application in September. One year after treatment, both summer and fall applications at 103 or 172 g a.i. ha^{-1} provided between 80 and 100 percent control of tall buttercup (Celestine Duncan, Personal Communication 2014).

While Milestone™ appears promising to control tall buttercup infestations in Montana meadows, further data is necessary for Milestone™ and for other herbicides that have the potential to provide adequate tall buttercup control. Moreover, in order to prevent herbicide resistant populations of tall buttercup and to encourage the competiveness of desired species, herbicides should be used in combination with other control methods.

Mowing. Mowing may promote the competiveness of desirable forage species while reducing tall buttercup flowering (Lamoureaux and Bourdôt 2007). Mowing pastures infested with tall buttercup reduced its flowering in red-fescue dominated meadows in Russia (Saurina 1972). However, in long term studies in Slovenia, tall buttercup increased after five years when mowing frequency was increased from two to four cuts per year, because the competitive tussock grasses were also reduced (Leskosek 1996). If mowing is used to control tall buttercup, proper timing and frequency must be employed to maintain other desirable competitive plant species (Lamoureaux and Bourdôt 2007).

Similarly, another invasive *Ranunculus*, lesser celandine (*Ranunculus ficaria*), may provide insight into tall buttercup management. It is also an herbaceous perennial occurring in moist settings (Chevallier 1996). Several studies have been conducted on lesser celandine management, including mechanical methods like mowing. Taylor and Markham (1978) found that repeatedly mowing stems of lesser celandine to ground level reduced both the growth and biomass of the plant.

Mowing has also been shown to effectively reduce populations of the invasive forb spotted knapweed (*Centaurea stoebe*). Repeated defoliation of spotted knapweed in greenhouse experiments was shown to reduce both root and crown weight (Kennett et al. 1992), which can reduce the long term survival of individual plants (Menke and Trlica 1981). Rinella and others (2001) tested the impact of mowing frequency and season on spotted knapweed density and found that applying a single fall mowing for three years when spotted knapweed was in the flowering or seed production stage was the most effective in decreasing spotted knapweed adult density. Mowing did appear to favor grasses over knapweed (Rinella et al. 2001).

Fertilization. Soil fertilization has the potential to be used as an integrated management approach in addition to or in replacement of herbicide use to control tall buttercup. The concept behind soil fertilization is to shift the competitive balance between the invasive plant to the crops or native species (Buhler 2002). By changing the ratios of essential plant resources in the soil, there is the potential to favor one species over another. This can be achieved by specific fertilization strategies designed to

maximize nutrient uptake by desired species, while minimizing nutrient availability to invasive plants (Tilman et al. 1999).

However, several studies on soil nutrient availability suggest that fertilization can competitively favor invasive plants over desired plants, and measures to reduce soil nutrient availability will benefit the native plant community (Vasquez et al. 2008, Ehrenfeld 2003, Huenneke et al. 1990). In North America, late-seral species typically have slow growth rates and are highly competitive for limiting nutrients in the soil (Arredondo et al. 1998, Daehler 2003, Harpole 2006), indicating that these species will dominate in habitats with low soil nutrients. In semi-arid and arid ecosystems nitrogen, followed by water, is the most limiting resource (Daehler 2003, James et al. 2005, Krueger-Mangold et al. 2004). Lowering a critical and limiting soil resource such as nitrogen to a level that is unavailable to invasive species will give native species a competitive advantage (Herron et al. 2001). Nevertheless, the use of fertilizer to manage tall buttercup populations in the already productive hayfield meadows where it occurs may be important. On these cultivated pastures and hay meadows where non-native perennial grasses and forbs are prevalent, nutrient management, including nitrogen addition is crucial to maintain the competitiveness of desired perennial grasses over tall buttercup (Jacobs et al. 2010).

Fertilizer applications to deteriorated pasturelands with tall buttercup infestations have been effective in several studies (Lamoureaux and Bourdôt 2007). Following the addition of nitrogen, phosphorous, and potassium fertilizers in a pasture dominated by tall buttercup in Moscow, Russia, there were higher proportions of desired palatable grass

species (Melnikov et al. 1975). Moreover, in an annual grassland in Greece infested with tall buttercup, the application of nitrogen fertilizer reduced the relative abundance of tall buttercup (Tzialla et al. 2002). In contrast, fertilizer applied to deteriorated pastures in Austria, Slovenia, and New Zealand had little effect on the abundance of tall buttercup (Lamoureaux and Bourdôt 2007).

The successful use of integrated management involving fertilizer was tested on spotted knapweed infestations in rangeland settings (Jacobs and Sheley 1999). The study combined an integrated management approach using the herbicide 2,4-D and nitrogen fertilizer. Two,4-D was applied in the mid-summer to target spotted knapweed while minimizing impacts to the early season forbs, and fertilizer was applied to increase competitiveness of grasses and reduce re-invasion by spotted knapweed. The response of the plant community to the combination of 2,4-D and fertilizer was dependent on initial composition of the plant community. On the site dominated by spotted knapweed, only 2,4-D was found to affect spotted knapweed, which resulted in an increase in grass density and biomass. However, on the site dominated by native grasses and forbs, the combination of 2,4-D and nitrogen increased grass density while minimizing impacts to the desired native forbs (Jacobs and Sheley 1999).

Biological Control. Tall buttercup hosts both insects and parasitic fungi (Harper 1957) that may be responsible for high rates of seedling mortality (Lamoureaux and Bourdôt 2007). There are two pathogenic fungi that occur naturally on tall buttercup in New Zealand, and some research has been conducted on their use as biological herbicides. The first fungus, *Gnomonia* spp., attacks the roots of herbaceous and woody

plants. *Gnomonia* was grown in culture and applied to tall buttercup plants in the field. However, only 30 to 40 percent of plants became infected, and no tall buttercup mortality occurred (Hardwick et al. 1993).

The second fungus, *Sclerotinia sclerotiorum*, results in soft-rot disease and death of infected tall buttercup plants. It is a wide host-range pathogen that produces spores that travel in air currents (Bourdôt and Lamoureaux 2002). It may present a disease risk to neighboring crops; however, it is harmless to pasture grasses and clovers, and is easy to grow in culture making it an ideal control agent for pasturelands and meadows (Lamoureaux and Bourdôt 2007). Several studies have examined its potential as a mycoherbicide for tall buttercup management. *Sclerotinia sclerotiorum* was applied to the axils of young rosette leaves of tall buttercup, resulting in 75 percent mortality within 21 days of inoculation (Green et al. 1993). Following a single application of the fungus to a dairy pasture, tall buttercup dry weight was reduced by 57 percent (Cornwallis et al. 1999). Another experiment by Verkaaik and others (2004) found greater than 60 percent mortality of tall buttercup and reduced growth of surviving plants following *Sclerotinia sclerotiorum* inoculation, however the impact was variable between sites. Despite the promising results suggesting little risk to desirable forage species, a commercial product is not available. Bourdôt and Lamoureaux (2002) speculate that this is due to the probable high costs of production for *Sclerotinia sclerotiorum* as a mycoherbicide, in addition to high field application rates with the current formulations. Further research is necessary to better understand the potential of both *Gnomonia* and *Sclerotinia*

sclerotiorum as biological herbicides. No known insect biological control agents are available.

Grazing Management. Grazing can either promote or reduce tall buttercup, depending on the intensity of grazing, amount of bare soil exposed, and timing (Lamoureaux and Bourdôt 2007). In a grassland in Finland, tall buttercup increased when sheep were introduced. This was likely due to a combination of factors including litter removal, exposure of bare soil which helped to promote tall buttercup germination, and the suppression of dominant competitive grasses (Hellstrom et al. 2003). Furthermore, in grasslands in Iceland, tall buttercup increased when the duration of spring grazing increased (Thorvaldsson 1996). Similar results occurred in Britain where tall buttercup increased in abundance following increased pasture age, cropping for hay, and overgrazing by livestock that avoided the plant in favor of more palatable species (Harper 1957). However, prescribed grazing to promote competitive forage plants may prevent tall buttercup invasion. Timing of grazing may be most important to manage tall buttercup invasions. For example, spring grazing is likely to favor tall buttercup by removing shade cover of competitive plants when tall buttercup leaf area is greatest (Jacobs et al. 2010). Similar to other invasive plants, grazing used as part of an integrated management strategy may also be successful for tall buttercup control. For example, the use of grazing in combination with biological control was tested on the invasive forb leafy spurge (*Euphorbia esula*). A study by Jacobs et al. (2006) tested an integrated management strategy that combined cattle and sheep grazing with the *Aphthona* flea beetle. They found that grazing combined with the *Apthona* biocontrol agent reduced

leafy spurge density and cover to a greater extent than just the use of the biocontrol alone (Jacobs et al. 2006). These results support the use of integrated management to provide better control of invasive plant species.

Irrigation. Tall buttercup is largely found in moist habitats, including irrigated and floor or sub-irrigated fields. Tall buttercup can tolerate up to 30 days of flooding (Harper 1957, He et al. 1999), therefore flood irrigation may favor tall buttercup over other forage species (Jacobs et al. 2010). Previous research has shown that tall buttercup has low drought tolerance (Sarukhan 1974, Harper 1973), so altering the timing or methods of irrigation in an infested site could potentially be a management option. Carefully planned irrigation management may reduce tall buttercup invasions while encouraging competitive forage species to increase if used with other control methods including herbicides, mowing and fertilizer.

Impacts of Invasive Plants on Species Richness and Diversity

In Montana it has been speculated that tall buttercup is reducing species richness and plant diversity in the hayfield meadows where it invades. In an effort to better understand how an invasive forb like tall buttercup may influence these factors, existing literature on the impact of other invasive plants on species richness and diversity with a focus on invasive wetland species are reviewed. While recent studies have focused on the negative effects of invasive plants on their respective invaded ecosystems and the mechanisms behind these invasions, few have examined community level effects of invasive plants, including the impact on species richness and diversity (Hejda et al.

2009). In fact, there is little evidence of competition from introduced invasive plants that result in competitive exclusion of native plants from the natural plant community (Dukes 2002). The evidence that does exist is largely from small-scale competition experiments (Boylen et al. 1999). However, studying the community level impacts by comparing both invaded and uninvaded sites can provide valuable information for management and conservation (Manchester and Bullock 2000).

A large-scale study conducted by Houlahan and Findlay (2004) in Ontario, Canada, sought to determine whether or not invasive plant species in temperate wetlands outcompeted resident native plant species. Across the 58 temperate wetlands included in their study, they found no evidence that invasive wetland plants competitively excluded native plant species. In fact, invasive and native species richness were positively related and the invasive species were no more likely to become dominant in the plant community than natives were. Moreover, when the effects of other wetland characteristics were statistically controlled, the impact of invasive dominants on species richness was indistinguishable from the impacts of native dominants (Houlahan and Findlay 2004).

Another study conducted in the Czech Republic examined the effect of 13 different invasive plants on species diversity (Hejda et al. 2009). Invasive species were found in a range of site conditions and vegetation types ranging from lowland alluvial meadows to sub-alpine meadows. The impact on the invaded community varied greatly among the 13 species, and differences in species richness were found for only five species. Of those five species, species richness was reduced by invasion; however, severity of impact depended almost exclusively on the identity and characteristics of the

invading species. Decreases in species richness were dependent on the differences in height between the invasive species and the dominant natives along with cover of the invading species. In particular, the invasive species that had the strongest impact on species richness and plant diversity were taller than resident species and had a vigorous growth rate which enabled them to achieve higher cover. Moreover, the greatest suppression of native plant species came from invasive plants that formed extensive rhizomatous root systems along with species that had the ability to form homogenous stands. Finally, Hejda and others (2009) noted that the type of invaded community largely influenced the response of the invaded community. In particular, invasive riparian species had relatively low impacts on the invaded community. A previous study by Hejda and Pysek (2006) explained that this finding was likely due to native riparian plant communities typically being occupied by tall, competitive species, thus the impact of the invader is not much different from the tall native dominants.

Tall buttercup displays some of the same traits as the invaders that were found to reduce species diversity in the studies outlined above (Hejda et al. 2009, Houlahan and Findlay 2004, Hejda and Pysek 2006), including its extensive rhizomatous system (Lamoureaux and Bourdôt 2007) and the ability to grow up to 100 cm in height (Harper 1957). However, tall buttercup is typically found in moist fields and meadows alongside other riparian vegetation, so it is possible that tall buttercup may have such similar traits to plants in the existing community that no impact will be observed (Hejda et al. 2009). Moreover, many of the perennial grasses and forbs in these plant communities are productive and may be more competitive than tall buttercup. Whether or not tall

buttercup is reducing species richness or plant diversity in Montana meadows and hayfields is currently unknown.

Project Justification and Objectives

Tall buttercup is of concern to landowners in western Montana where it has invaded irrigated hayfield meadows. It was first reported in Montana in Gallatin County in 1916 and has since been reported in 23 additional counties. Approximately 8300 ha in 13 counties are now infested with tall buttercup (Rice, INVADERS Database). It was listed as a Montana state noxious weed in 2003 as a priority 2A species (Montana Noxious Weed Summit Advisory Council 2008). As a state listed noxious weed, landowners with tall buttercup on their property are required by law to control the species. However, despite its noxious status, little information is available for landowners about how to best manage the species while maintaining plant diversity and productivity.

A better understanding of tall buttercup ecology and its potential to reduce forage productivity in Montana is necessary. Additionally, knowledge of the best integrated management strategies is warranted to provide land managers with guidelines to eradicate or contain existing infestations and to prevent tall buttercup from becoming even more widespread. In an effort to obtain additional information on the impacts of tall buttercup and to develop an effective integrated management strategy to control existing infestations, a two-year, multi-study research program was initiated in summer 2012. This is the first known tall buttercup research project in the United States. The information gained through this research will contribute to existing knowledge from New

Zealand and Europe and further explain how the invasion of tall buttercup is impacting plant communities in a different region of the world. Specifically, the first study in this project investigated the association of tall buttercup with species richness, plant diversity and forage production in hayfield meadows where it is most problematic. The second study tested various integrated management strategies including herbicides, mowing and fertilization alone and in combination with each other with the goal of controlling the species while maintaining forage productivity and plant diversity. The third study evaluated the role of soil moisture in tall buttercup seedling emergence and growth.

CHAPTER TWO

ASSESSING THE ASSOCIATIONS OF TALL BUTTERCUP ON SPECIES RICHNESS, PLANT DIVERSITY AND FORAGE PRODUCTION

Introduction

The impacts of tall buttercup (*Ranunculus acris* L.) on plant community diversity and forage production in North America have been speculated upon based on research in other regions of the world. Tall buttercup has been in North America since the early 1900's and is currently found in all but eight states and one Canadian Province (USDA PLANTS Database). While there is no published research on the impacts of tall buttercup in North America, extensive research has been conducted in New Zealand where the species has been problematic for many years (Bourdôt et al. 2003). In particular, tall buttercup has been of concern in the dairy farming regions of New Zealand, where it competitively excludes palatable pasture grasses and clovers (Bourdôt and Lamoureaux 2002). In New Zealand tall buttercup canopy cover can range from five to 50 percent during peak standing biomass (Bourdôt et al. 2003). The species persists in infested areas due to its annual recruitment of new plants from seed (Lusk et al. 2009). Additionally, stout rhizomes of tall buttercup provide resilience to adverse environmental conditions and higher regenerative capabilities when compared to other pasture plants (Lamoureaux and Bourdôt 2007).

In western Montana tall buttercup commonly invades moist hayfield meadows that are used for forage production. These hayfield meadows are often grazed by cattle in

spring, flood or sub-irrigated in early summer, and hayed in late summer. Each of these management practices may encourage the growth and spread of tall buttercup in the region. Cattle typically avoid tall buttercup due to its bitter flavor. If it is not grazed, tall buttercup gains a competitive advantage over desired pasture grasses and forbs that are more palatable (Lamoureaux and Bourdôt 2007). Flood and sub-irrigation in early summer creates a favorable habitat for tall buttercup and increases the spread of the species to non-infested lands via seed transport in irrigation water. Finally, hay contaminated with tall buttercup is likely to increase its prevalence in the region as seeds are transported to new areas through hay bales (Tuckett 1961, Jacobs et al. 2010).

Tall buttercup was listed on the Montana noxious weed list in 2003 as a priority 2A species (Montana Noxious Weed Summit Advisory Council 2008). Local producers and landowners speculate that tall buttercup has reduced plant diversity and forage production in hay meadows, however there is little to no information about its associations with plant community diversity or production in the region. The objective of this study was to determine whether tall buttercup is associated with a reduction in diversity, species richness, and forage production to provide a better understanding of its function the hayfield meadow plant community where it invades in western Montana. Species richness, plant diversity and forage production were predicted to decrease as tall buttercup biomass and cover increased.

Materials and Methods

Site Description

Two sites were selected in Madison County approximately 16 km southwest from Twin Bridges, Montana. Sites were located in either flood or sub-irrigated hayfield meadows managed primarily for forage production and were chosen based upon tall buttercup cover and landowner cooperation. Sites will be referred to as Novich and Ashcraft, in reference to the names of the landowners of either site. The land management practices on the study sites include cattle grazing in the early summer, and the Novich site is flood irrigated while the Ashcraft site is sub-irrigated in the mid-summer. Both sites are hayed in the late summer. These management practices occurred as normal throughout the project's duration. The Novich and Ashcraft sites are located approximately three km from one another.

The elevation at the Novich site is 1448 m (45°29'51.90", 112° 24'06.77"). The soil is a Rivra-Ryell Havre complex and has a pH of 7.8. The elevation at the Ashcraft site is 1436 m (45°29'25.07", 112°26'26.76'). Soil at this site is also a Rivra-Ryell Havre complex with a pH of 7.6. The soil texture at both sites is comprised of a sandy clay loam (Appendix B). At both sites the mean annual average precipitation is 24.2 cm with an average annual temperature of 14.6°C. Weather data for the past 63 years was compiled from the Western Regional Climate Center from a weather station in Twin Bridges, Montana (NOAA National Climactic Data Center 2002).

Experimental Design

Three 100 meter transects were permanently established at both sites. Transects were placed along a gradient of increasing tall buttercup cover starting at zero percent cover. Transects were placed parallel to each other with at least five meters distance between them (Figure 2.1).

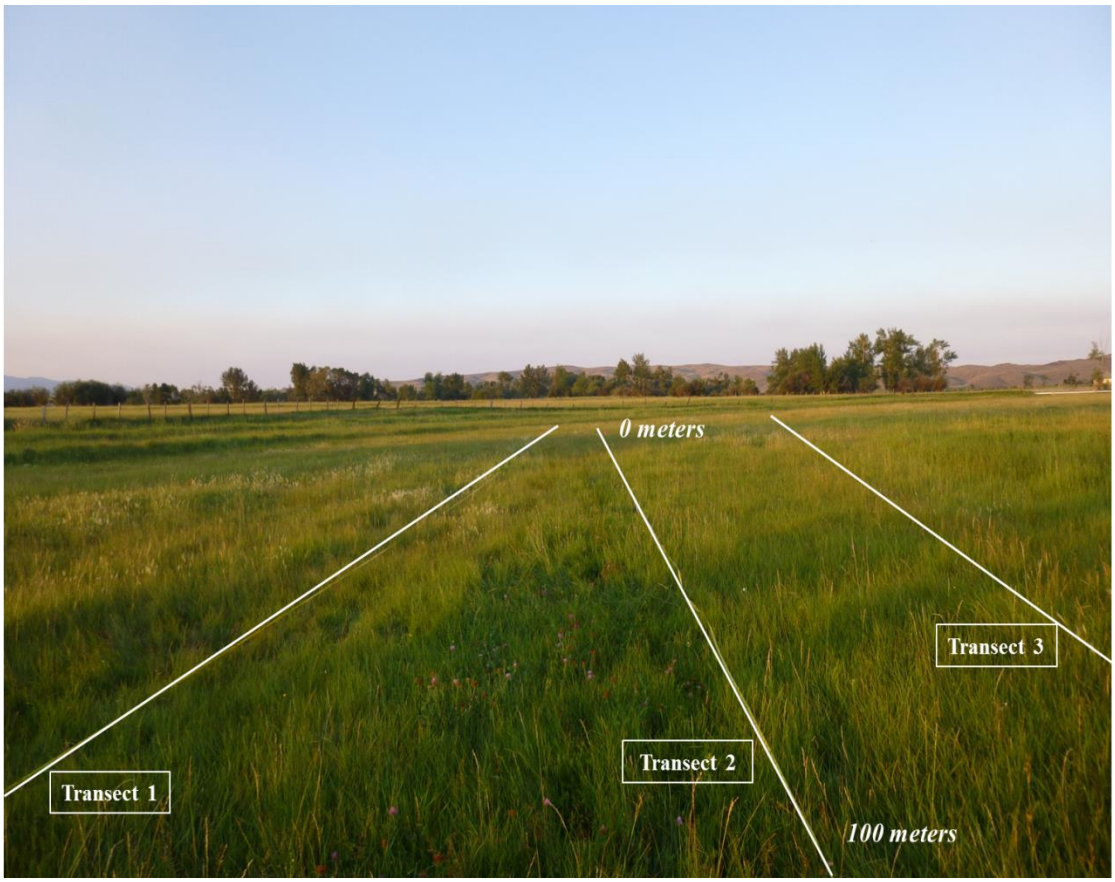


Figure 2.1. Experimental design including three permanent 100 m transects placed from low to high density of tall buttercup. Transects were placed at least five meters apart.

Vegetation and Soil Moisture Sampling

Transects were sampled 16-18 July and 27-30 July 2012 at the Ashcraft and Novich site, respectively. In 2013, the Ashcraft site was sampled 8-9 July and the Novich

site was sampled 15-17 July. For both years sites were sampled following spring grazing. Transects were sampled using a 20 cm x 50 cm Daubenmire (1959) frame placed every five meters along each transect starting at zero meters. In 2012 vegetation was sampled on the right side of each transect and in 2013 vegetation was sampled on the left side to avoid clipping the same area two years in a row. Within each frame, canopy cover and biomass were estimated by species. Vegetation was clipped to a height of two cm, bagged and transported to the Plant Growth Center (PGC) at Montana State University, Bozeman, Montana. Biomass was dried at 65°C for a minimum of 72 hours and weighed to the nearest 0.01 gram.

To quantify the role of soil moisture on the biomass and cover of tall buttercup, soil moisture was sampled on 24 June 2013 prior to any flood or sub-irrigation events and again on 1 July 2013 during the first week of irrigation. Soil moisture was sampled on the right side of each transect where vegetation was clipped in 2012 to prevent any damage to the vegetation for sampling in 2013. A 20 cm x 50 cm Daubenmire (1959) frame was placed every five meters along each transect and soil moisture samples were taken from the center of the frame. A tulip bulb planter was used to sample approximately 2330 cm^3 of the soil to a depth of 15 cm. Soil samples were transported to the PGC and weighed to the nearest 0.01 gram prior to being dried at 65 °C for a week. Soils were removed from the drier and weighed again to obtain dry soil weight. Gravimetric soil moisture was calculated using Equation 2.1.

$$\frac{(wt\ of\ wet\ soil\ ) - (wt\ of\ dry\ soil\ )}{(wt\ of\ dry\ soil\ )}$$

Equation 2.1. Gravimetric Soil Moisture.

Statistical Analysis

The biomass of each species clipped within the Daubenmire frames was separated into five different functional groups for analysis including tall buttercup (TB), perennial grass (PG), grass-like species (GL), exotic forb (EF), and native forbs (NF). Certain forage species of interest including meadow brome (*Bromus bieberstenii*), Canada bluegrass (*Poa compressa*), meadow foxtail (*Alopecurus pratensis*), sedges (*Carex* spp.), rushes (*Juncus* spp.), and red and white clover (*Trifolium* spp.) were also individually analyzed.

Shannon-Wiener Diversity Index (Shannon 1948) was used to calculate plant diversity. The biomass of all species present, not including tall buttercup, was used as a measure of the relative abundance and evenness of species along each transect. The proportion of each species (*i*) relative to the total number of species (*pi*) was calculated and multiplied by the natural logarithm of the proportion (ln(*pi*)). Shannon-Weiner Diversity was calculated using Equation 2.2.

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Equation 2.2. Shannon-Wiener Diversity.

Linear regression was conducting using RStudio 0.98.501 statistical software to predict the response of functional group biomass (PG, GL, EF and NF) and the biomass of species of interest as a function of tall buttercup biomass (R Core Team 2013). Separate regression models were fit to predict the response of each functional group. Moreover, linear regression was used to predict percent cover of each functional group and species of interest as a function of tall buttercup percent cover. Linear regression was also used to assess the relationship between plant diversity and tall buttercup biomass and cover. Finally, linear regression was used to predict tall buttercup biomass and percent cover in 2013 as a function of soil moisture. The two different soil moisture sampling events that occurred in June and July of 2013 were fit in separate regression models.

Preliminary regression analyses indicated differences between sites ($P<0.001$), so sites were analyzed separately. Year was included in each model as a categorical variable. All response variables and explanatory variables (tall buttercup biomass and cover) were ln transformed to meet the assumptions of linearity and constant variance. The number one was added to any functional groups containing zeroes to avoid undefined log transformations.

I speculated that spatial correlation existed among the data due to the study design. If positive spatial correlation exists, but is not accounted for in the model, it can lead to underestimation of the standard errors (Ramsey and Schafer 2002). A naïve model was fit without accounting for spatial correlation by ignoring the effect of transect and frame (Equation 2.3), and a semi-variogram of the residuals was used to test for spatial correlation.

$$lm(Log\ PG \sim Log\ RAAC + Year)$$

Equation 2.3. A naïve model fit to examine the presence of spatial correlation.

The semi-variogram confirmed the presence of spatial correlation, therefore transect and frame were included in the final models used for inference. Equation 2.4 provides an example of a final model that explicitly included the presence of spatial correlation.

$$lm(Log\ PG \sim Log\ RAAC + (Year/(Frame)) + (Year/(Transect)))$$

Equation 2.4. An example of a final model used that includes both transect and frame to account for spatial correlation.

Binomial regression was used to correlate species richness as a function of tall buttercup biomass, because species richness is a count with an upper bound (the total number of species found among transects at each site). The response variable, species richness, was considered binomial with the probability *p* for a species to be observed among a total of 16 species at the Ashcraft site across both years and a total of 28 species at the Novich site across both years. The glmPQL function in the R package MASS was used to combine both binomial logistic regression and to account for the presence of spatial correlation (Venables and Ripley 2002). In cases where models are significant at $P \leq 0.05$, non-transformed coefficients and regression equations are presented for ease of interpretation.

Results

Perennial Grass

At the Ashcraft site, perennial grass biomass was negatively associated with tall

buttercup biomass (Table 2.1). Each g m^{-2} increase in tall buttercup was associated with a 2.6 g m^{-2} decrease in perennial grass biomass (Figure 2.2). Perennial grass cover was not associated with tall buttercup cover (Table 2.1). In contrast to the Ashcraft site, at the Novich site there was no correlation between perennial grasses and tall buttercup (Table 2.1).

Table 2.1. *P*-values for the regression models predicting functional group biomass and cover based on tall buttercup biomass and cover from the Ashcraft and Novich sites. Year was included in the model as a predictor variable. *P*-values were generated from models using the natural log transformed data. Significant *P*-values (≤0.05) are indicated in bold.

	Ashcraft: Biomass				**Novich: Biomass**			
	Response variables				*Response variables*			
Predictor variables	PG	GL	EF	NF	PG	GL	EF	NF
TB*	**0.015**	**0.019**	0.115	0.798	0.688	0.452	**0.001**	0.236
Year	0.472	0.508	0.253	0.947	0.692	0.221	0.287	0.236
	Ashcraft: Cover				**Novich: Cover**			
	PG	GL	EF	NF	PG	GL	EF	NF
TB	0.771	**0.005**	**0.013**	0.680	0.142	0.067	**0.003**	**0.033**
Year	0.624	0.633	0.225	0.765	0.311	0.924	0.116	**<0.001**

*TB= tall buttercup, PG=perennial grass, GL=grass-like species, EF=exotic forbs, NF=native forbs

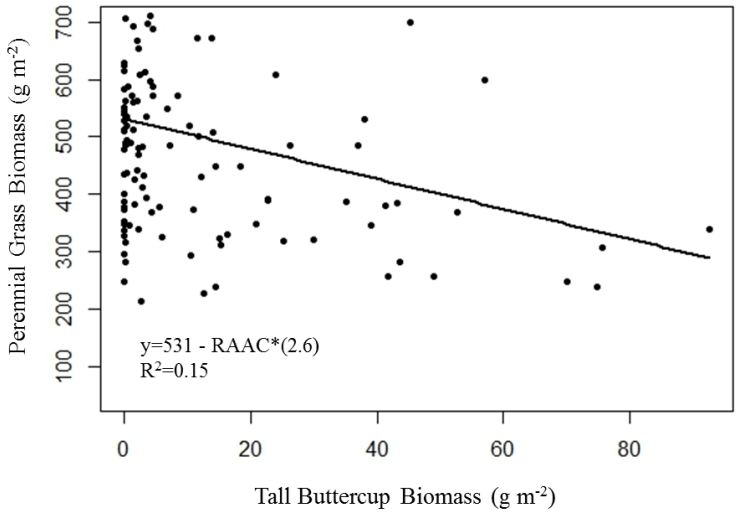


Figure 2.2. Regression model predicting perennial grass biomass as a function of tall buttercup biomass at the Ashcraft site.

Grass-like Species

Predicted grass-like biomass was positively related to tall buttercup biomass at the Ashcraft site (Table 2.1). Specifically, each g m^{-2} increase in tall buttercup was associated with a 0.9 g m^{-2} increase in biomass of grass-like species (Figure 2.3). Predicted grass-like cover was also positively related to tall buttercup cover. Every percent increase in tall buttercup was predicted to increase grass-like cover by 0.5% (Figure 2.4). At the Novich site, grass-like species were not associated with tall buttercup (Table 2.1).

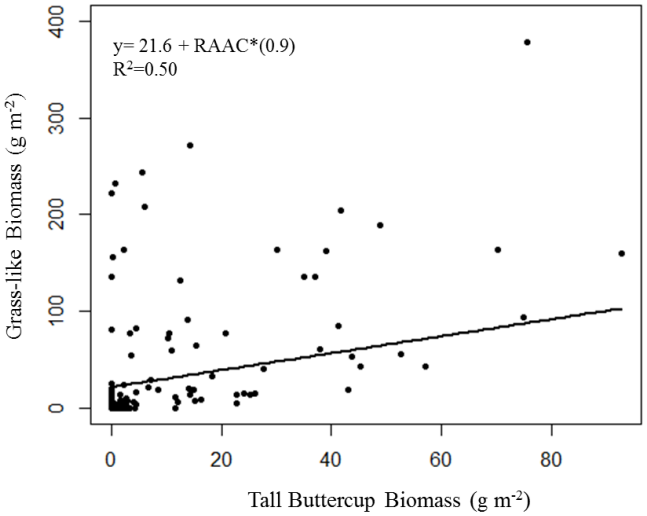


Figure 2.3. Regression model predicting grass-like biomass as a function of tall buttercup biomass at the Ashcraft site.

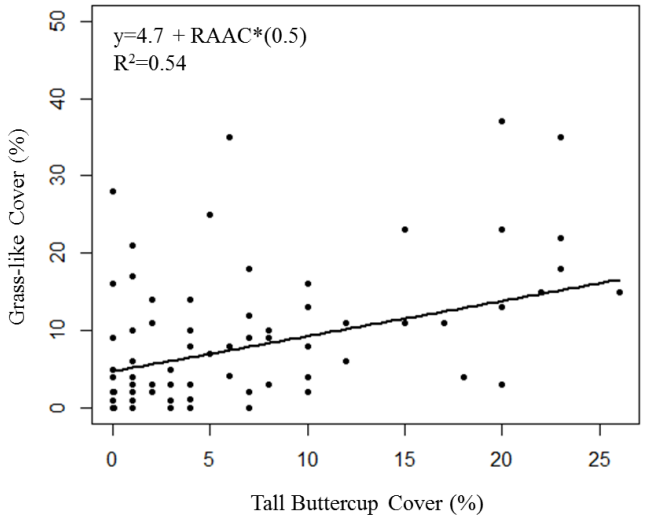


Figure 2.4. Regression model predicting grass-like cover as a function of tall buttercup cover at the Ashcraft site.

Exotic Forbs

Exotic forb biomass was not related to tall buttercup biomass at the Ashcraft site. However, exotic forb cover was positively related to tall buttercup cover (Table 2.1). Each percent increase in tall buttercup was associated with an increase in exotic forb cover of 0.1% (Figure 2.5).

At the Novich site, both exotic forb biomass and cover were positively associated with tall buttercup biomass and cover (Table 2.1). With each g m^{-2} increase in tall buttercup, exotic forb biomass increased by 0.9 g m^{-2} (Figure 2.6). Furthermore, each percent increase in tall buttercup cover was associated with a 0.5% increase in exotic forb cover (Figure 2.7).

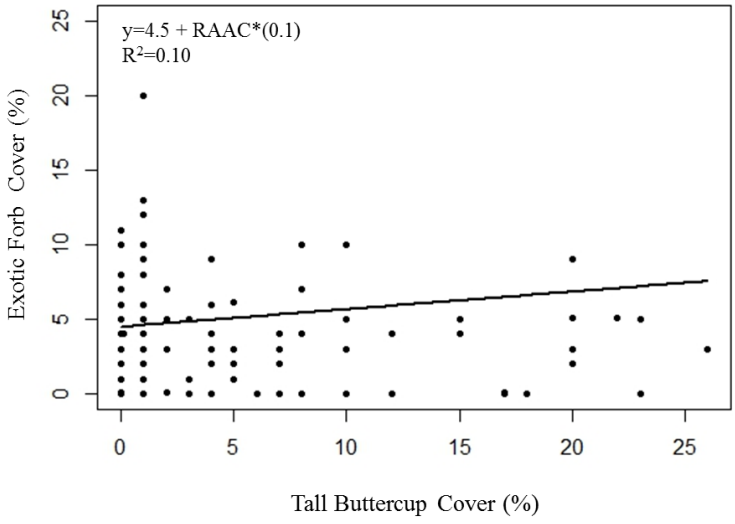


Figure 2.5. Regression model predicting exotic forb cover as a function of tall buttercup cover at the Ashcraft site.

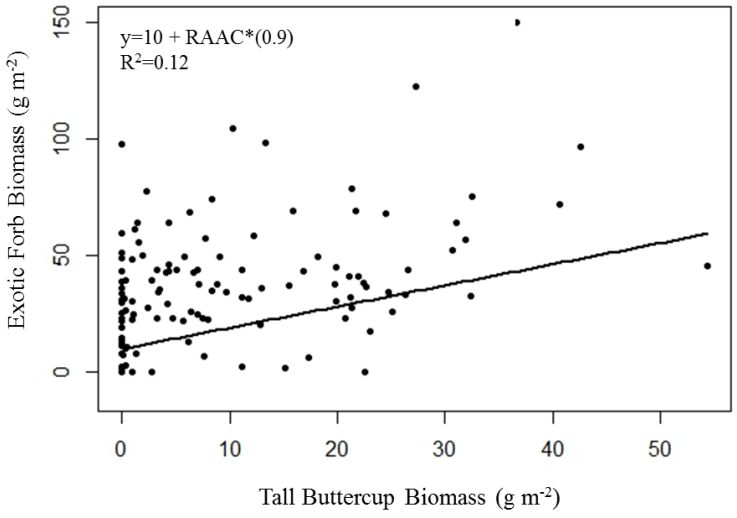


Figure 2.6. Regression model predicting exotic forb biomass as a function of tall buttercup biomass at the Novich site.

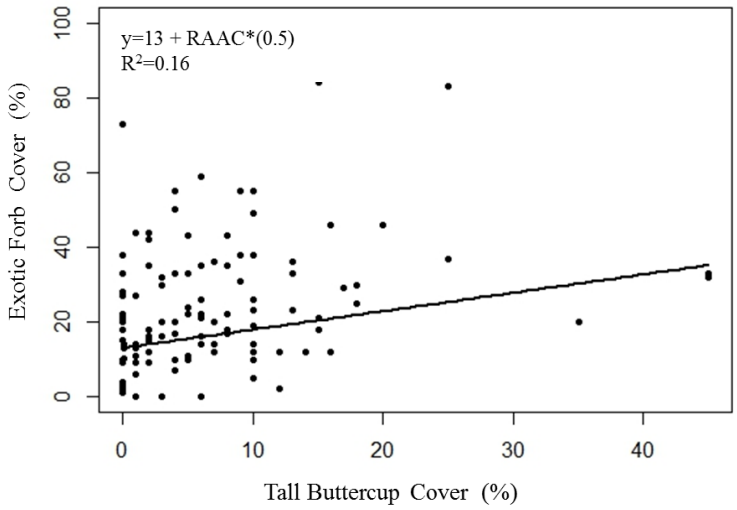


Figure 2.7. Regression model predicting exotic forb cover as a function of tall buttercup cover at the Novich site.

Native Forbs

There was no association between native forbs and tall buttercup at the Ashcraft site (Table 2.1). At the Novich site there was no association between native forb biomass and tall buttercup biomass. However, there was a negative relationship between native forb cover and tall buttercup cover. Native forb cover was also related to year (Table 2.1). Native forb cover was only negatively related to tall buttercup cover in 2012. In 2012, each percent increase in tall buttercup resulted in a 0.1% decrease in native forb cover (Figure 2.8).

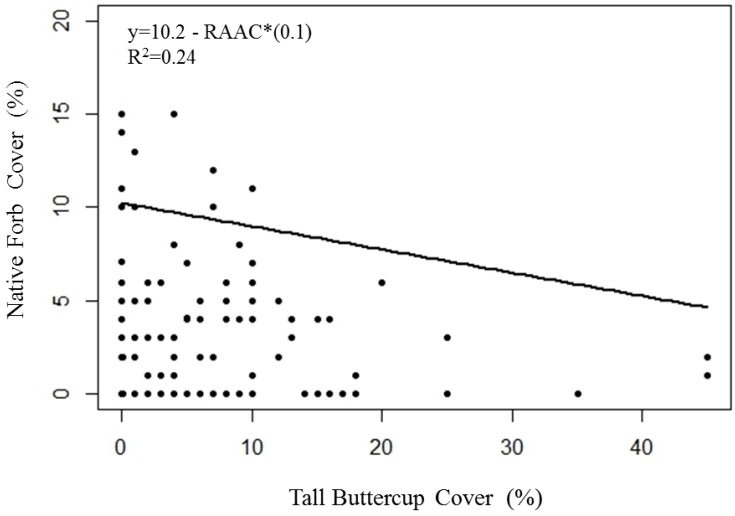


Figure 2.8. Regression model predicting native forb cover as a function of tall buttercup cover in 2012 at the Novich site. There was no association with native forb cover and tall buttercup in 2013.

Individual Species of Interest

For both sites, the species of interest were regressed against tall buttercup to

explore whether they had the same association with tall buttercup biomass and cover as their respective functional groups. Species of interest used in regression models are listed in Table 2.2. At the Ashcraft site, the perennial grass species meadow brome, Canada bluegrass, red top, smooth brome, and foxtail were not related to tall buttercup biomass or cover. However, similar to the association between perennial grass biomass and tall buttercup, creeping meadow foxtail biomass was predicted to decrease as tall buttercup biomass increased (P=0.04, R^2=0.22). Each g m^{-2} increase of tall buttercup was associated with a 2.7 g m^{-2} decrease of creeping meadow foxtail.

Furthermore, the grass-like species horsetail, rushes and arrowgrass were not related to tall buttercup biomass or cover at the Ashcraft site. However, sedge biomass and cover was predicted to increase as tall buttercup biomass and cover increased (P=0.01 and P=0.03, respectively). Biomass of exotic forb species including red and white clover and dandelion were not related to tall buttercup biomass. Predicted dandelion cover was positively associated with tall buttercup cover (P=0.04). Similar to the native forb functional group, the biomass and cover of individual native forb species were not association with tall buttercup biomass and cover.

At the Novich site, biomass and cover of perennial grass species including red top, Canada bluegrass, meadow brome, timothy, foxtail and quackgrass were not related to tall buttercup biomass or cover. However, creeping meadow foxtail was negatively associated with tall buttercup biomass (P=0.008, R^2=0.04). Each g m^{-2} increase in tall buttercup was associated with a 0.2 g m^{-2} decrease in creeping meadow foxtail biomass. Grass-like species including sedges, rushes and arrowgrass were not related to tall

buttercup biomass or cover. Similarly to exotic forb biomass, dandelion and red and white clover were positively associated with tall buttercup biomass (P=0.003 and P=0.023, respectively). Greater plantain, field sowthistle, alfalfa, and yellow sweet clover were not related to tall buttercup biomass. Predicted cover of dandelion, red and white clover were positively related to tall buttercup cover (P<0.001 and P=0.001, respectively). Native forb biomass was not associated with tall buttercup biomass.

Species Richness

Species richness was not associated with tall buttercup biomass (P=0.108) or year (P=0.895) at the Ashcraft site. Similarly at the Novich site, species richness was not related to tall buttercup biomass (P=0.767) or year (P=0.063). The Ashcraft site had a total of 16 species while the Novich site had a total of 28 species (Table 2.2).

Plant Diversity

At the Ashcraft site, plant diversity was positively related to tall buttercup biomass (P=0.013). Each g m^{-2} increase in tall buttercup was associated with a 0.003 increase in plant diversity (Figure 2.9a). Similarly, at the Novich site, plant diversity was positively associated with tall buttercup biomass (P<0.001). Each g m^{-2} increase of tall buttercup is associated with a 0.004 increase in plant diversity (Figure 2.9b).

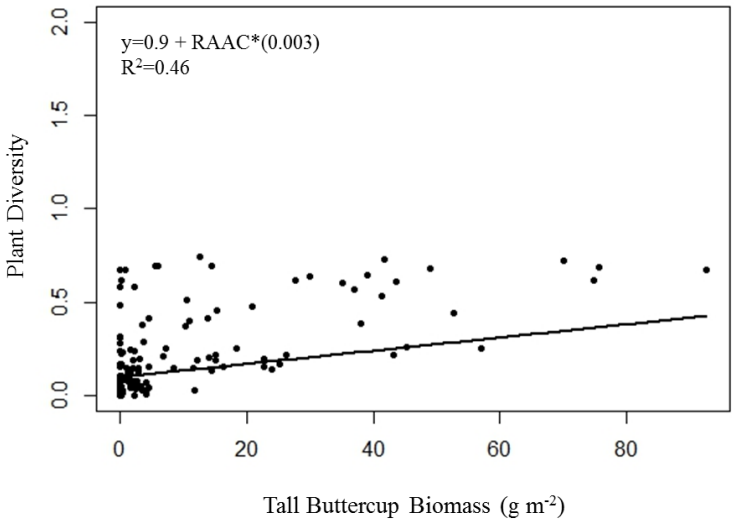
Gravimetric Soil Water and Tall Buttercup

For the two different soil moisture sampling events, tall buttercup biomass and cover were not related to gravimetric soil moisture at the Ashcraft site (P=0.847 and P=0.602, respectively). Likewise, there was no association of tall buttercup biomass and

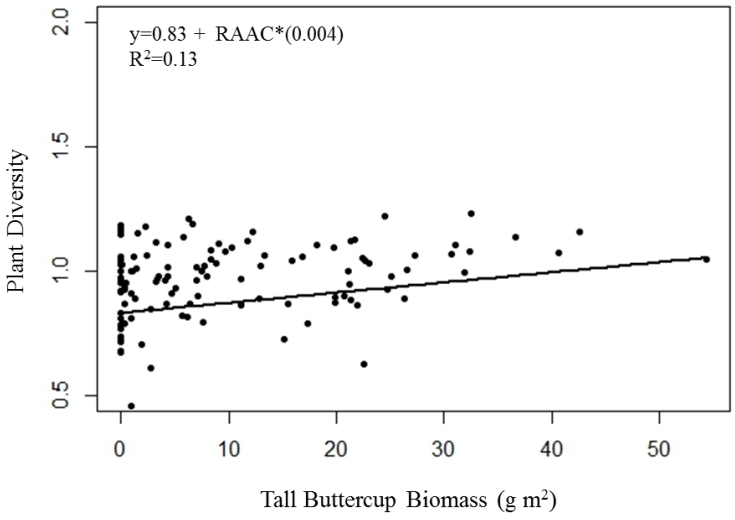
cover with gravimetric soil moisture at the Novich site (P=0.621 and P=0.530, respectively).

Table 2.2. Individual species of interest at each site by plant functional group. An "X" in the column under Ashcraft or Novich indicates that the species was found at that site.

Common Name	**Scientific Name**	**Ashcraft**	**Novich**
	Perennial Grasses (PG)		
Timothy	*Phleum pretense*	**X**	**X**
Meadow brome	*Bromus bieberrsteninii*	**X**	**X**
Creeping meadow foxtail	*Alopercurus arundinaceus*	--	**X**
Canada bluegrass	*Poa compressa*	**X**	**X**
Red top	*Agrostis stolonifera*	--	**X**
Foxtail	*Hordeum jubatum*	**X**	**X**
Quackgrass	*Elymus repens*	--	**X**
Intermediate wheatgrass	*Agropyron intermedium*	**X**	**X**
Smooth brome	*Bromus inermis*	**X**	**X**
Orchard grass	*Dactylis glomerata*	--	**X**
	Grass-Like Species (GL)		
Arrowgrass	*Triglochin maritimum*	**X**	**X**
Rush	Genus *Juncus*	**X**	**X**
Sedge	Genus *Carex*	**X**	**X**
Common spikerush	*Eleocharis palustris*	**X**	**X**
Nebraska sedge	*Carex nebrascensis*	**X**	**X**
Horsetail	Genus *Equisetum*	**X**	**X**
	Exotic Forbs (EF)		
Red and white clover	*Trifolium* spp.	**X**	**X**
Field sowthistle	*Sonchus arvensis*	--	**X**
Dandelion	*Taraxacum officinale*	**X**	**X**
Greater plantain	*Plantago major*	--	**X**
Yellow sweet clover	*Melilotus officinalis*	--	**X**
	Native Forbs (NF)		
Potentilla	*Cinquefoil* spp.	**X**	**X**
Aster spp.	*Symphyotrichum* spp.	**X**	**X**
Golden banner	*Thermopsis divaricarpa*	--	**X**
Unknown native forbs 1-4	--	--	**X**



a) Ashcraft site.



a) Novich site.

Figure 2.9. Regression models predicting plant diversity as associated with tall buttercup biomass at the a) Ashcraft site and b) Novich site. Models are presented on the non-transformed scale.

Discussion

Invasive plants are generally defined as species that can establish, naturalize and spread to habitats without further assistance by humans (Randall 1997). Some of the ecological impacts caused by invasive plants include a reduction in biodiversity, loss of habitat and food sources for wildlife, and loss or encroachment of endangered and threatened species and their habitats (Parker et al. 1999, Alien Plant Working Group 2002). In Montana it has been speculated that the invasive plant tall buttercup is reducing species richness, plant diversity, and forage production in the hayfield meadows where it invades (Appendix A). Tall buttercup has the potential to be a problematic invasive species in North America given its ability to outcompete clovers and perennial grasses and reduce forage production in New Zealand dairy pastures (Lamoureaux and Bourdôt 2007). However, prior to this study there was minimal data to support its assumed detrimental effects on plant community productivity and diversity in North America.

In general, few associations were found between the biomass and cover of tall buttercup and each functional group across my two study sites in western Montana. The only negative association found for forage production was between biomass of perennial grasses and tall buttercup at the Ashcraft site. At this site, a one gram m^{-2} increase in tall buttercup biomass predicted a decrease perennial grass biomass of 2.7 g m^{-2}. In New Zealand tall buttercup reduced forage production in dairy pastures where it invades, and the species replaces important pasture grasses through competition (Bourdôt and Lamoureaux 2002). My regression results support the hypothesis that tall buttercup reduces perennial grass biomass in Montana as it does in New Zealand.

Interestingly, creeping meadow foxtail was the only perennial grass species negatively associated with tall buttercup, and this negative association was present at both sites. At the Ashcraft site, creeping meadow foxtail was one of the most common perennial grass species in addition to meadow brome and Canada bluegrass. It is likely that the perennial grass response to tall buttercup at the Ashcraft site came from the negative association between creeping meadow foxtail and tall buttercup (P=0.04, R^2=0.22) While creeping meadow foxtail was also negatively associated with tall buttercup at the Novich site, the majority of perennial grass biomass was not comprised of the species. The perennial grasses with the highest biomass included meadow brome, Canada bluegrass, and red top. Furthermore, the regression model predicting creeping meadow foxtail as a function of tall buttercup did not explain a large percentage of the variation as it had a low R^2 value of 0.04 (P=0.008). This indicates that there are likely many other factors influencing the occurrence of creeping meadow foxtail at the Novich site rather than just tall buttercup including the other productive perennial grass species. For example, perhaps creeping meadow foxtail did not recover as quickly as other perennial grasses following grazing. Despite this, creeping meadow foxtail may be sensitive grass species to tall buttercup invasion.

Grass-like species were positively related to tall buttercup at the Ashcraft site. This finding may be a direct outcome of the habitat requirements of grass-like species and tall buttercup. In Montana grass-like species including sedges and rushes are considered Obligate Wetland species (greater than 99% occurrences are in wetlands); tall buttercup has similar habitat requirements and is considered a Facultative Wetland

species (67 to 99% occurrences are in wetlands) (Lesica and Husby 2006). It is likely that along each transect there were micro-habitats that provided more favorable conditions in terms of soil moisture and nutrients, allowing both tall buttercup and grass-like species to thrive.

From a management perspective, sedges and rushes are considered low quality forage plants (Torell et al. 2009). However, ecologically, this is important because tall buttercup has the potential to invade natural wetland habitats. Because it was positively associated with important wetland species like sedges and rushes, it is promising that grass-like plants in a natural wetland system may not be negatively affected by tall buttercup infestations of a similar nature to those encountered in this study. However, with a more severe infestation of tall buttercup, the impacts may be greater. Results may have been different if both sites were more severely infested.

Similarly, it is not surprising that as tall buttercup increased, exotic forb biomass and cover also increased. Like grass-like species, the exotic forbs found at both sites may have similar habitat requirements as tall buttercup. Both exotic forbs and tall buttercup likely had higher biomass and cover in areas along each transect where there was less competition from the large rhizomatous perennial grass species. From a forage production standpoint, the fact that tall buttercup was not associated with a decrease in red and white clovers is ideal. Clovers and other legumes produce high quality forage that is typically higher in crude protein, minerals and vitamins than perennial grasses. In particular, white clover has low levels of sugar and fiber which gives it a high digestibility rate and a higher energy value than perennial grasses (Dewhurst et al. 2009).

The presence of clovers in pastures can enhance the growth rate and milk yield of cattle in comparison to a pasture of pure grasses (Dewhurst et al. 2009, Russell and Johnson 2007). Because of this, landowners often want to increase clover production in their hayfield meadows. Management practices targeted to control tall buttercup will also reduce the prevalence of clovers (see Chapter Three), so landowners need to carefully consider the costs and benefits of controlling tall buttercup since it is not negatively associated with clover species.

The biomass and cover of native forb species was minimal across both sites. Even though hayfield meadows are not managed for high native forb diversity, at the Novich site native forb biomass and cover were negatively associated with tall buttercup. This finding is important because in a more natural, unmanaged habitat infested by tall buttercup, any reduction in native forbs may be detrimental. Increased native species diversity has been shown to decrease susceptibility to invasion by exotic species (Tilman 1997). Pokorny and others (2005) removed certain functional groups from a native plant community including shallow-rooted forbs, deep-rooted forbs, all forbs, or grasses. Following removal, the invasive forb spotted knapweed (*Centaurea stoebe*) was seeded. They found that native forbs resisted invasion of spotted knapweed better than grasses. The authors concluded that the loss of an important functional group like native forbs may increase the susceptibility of a plant community to invasion by an exotic forb (Pokorny et al. 2005). The results from this study are important to consider for tall buttercup invasions in both natural and managed systems. The minimal native forb biomass and cover in hayfield meadows may be contributing to the invasion of tall

buttercup. Furthermore, in natural systems susceptible to invasion by tall buttercup, it may be crucial to maintain a diversity of forb species to minimize or prevent tall buttercup invasion.

Species richness was not correlated with tall buttercup biomass at either site. My study system is highly managed and designed to have a low richness of a few very productive perennial grasses and clovers. Because the study system supports high productivity of a variety of species among four different plant functional groups, it is not surprising that tall buttercup biomass was not associated with species richness. In addition, it is possible that the characteristics of tall buttercup make it an invasive species that cannot easily compete with the surrounding plants in the hayfield meadows where it is found. A study by Hejda and others (2009) examined the effects of 13 different invasive plants on species richness and plant diversity. Of those 13 species, differences in species richness between invaded and uninvaded sites were found for five species, but most of the invasive plants in the study exhibited low to no impact on richness or diversity. The authors suggested that the impact of invasion is species-specific, and the severity of impact depends on the identity of the invading species. Species that had the strongest impact on species richness and diversity were taller than the resident species and had a vigorous growth rate which allowed them to achieve a higher cover. To measure the impact of species invasion, the traits of the invading species rather than the number of species lost should be examined (Hejda et al. 2009). Conclusions from my study can be applied to better understand the associations of tall buttercup infestations. It is possible that tall buttercup did not have any influence on species richness because it

was typically shorter than the surrounding vegetation and not able to achieve cover as high as that of the highly productive rhizomatous perennial grasses at the sites. The ability of tall buttercup to achieve higher cover may depend on the community type where it invades; however, at my study sites the association between tall buttercup and important forage species was minimal.

Tall buttercup was positively associated with plant diversity at both sites. This finding may be attributed to the fact that the hayfield meadows where tall buttercup invades are highly productive systems. Habitat conditions that were favorable for tall buttercup to thrive may have been equally as or more favorable for other species at both sites. Alternatively, other studies have found that productive and diverse plant communities are conducive to invasion by new species due to high resource availability (Stohlgren et al. 1999, Wiser et al. 1998, Lonsdale 1999). A large-scale study conducted by Houlahan and Findlay (2004) examined whether or not invasive plants in wetlands outcompeted the resident native plant species. Fifty-eight wetlands were included in the study, and it was found that invasive and native species richness were positively related, and that invasive plants were no more likely to become dominant than the native resident species. The authors did not suggest that invasive species are not a problem, rather that the evidence for the negative effects of invasive species on species richness is much less convincing and that exotic plant species are perhaps a minor threat to native wetland plant diversity. To conserve wetland plant diversity, they recommended focusing efforts on discouraging the spread of community dominants (Houlahan and Findlay 2004). Similarly to the invasive wetland plants in the Houlahan and Findlay (2004) study, it is

possible that because tall buttercup is also found in wetland-type habitats and was not a community dominant relative to the perennial grass and grass-like species at both sites, it was not associated with richness or diversity.

Generally it is assumed that all invasive plants have negative impacts that justify their control; however, there are some cases where the invasive plant may exert negligible effects on the surrounding plant community (Hejda et al. 2009, Houlahan and Findlay 2004, Skurski et al. 2013). Overall, tall buttercup was either not associated with or positively associated with the surrounding plant community. While at the Ashcraft site it was negatively associated with perennial grasses, specifically creeping meadow foxtail, it was not associated with perennial grasses at the Novich site. Tall buttercup was not associated with exotic forbs, species richness, or plant diversity at either site. Because of this, producers should consider both the costs and benefits to managing tall buttercup if it is not associated with forage loss. In a study conducted by Skurski et al. (2013), the community level impact of spotted knapweed in sage-brush grassland communities in southwestern Montana was examined. It was found that in some plant communities, spotted knapweed appears to have a negligible effect on the cover, richness and biomass of native forbs and grasses. These results indicated that despite the presence of the invasive spotted knapweed, it is possible for plant communities to maintain ecological values like forage production or wildlife habitat. The authors concluded that control efforts for spotted knapweed should not be abandoned, but further consideration should be given to the costs and benefits of management (Skurski et al. 2013). Likewise, if tall buttercup is not negatively associated to clovers and other forage species, perhaps it

doesn't have a negative effect, and the use of herbicides may be more detrimental than beneficial because clovers, and even grasses, may experience non-target effects of the herbicides (see Chapter Three). The magnitude of infestation should be assessed to determine if control efforts will favor hay production over tall buttercup.

The association of soil moisture on the biomass and cover of tall buttercup was explored through soil samples taken along each transect on two different occasions in the 2013 field season. I hypothesized that as soil water increased, tall buttercup biomass and cover would increase. No association was found between tall buttercup and soil moisture. While tall buttercup biomass or cover was not related to soil moisture, these findings do not suggest that tall buttercup does not require high soil moisture conditions. The amount of soil water at these sites varies greatly throughout the summer via rainfall and flood/sub-irrigation events. The soil moisture samples were only taken two times throughout the growing season, which was likely not enough data to find an association with tall buttercup. Further, sampling only the top several centimeters of the soil profile may not have been deep enough to obtain accurate soil moisture information. While gravimetric soil water can be an accurate and simple way to estimate soil moisture, it has drawbacks and can result in serious errors. This method only provides soil moisture information from the day samples were taken. Other methods that measure soil moisture continuously like Time-Domain Reflectrometry (TDR) may provide a more accurate representation of changing soil moisture conditions throughout the growing season (Schneekloth et al. 2014), and may provide deeper insight into the relationship between tall buttercup and soil moisture.

Management Implications

To my knowledge, this was the first study examining plant community level effects of tall buttercup in North America, and the results from my predictive models will be a valuable tool for weed managers and landowners to use to guide their management decisions (Archer 1989). The findings from my study were varied regarding the association of tall buttercup on forage production. Because tall buttercup was negatively associated with perennial grasses at the Ashcraft site, it has the potential to have the same effect in similar hayfield meadow habitats, especially in areas with more severe infestations. Moreover, because tall buttercup was negatively associated with the perennial grass species creeping meadow foxtail, hayfield meadows with a greater prevalence of this grass may experience increased negative impacts on forage production.

Hayfield meadows and grasslands where tall buttercup invades are dynamic systems that can vary greatly in productivity from year to year (Foster and Dickson 2004). Tall buttercup growth and its ability to outcompete desirable species may be dependent on the productivity of the surrounding plant community where it invades. In addition, land management practices may also strongly influence the association of tall buttercup on the surrounding plant community. For example, landowners may be able to alter the method and timing of their irrigation or grazing practices to achieve tall buttercup control (see Chapter Four). Producers must consider the tradeoffs to managing tall buttercup. If tall buttercup is not associated with reductions in forage production, species richness, or plant diversity, the risk of decreasing important forage species like clovers while controlling tall buttercup is likely not a desirable outcome.

While my study indicated tall buttercup has minimal associations on the plant community, it is still a Montana state listed noxious weed, which means that landowners are required to have a management plan for the species if it is on their property to prevent its spread. Further, control efforts are important and warranted in hayfield meadows due to the plant's toxicity and because hay can serve as a mechanism for seed dispersal for the species (Tuckett 1961, Jacobs et al. 2010). It is also possible that if tall buttercup infestations had been more severe at my study sites that increased negative associations between plant functional groups and tall buttercup would have been observed. Finally, because tall buttercup has been reported throughout North America (USDA PLANTS Database), and it is known to be an ecologically significant invader in New Zealand (Lamoureaux and Bourdôt 2007), further research is necessary in Montana and throughout North America. Particular emphasis should be placed on infestations found in different habitat types to better understand its breadth and invasion potential in both hayfield meadows and natural systems.

CHAPTER THREE

INTEGRATED MANAGEMENT OF TALL BUTTERCUP IN MONTANA HAYFIELD MEADOWS

Introduction

Tall buttercup (*Ranunculus acris* L.) is a perennial invasive forb native to central and northeastern Europe (Coles 1971). It occurs in moist habitats including pastures, grasslands and irrigated meadows (Lamoureaux and Bourdôt 2007). Tall buttercup was first reported in North America in the early 1900's. It has since been found in all but eight states and one Canadian Province (USDA PLANTS DATABASE). Tall buttercup has been invasive in New Zealand for many years. In particular, the species is problematic in the dairy farming regions of South Auckland, Hawke's Bay and in the Taranaki regions as well as the districts of Wairarapa, Horowhenua and Tasman (Lamoureaux and Bourdôt 2007). In these regions, tall buttercup is highly competitive and has been reported to infest over 50 percent of pasture areas. The plant produces the toxic glycoside ranunculin which gives it a bitter flavor, so it is typically avoided by grazing cattle (Lamoureaux and Bourdôt 2007); reducing the carrying capacity (Harper 1957). In 2001-2002 tall buttercup caused a national loss in dairy-farm income of $NZ 156 million (Bourdôt et al. 2003).

Tall buttercup has been controlled with herbicides in New Zealand since the 1950's. Phenoxy herbicides like MCPA and MCPB were used by dairy farmers between the 1950s and 1960s with adequate control (Tuckett 1961). Herbicides including paraquat, diquat, 2,4-D, and MCPA have been used in field experiments in

Czechoslovakia (Hrazdira 1970), Russia (Babenko 1991), Norway (Vidme 1973), and Slovakia (Lackovic 1974) and were shown to reduce tall buttercup and encourage the growth of more nutritional species in pasturelands. However, 30 years later tall buttercup has developed resistance to the herbicides MCPB and MCPA. The degree of MCPB and MCPA herbicide resistance is positively correlated with frequency of historical applications of both herbicides (Bourdôt et al. 1990). Genetic work concluded that MCPA resistance is a heritable trait in tall buttercup following high selection pressure (Bourdôt and Hurrell 1991).

Because of the potential of tall buttercup to develop herbicide resistance, an integrated management strategy incorporating various methods of control is warranted. Furthermore, integrated management encourage the growth and fitness of desired species in a way that meets environmental and economic priorities (Jacobs et al. 2006). Additional control methods that have been tested on tall buttercup include mowing and fertilizer application (Lamoureaux and Bourdôt 2007). Mowing can reduce tall buttercup flowering in red fescue dominated meadows in Russia (Saurina 1972). Additional studies in Scotland (Ford 1996), Austria (Buchgraber and Sobotik 1995), and Dagastan (Andreev and Gamidov 1985) have also shown reductions in tall buttercup flowering following cutting. Fertilizing with nitrogen (N), phosphorus (P), and potassium (K) increased palatable grasses, and decreased tall buttercup in deteriorated pastures in Russia (Melnikov et al. 1975). The addition of nitrogen fertilizer in grasslands in northwestern Greece reduced the relative abundance of tall buttercup (Tzialla et al. 2002). However, the addition of fertilizer to tall buttercup infested pastures in Austria (Buchgraber and

Sobotik 195), Slovenia (Leskosek 1996), and New Zealand (Brown 1993) had little impact on tall buttercup abundance. While these studies suggest that fertilizer application may not be effective in reducing tall buttercup abundance or growth, it does have the potential to promote more palatable species (Lamoureaux and Bourdôt 2007).

Tall buttercup has more recently been of concern in North America, especially in Montana where it has invaded over 8300 hectares (Montana Noxious Weed Summit Advisory Council 2008). It was listed as a priority 2A noxious weed in Montana in 2003 based upon their abundance across the state. Priority 2A species are common in isolated areas, and management practices are focused on eradication and containment (Montana Noxious Weed Summit Advisory Council 2008). As a state listed noxious weed, landowners with tall buttercup on their property are required to develop a management plan to control the species to prevent its spread. However, because there has never been a study on tall buttercup management in North America, landowners have little guidance about how to control the species while still maintaining productivity and plant diversity. Further, along with tall buttercup's history of developing herbicide resistance in New Zealand (Lamoureaux and Bourdôt 2007), research on integrated management is necessary to provide land managers with guidelines to control the species and to prevent it from becoming even more widespread across the state.

The objective of this two year study was to test various tall buttercup management approaches including herbicides, mowing, and fertilization alone and in combination with each other. These management strategies were selected to specifically target tall buttercup while encouraging the growth of more desirable pasture plants. Tall buttercup

was predicted to decrease in response to control treatments, with the greatest control coming from the combination of herbicides, mowing and fertilization. Desired perennial grasses were predicted to increase following treatments. Response of desired forbs would vary depending on species and control treatment.

Materials and Methods

Site Description

Two study sites were selected approximately 16 km southwest from Twin Bridges, Montana. Sites were located in flood and sub-irrigated hayfield meadows managed primarily for forage production and were chosen based upon tall buttercup density and landowner cooperation. Sites are referred to as Novich and Ashcraft, in reference to the names of the landowners of either site. The land management practices on both study sites include cattle grazing in the early summer and haying in the late summer. In the mid-summer the Novich site is flood irrigated and the Ashcraft site is sub-irrigated. These management practices occurred as normal throughout the project's duration. The Novich and Ashcraft sites are located approximately three km from one other.

Elevation at the Novich site is 1448 m (45°29'51.90", 112° 24'06.77"). The soil is a Rivra-Ryell Havre complex and has a pH of 7.8. Elevation at the Ashcraft site is 1436 m (45°29'25.07", 112°26'26.76'). Soil at this site is also a Rivra-Ryell Havre complex with a pH of 7.6 (45°29'25.07", 112°26'26.76'). Soil texture at both sites consists of a sandy clay loam (Appendix B). At both sites the mean annual average precipitation is

24.2 cm with an average annual temperature of 14.6°C. Weather data was compiled from 6/1/1950 to 2/28/2013 and was obtained from the Western Regional Climate Center from a weather station in Twin Bridges, Montana (NCDC 2002).

Experimental Design

The study followed a split-plot design with four replicates at each site. Sites were selected in spring 2012 and treatments were applied once during a two day time period between 06/20/12 and 06/21/12. Whole-plots were 12.2 m x 9.1 m and placed five m apart from one another to minimize border effects. One of four different herbicide treatments was randomly assigned to each whole plot including 1) non-sprayed; 2) aminopyralid (AMP) [172 g a.i. ha^{-1} applied as Milestone™ at 5 oz $acre^{-1}$]; 3) dicamba (DIC) [981 g a.i. ha^{-1} applied as Vanquish® at 24 oz $acre^{-1}$]; and 4) aminocylopyrachlor + chlorsulfuron (AMCP+CHL) [83 g a.i. ha^{-1} + 33 g a.i. ha^{-1} applied as Perspective™ at 3 oz $acre^{-1}$]. Within each whole-plot were four 3 m x 9 m split-plots. One of four different treatments were randomly assigned to each split-plot including 1) mowing, 2) fertilization (28 kg N ha^{-1} using Scott's Lawn Pro™ 26-0-3), 3) mowing plus fertilization, and 4) the non-treated control (Figure 3.1). Mowing was applied before any other treatment on 6/20/12 using a push mower (Husqvarna model 5521P). Vegetation was mowed to a height of 15 cm, and clippings were removed from each plot. Following mowing, fertilizer was hand broad-cast in the assigned split-plots. Herbicides were applied last using a CO_2 powered 3.3 m boom sprayer delivering 157 L ha^{-1} water at three kg cm^{-2} pressure.

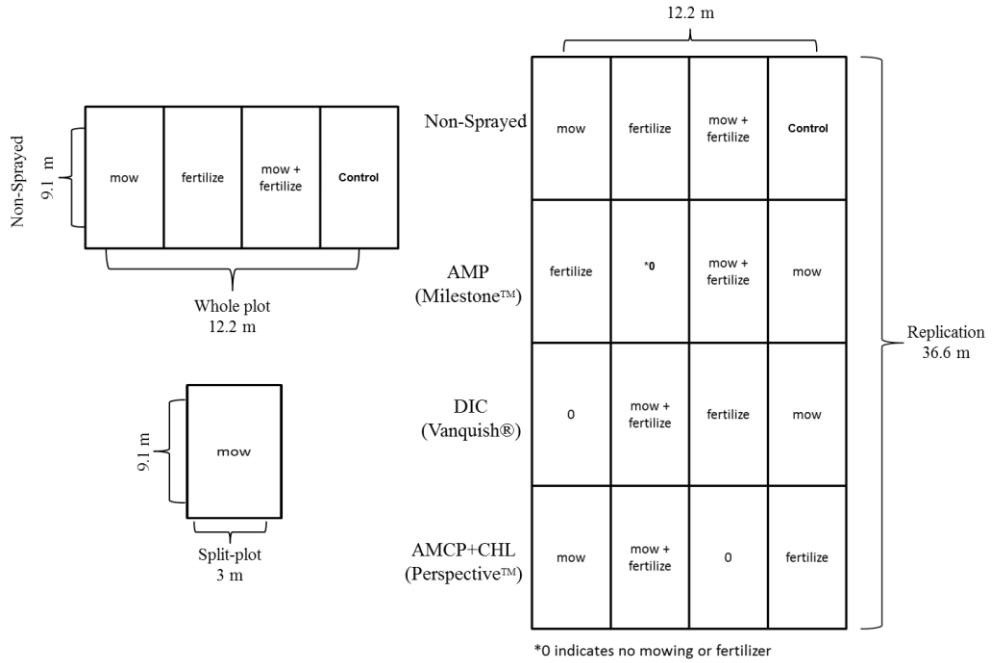


Figure 3.1. Split-plot design.

Vegetation Sampling

Plots were sampled during peak standing biomass in late July 2012 and 2013, approximately one month and one year, respectively, after treatment. Three 20cm x 50cm Daubenmire (1959) frames were randomly placed in each split-plot. Percent canopy cover was estimated by species, and biomass was clipped by functional group. Functional groups included tall buttercup (TB), perennial grass (PG), exotic forb (EF), grass-like species including sedges and rushes (GL), and native forb (NF). Species found at each site within each functional group are listed in Table 3.1. Vegetation samples were taken to the Plant Growth Center (PGC) at Montana State University, Bozeman, Montana. Biomass samples were dried at 65°C for a minimum of 72 hours to obtain dry plant biomass to the nearest 0.1 gram.

Table 3.1. Species found at each site by plant functional group. An "X" in the column under Ashcraft or Novich indicates that the species was found at that site.

Common Name	Scientific Name	Ashcraft	Novich
	Perennial Grasses (PG)		
Timothy	*Phleum pretense*	**X**	**X**
Meadow brome	*Bromus bieberrsteninii*	**X**	**X**
Creeping meadow foxtail	*Alopercurus arundinaceus*	--	**X**
Canada bluegrass	*Poa compressa*	**X**	**X**
Red top	*Agrostis stolonifera*	--	**X**
Foxtail	*Hordeum jubatum*	**X**	**X**
Quackgrass	*Elymus repens*	--	**X**
Intermediate wheatgrass	*Agropyron intermedium*	**X**	**X**
Smooth brome	*Bromus inermis*	**X**	**X**
Orchard grass	*Dactylis glomerata*	--	**X**
Blue wildrye	*Elymus glaucus*	**X**	**X**
	Grass-Like Species (GL)		
Arrowgrass	*Triglochin maritimum*	**X**	**X**
Rush	Genus *Juncus*	**X**	**X**
Sedge	Genus *Carex*	**X**	**X**
Common spikerush	*Eleocharis palustris*	**X**	**X**
Nebraska sedge	*Carex nebrascensis*	**X**	**X**
Horsetail	Genus *Equisetum*	**X**	**X**
	Exotic Forbs (EF)		
Red and white clover	*Trifolium* spp.	**X**	**X**
Field sowthistle	*Sonchus arvensis*	--	**X**
Dandelion	*Taraxacum officinale*	**X**	**X**
Greater plantain	*Plantago major*	--	**X**
Yellow sweet clover	*Melilotus officinalis*	--	**X**
Alfalfa	*Medicago sativa*	--	**X**
	Native Forbs (NF)		
Potentilla	*Cinquefoil* spp.	**X**	**X**
Aster spp.	*Symphyotrichum* spp.	**X**	**X**
Golden banner	*Thermopsis divaricarpa*	--	**X**
Rocky Mountain Iris	*Iris missouriensis*	--	**X**

Soil Nutrient Sampling

Both sites had been fertilized with N by the landowners each year prior to the start of the study. Because of this, it was important to obtain baseline nutrient content in the

soil under non-sprayed split-plots that were not treated by mowing or fertilization, and under non-sprayed split-plots that had only received a fertilizer treatment. Soil sampling helped to determine whether the fertilizer treatment had any measurable effect on soil N concentration beyond that of standard fertilization practices by landowners in the vicinity of the study.

In June 2013, soil was sampled for nutrient analysis in each of the control split-plots (no herbicide, mowing or fertilizer) and in the non-sprayed/fertilize split-plot. A tulip bulb planter was used to collect 2,330 cm^3 of the soil to a depth of 15 cm in five random soil samples within each split-plot. Soils were composited and sent to a third-party soil analytics lab (AGVISE Laboratories, Northwood, ND) for analysis of N, P, and K, pH, percent soil organic matter, soil type and texture.

Statistical Analysis

To determine the effects of herbicide, mowing and fertilizer treatments on biomass and percent cover of each functional group, and year, split-plot repeated measures analysis of variance (ANOVA) was conducted using R Studio 0.98.501 statistical software. This analysis was used because data was collected on the same split-plots across both years. Response variables were ln transformed to meet assumptions of normality and constant variance. Sites were analyzed separately. When possible, percent cover of forage species of interest and the most commonly found species at each site were also analyzed to determine treatment effects. Species of interest included meadow brome (*Bromus biebersteinii*), Canada bluegrass (*Poa compressa*), creeping meadow foxtail (*Alopecurus arundinaceus*), timothy (*Phleum pretense*), red top (*Agrostis*

stolonifera), arrowgrass (*Triglochin maritima*), sedges (*Carex* spp.), rushes (*Juncus* spp.), clover (*Trifolium* spp.), and a native cinquefoil (*Potentilla* spp.). When significant main effects or interactions were found ($P \leq 0.05$), means separation tests were performed using Conventional Tukey's Test using the R package TukeyC ($\alpha \leq 0.05$) (Faria et al. 2013). Non-transformed means are presented for ease of interpretation. Soil nutrient results were averaged together by split-plot (control or non-sprayed/fertilized split-plot) for both sites. Two-sample t-tests were used to determine if the fertilization treatments had any effect on the levels of soil N, P, or K.

Results

Tall Buttercup (TB)

Ashcraft Site. The main effect of herbicide and the interaction between herbicide and year influenced tall buttercup biomass and cover. Tall buttercup biomass was also affected by the main effect of fertilize and mow (Table 3.2). In 2012, all three herbicides resulted in tall buttercup biomass similar to the non-sprayed treatment. The AMCP+CHL treatment had 8.4 ± 1.5 g m^{-2}, AMP had 10.1 ± 3.1 g m^{-2}, DIC had 6.6 ± 1.7 g m^{-2}, and the non-sprayed treatment had 11.7 ± 6.9 g m^{-2} of tall buttercup (Figure 3.2). However, in 2013 all three herbicides reduced biomass relative to the non-sprayed treatment. The AMCP+CHL treatment had 0.5 g m^{-2} ± 0.4 g m^{-2} of tall buttercup, while plots treated with AMP had 0.5 ± 0.3 g m^{-2}. DIC also reduced tall buttercup, but plots treated with DIC had a higher biomass than the AMCP+CHL and AMP treatments. DIC treated plots

had 8.1 ± 2.3 g m^{-2}, while the non-sprayed treatment had 14.5 ± 3.0 g m^{-2} of tall buttercup (Figure 3.2). When examining the fertilizer and mow treatments across both years, mowing reduced tall buttercup biomass to 4.1 ± 0.9 g m^{-2} relative to the control that had 14.1 ± 4.6 g m^{-2} of tall buttercup. The fertilizer treatment was similar to the mow treatment, and reduced tall buttercup to 6.1 ± 1.2 g m^{-2}. The treatment combination of fertilizer and mow had biomass comparable to the control (7.5 ± 1.8 g m^{-2}).

Table 3.2. *P*-values for treatment effects on functional group biomass and cover from the Ashcraft site. Significant *P*-values (<0.05) are indicated in bold.

		Biomass			
Parameter	**Df**	**TB**	**PG**	**GL**	**EF**
Herb	3	**<0.001**	**<0.001**	**0.021**	0.118
Fert Mow	3	**0.037**	0.242	0.112	0.069
Herb*Fert Mow	9	0.207	0.261	0.763	0.266
Year	1	**<0.001**	**<0.001**	0.626	**<0.001**
Herb*Year	3	**<0.001**	**<0.001**	0.053	0.156
Fert Mow*Year	3	0.708	0.239	0.277	0.372
Herb*Fert Mow*Year	9	0.686	0.171	0.784	0.165
		Cover			
Parameter	**Df**	**TB**	**PG**	**GL**	**EF**
Herb	3	**<0.001**	0.327	0.155	**0.023**
Fert Mow	3	0.276	0.582	0.375	**0.014**
Herb*Fert Mow	9	0.835	0.880	0.868	0.201
Year	1	**<0.001**	**<0.001**	**0.006**	**<0.001**
Herb*Year	3	**<0.001**	**0.002**	0.112	0.658
Fert Mow*Year	3	0.076	0.099	0.729	0.066
Herb*Fert Mow*Year	9	0.832	0.561	0.788	0.387

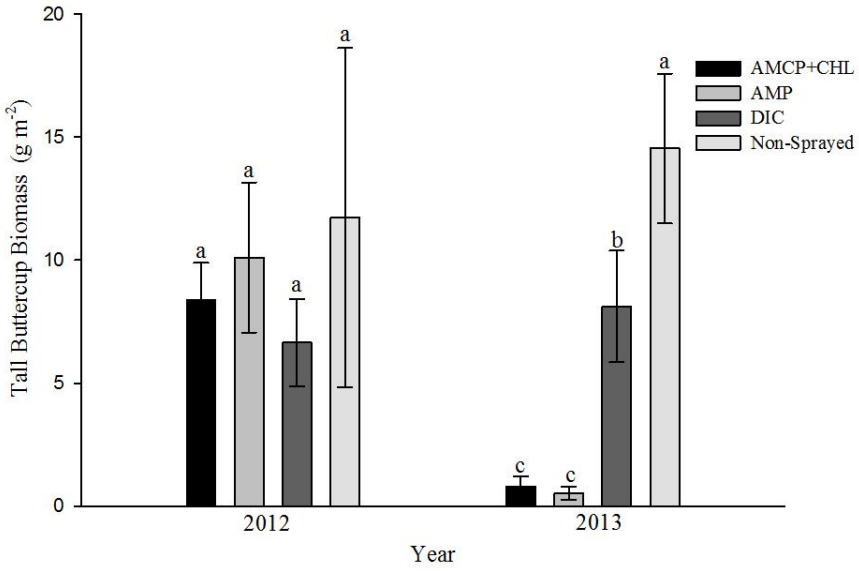


Figure 3.2. Tall buttercup biomass as affected by herbicide and year at the Ashcraft site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

Herbicide effect on tall buttercup cover depended on year (Table 3.2). In 2012, all herbicide treatments had similar tall buttercup cover; however, in 2013 both AMCP+CHL and AMP reduced cover relative to the non-sprayed treatment. Cover in the AMCP+CHL and AMP treatments was 0.10 ± 0.05 % and 0.13 ± 0.06 %, respectively, while cover in the non-sprayed treatment was 2.2 ± 0.66 %. The DIC treatment resulted in cover of 1.5 ± 0.35 %, which was similar to the non-sprayed treatment (Figure 3.3).

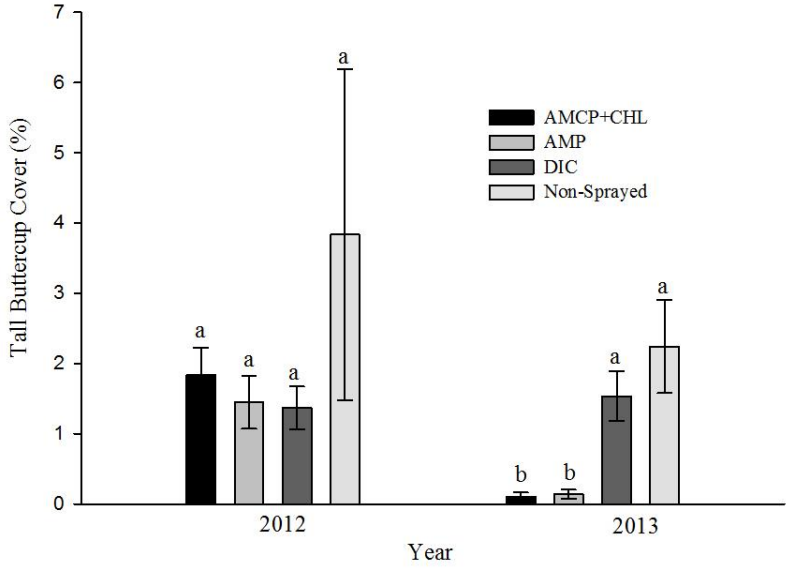


Figure 3.3. Tall buttercup cover as affected by herbicide and year at the Ashcraft site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

Novich Site. Tall buttercup biomass and cover were influenced by herbicide and the interaction of year and herbicide (Table 3.3). In 2012, AMCP+CHL, AMP and DIC reduced tall buttercup biomass to 6.5 ± 2.1 g m^{-2}, 6.3 ± 0.99 g m^{-2}, and 11 ± 2.1 g m^{-2}, respectively; biomass in the non-sprayed treatment was 42 ± 8.1 g m^{-2} (Figure 3.4). In 2013 the non-sprayed treatment had 28 ± 7.9 g m^{-2} of tall buttercup. AMCP+CHL treatment reduced biomass to 0.2 ± 0.1 g m^{-2} while AMP reduced biomass to 0.3 ± 0.2 g m^{-2}. Tall buttercup in the DIC treatment was higher than in the other two herbicide treatments, yet still less than in the non-sprayed treatment (12 ± 3.4 g m^{-2}) (Figure 3.4).

Table 3.3. *P*-values for treatment effects on functional group biomass and cover from the Novich site. Significant *P*-values (<0.05) are indicated in bold.

		Biomass				
Parameter	**Df**	**TB**	**PG**	**GL**	**EF**	**NF**
Herb	3	**<0.001**	**<0.001**	0.675	**<0.001**	0.060
Fert Mow	3	0.789	0.546	0.065	0.721	0.187
Herb*Fert Mow	9	0.444	0.989	0.312	0.241	0.549
Year	1	**<0.001**	**<0.001**	0.731	**<0.001**	0.941
Herb*Year	3	**<0.001**	**<0.001**	0.770	**<0.001**	0.219
Fert Mow*Year	3	0.210	0.142	0.698	0.682	0.525
Herb*Fert Mow*Year	9	0.876	0.951	0.829	0.787	0.293
		Cover				
Parameter	**Df**	**TB**	**PG**	**GL**	**EF**	**NF**
Herb	3	**<0.001**	0.347	**0.013**	**0.012**	0.684
Fert Mow	3	0.721	0.861	0.879	0.436	0.321
Herb*Fert Mow	9	0.083	0.069	0.662	0.694	0.062
Year	1	**<0.001**	**<0.001**	0.244	**<0.001**	0.502
Herb*Year	3	**<0.001**	0.519	0.292	**<0.001**	0.684
Fert Mow*Year	3	0.576	0.942	0.673	0.520	0.241
Herb*Fert Mow*Year	9	0.356	0.411	0.419	0.792	0.367

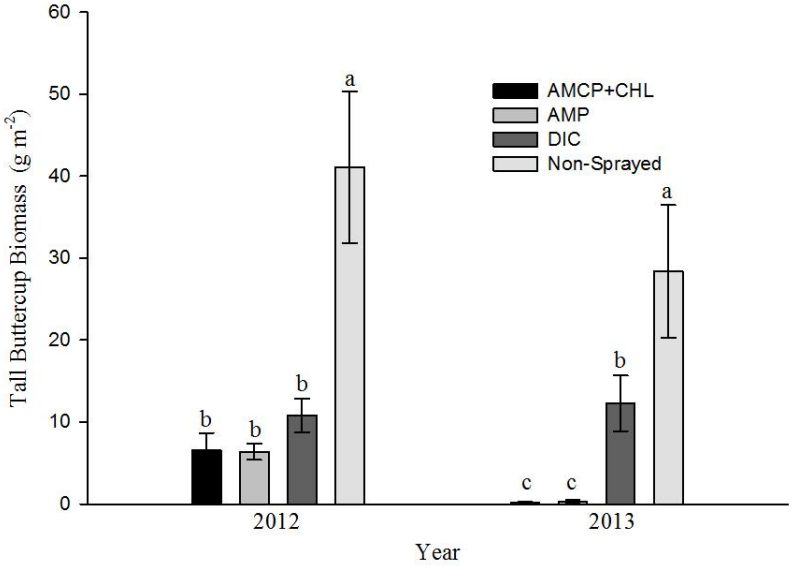


Figure 3.4. Tall buttercup biomass as affected by herbicide and year at the Novich site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

The effect of herbicide and year on tall buttercup cover was similar to that of biomass (Table 3.3). In 2012, treatments sprayed with any of the three herbicides resulted in lower tall buttercup cover than the non-sprayed treatment. In 2012, cover in the AMCP+CHL was the lowest at 1.4 ± 0.40 %. This was similar to the cover of AMP at 1.8 ± 0.32 % but less than that of DIC at 2.3 ± 0.43 % (Figure 3.5). In 2013, cover in the AMCP+CHL and AMP treatments was about 0.1 ± 0.1%, which was lower than both the DIC and non-sprayed treatments. Cover in the DIC treatment was 2.6 ± 0.5%, which was lower than that of the non-sprayed treatment at 5 ± 1.1% (Figure 3.5).

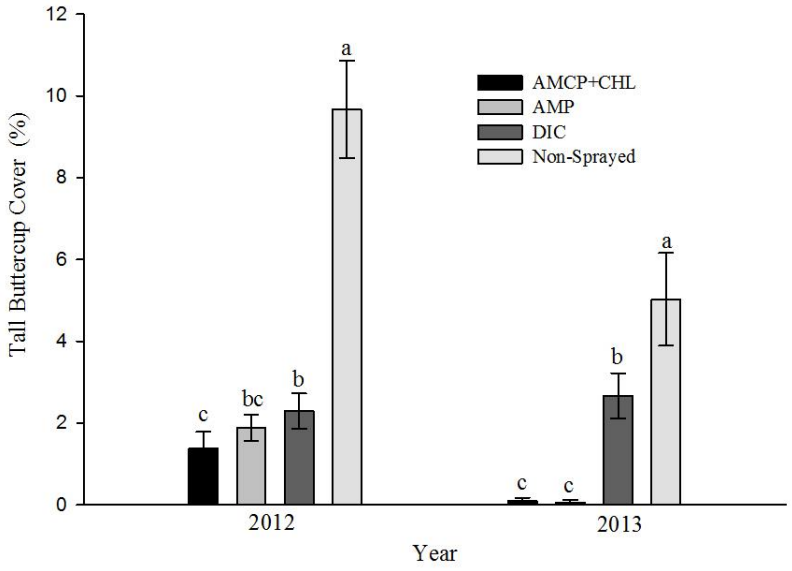


Figure 3.5. Tall buttercup cover as affected by herbicide and year at the Novich site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

Perennial Grass (PG)

Ashcraft Site. Year and herbicide interacted to affect perennial grass biomass and cover (Table 3.2). In 2012, AMCP+CHL treatment reduced perennial grass biomass to 824 ± 83 g m^{-2} relative to the non-sprayed treatment where biomass was 1,183 ± 107 g m^{-2}. AMP and DIC treatment plots had similar perennial grass biomass as the non-sprayed treatment with 1,009 ± 68 g m^{-2} and 1,053 ± 92 g m^{-2}, respectively. However, in 2013 AMP and DIC treatment plots had increased biomass to 1,031 ± 92 g m^{-2} and 1,026 ± 60 g m^{-2}, respectively, compared to the non-sprayed treatment at 952 ± 105 g m^{-2}. AMCP+CHL treatment reduced biomass in 2013 to 800 ± 64 g m^{-2} (Figure 3.6).

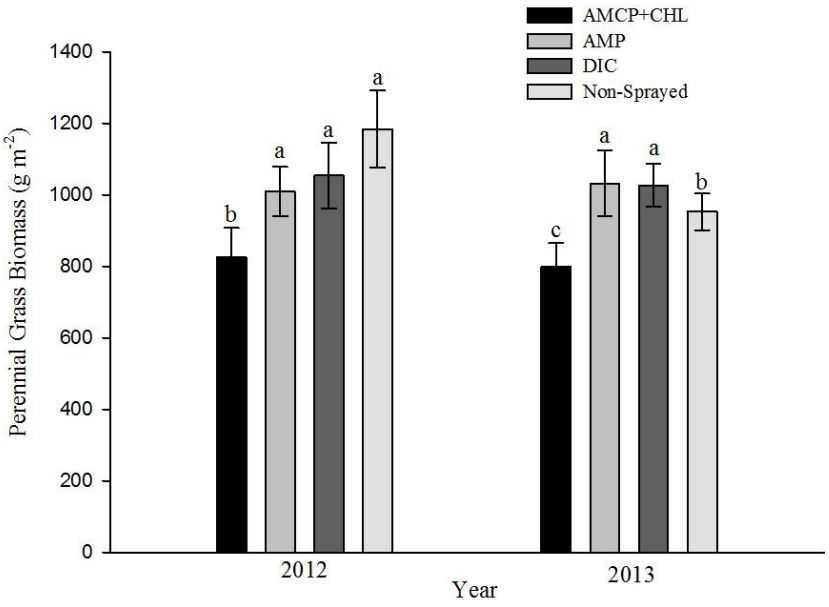


Figure 3.6. Perennial grass biomass as affected by herbicide and year at the Ashcraft site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

Perennial grass cover resulted in somewhat similar findings as biomass in relation to the herbicide treatments. In 2012 cover in the non-sprayed treatment was 79 ± 2.9%. AMCP+CHL treatment reduced cover to 68 ± 3.6 %, while AMP and DIC treated plots had similar cover as the non-sprayed treatment plots (77.2 ± 2.2 % and 77.4 ± 3.4%, respectively; Figure 3.7). In 2013, AMP was the only herbicide that had an effect on cover compared to the non-sprayed treatments. AMP increased cover to 70 ± 3.1 % versus 54 ± 9.9 % in the non-sprayed treatment. AMCP+CHL and DIC had the same cover of 63 ± 3.4% and were not different than the non-sprayed treatment.

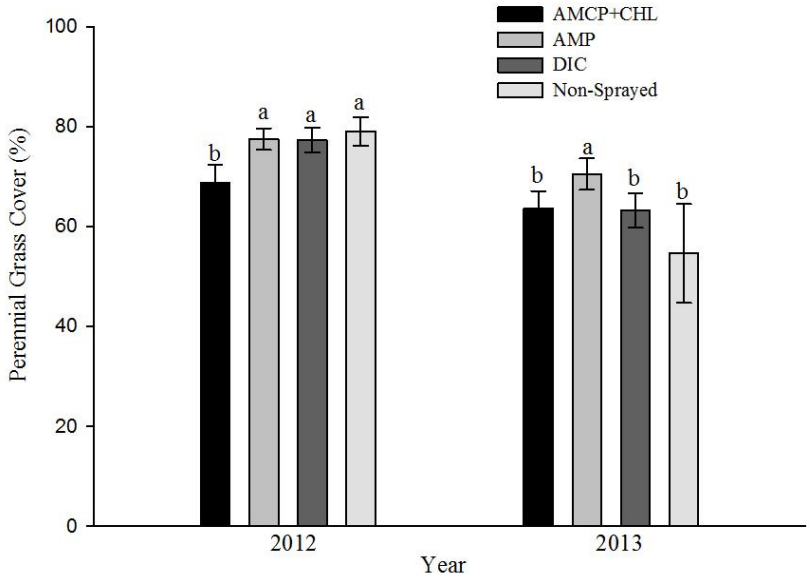


Figure 3.7. Perennial grass cover as affected by herbicide and year at the Ashcraft site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

Novich Site. Perennial grass biomass was influenced by the main effect of year and the interaction between herbicide and year. Only year influenced perennial grass cover (Table 3.3). In 2012, perennial grass cover was 57 ± 1.8%, while in 2013 cover was 50 ± 2.5%. Perennial grass biomass in the non-sprayed treatment was 605 ± 104 g m^{-2} in 2012. Relative to the non-sprayed treatment, AMCP+CHL decreased biomass to 405 ± 38g m^{-2}. AMP and DIC had similar perennial grass biomass as the non-sprayed treatment with 622 ± 49 g m^{-2} and 600 ± 53 g m^{-2}, respectively (Figure 3.8). In 2013, AMCP+CHL reduced biomass to 477 ± 49 g m^{-2} compared to the non-sprayed treatment at 556 ± 57 g m^{-2}. AMP and DIC remained similar to the non-sprayed treatment at 697 ± 60 g m^{-2} and 598 ± 35 g m^{-2}, respectively (Figure 3.8).

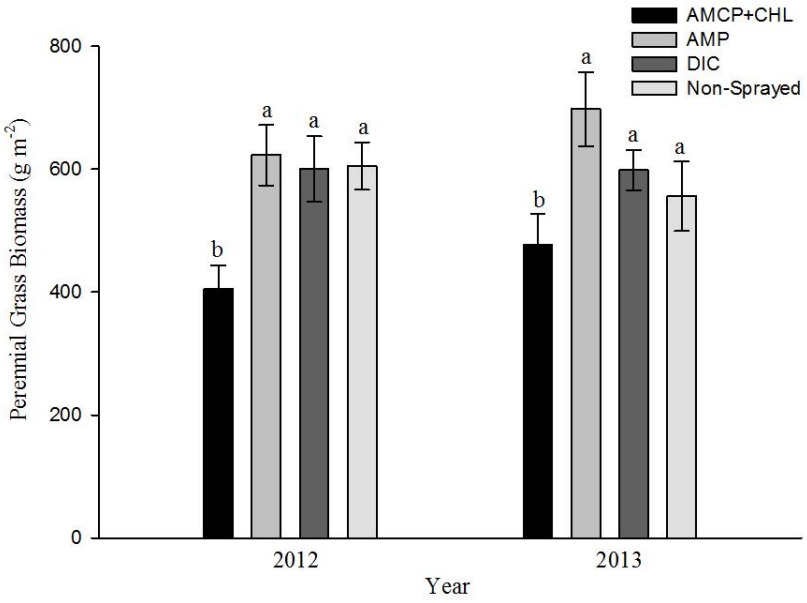


Figure 3.8. Perennial grass biomass as affected by herbicide and year at the Novich site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

Grass-like Species

Ashcraft Site. The main effect of herbicide influenced the biomass of grass-like plants (Table 3.2). The non-sprayed treatment had 164 ± 32 g m^{-2} of grass-like species. AMP and DIC treated plots had similar grass-like biomass as the non-sprayed treatment with 126 ± 10 g m^{-2} and 133 ± 20 g m^{-2}, respectively. Grass-like biomass was the highest in the AMCP+CHL treatment plots with 207 ± 25 g m^{-2}. Further, there was a main effect of year on grass-like cover; in 2012 cover was 7 ± 0.44%, and in 2013 grass-like cover was 9.5 ± 0.84%.

Novich Site. Grass-like cover was influenced by herbicide treatment (Table 3.3). Cover was 13.0 ± 0.9% in the DIC treatment, which was similar to the non-sprayed treatment where cover was 14.8 ± 1.2%. AMCP+CHL and AMP treatments increased grass-like cover relative to the other two treatments. AMCP+CHL treated plots had a grass-like cover of 21.6 ± 2.1% while AMP treated plots had a cover of 21.0 ± 2.6%.

Exotic Forbs (EF)

Ashcraft Site. Exotic forb biomass was only influenced by the main effect of year (Table 3.2). In 2012, exotic forb biomass was 24 ± 3.2 g m^{-2} and in 2013 biomass decreased to 19 ± 3.1 g m^{-2}. The main effect of herbicide influenced exotic forb cover (Table 3.2). All three herbicides resulted in a decrease in cover when compared to the non-sprayed treatment. The non-sprayed treatment plots had 9.8 ± 1.0% cover while AMCP+CHL, AMP, and DIC reduced cover to 8.0 ± 0.4, 7.0 ± 0.3 and 6.0 ± 0.6%, respectively.

The main effect of the split-plot treatments fertilize and mow influenced tall buttercup cover (Table 3.2). The control plots had 10 ± 1.9% cover of exotic forbs which was similar to the fertilizer treatment plots that had a cover of 7 ± 1.8%. The mow and combination of fertilizer and mow treatments reduced exotic forb cover relative to the control (4 ± 1.3% and 3.8 ± 1.7 %, respectively).

Novich Site. Exotic forb biomass and cover were influenced by the interaction between herbicide and year (Table 3.3). In 2012, the non-sprayed treatment had 102 ± 16 g m^{-2} of exotic forbs while AMCP+CHL, AMP and DIC all reduced exotic forb biomass

(93 ± 1.8 g m^{-2}, 85 ± 3.0 g m^{-2}, 83 ± 3.8 g m^{-2}, respectively; Figure 3.9). In 2013, all herbicides decreased biomass relative to the non-sprayed treatment. The non-sprayed treatment plots had a biomass of 86 ± 25 g m^{-2} while AMCP+CHL reduced exotic forbs to the greatest extent with 1.8 ± 1.6 g m^{-2}. AMP and DIC also reduced biomass, relative to the non-sprayed treatment, but were different than the AMCP+CHL treatment (11 ± 2.7 g m^{-2} and 12.5 ± 1.9 g m^{-2}, respectively; Figure 3.9).

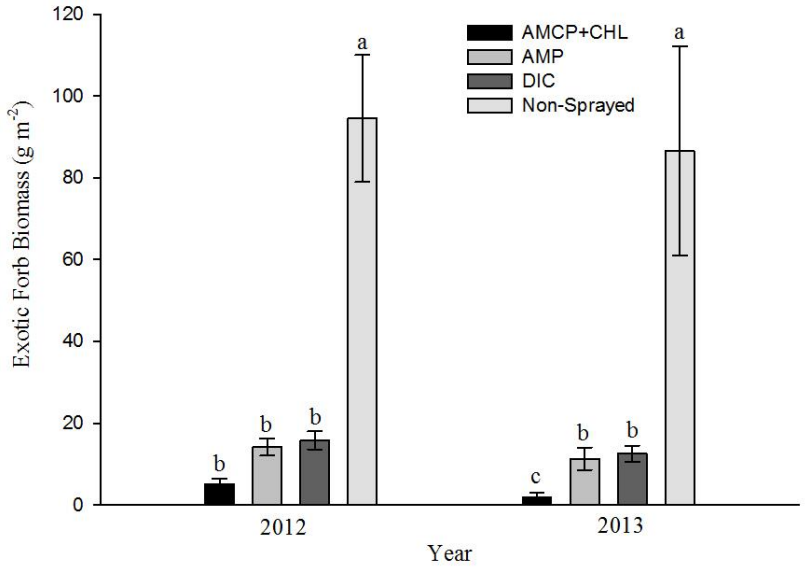


Figure 3.9. Exotic forb biomass as affected by herbicide and year at the Novich site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide among year 2012 or 2013.

Exotic forb cover decreased following any of the three herbicide applications in 2012. Cover in the non-sprayed treatment was 31 ± 3.5 % in 2012. AMCP+CHL, AMP, and DIC reduced cover to 4 ± 1.1 %, 5.4 ± 0.7%, and 3.8 ± 0.6 %, respectively (Figure 3.10). In 2013 the non-sprayed treatment plots had an exotic forb cover of 22 ± 2.9 %.

Relative to the non-sprayed treatment, AMCP+CHL and AMP reduced cover to the greatest extent followed by DIC (1.4 ± 0.8 %, 3.9 ± 0.9%, 9 ± 1.2%, respectively; Figure 3.10).

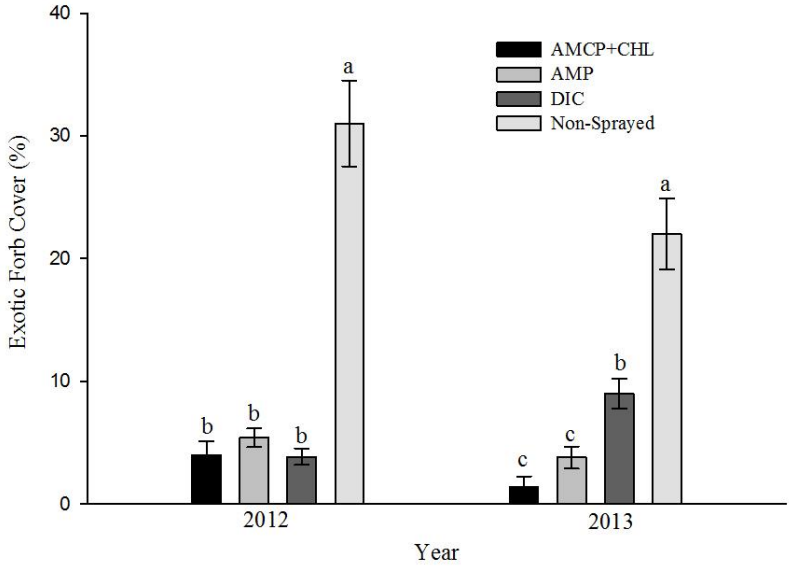


Figure 3.10. Exotic forb cover as affected by herbicide and year at the Novich site. Error bars indicate 1 SE of the mean. Lower case letters indicate means that are different within a herbicide treatment among year 2012 or 2013.

Native Forbs (NF)

Ashcraft Site. No analysis was performed as native forbs were found in less than 20% of the plots across both years.

Novich Site. There were no treatment effects on native forb biomass or cover for both years (Table 3.3).

Individual Species of Interest

When examining the cover of individual species of interest across both sites, each species responded similarly to its respective functional group, with the exception of the perennial grass red top at the Novich site. This species became more prevalent in 2013 in the AMCP+CHL and AMP treatment plots. Red top was reported in less than 50% of the plots in 2012, but was recorded in over 70% of the plots in 2013. The non-sprayed treatment in 2013 had 2.5 ± 0.7 % of red top, while cover was 12 ± 2.9% and 4.5 ± 1.3 %, respectively, in the AMCP+CHL and AMP treatments.

Soil Nutrient Analysis

Ashcraft Site. Means for the control (0+0) and non-sprayed/fertilized (0+F) split-plots from the soil nutrient analysis are listed in Tables 3.4 and 3.5. There was no effect of the fertilize treatments on levels of soil nitrate-N (P=0.646), Olsen-P (P=0.111), or K (P=0.528). Further, there were no differences in pH, levels of salts, percent organic matter, and percent sand, silt and clay between the control and non-sprayed/fertilized split-plots. Across the control and non-sprayed/fertilized split-plots soil texture was a sandy clay loam.

Novich Site. Means for the control and non-sprayed/fertilized split-plots from the soil nutrient analysis are listed in Tables 3.4 and 3.5. Similarly to the Ashcraft site, there was no effect of the fertilize treatments on levels of soil nitrate-N (P=0.561), Olsen-P (P=0.755), or K (P=0.727). In addition, pH, levels of salts and percent organic matter, sand, silt and clay were the same between the control and non-sprayed/fertilized split-

plots. Soil texture across the control and non-sprayed/fertilized split-plots ranged between a loam to a sandy clay loam.

Table 3.4. Means and standard errors for the soil nutrient analysis from both the Ashcraft and Novich sites. Samples were taken at split-plot level (0+0 or 0+F) on 29 May 2013. Soils were sent to AGVISE Laboratories for analysis (AGVISE Laboratories, Northwood, ND).

Ashcraft

Split-plot	pH	% OM	Salts mmhos cm^{-1}	Nitrate-N ppm	P-Olsen ppm	K-ppm
0+F	8.2 ± 0.2	16.2 ± 2.2	1.2 ± 0.2	14 ± 2.5	26.5 ± 2.1	116 ± 26
0+0	8 ± 0.08	19.8 ± 4.7	0.8 ± 0.1	12.6 ± 2	43.7 ± 10.8	114 ± 11

Novich

Split-plot	pH	% OM	Salts mmhos cm^{-1}	Nitrate-N ppm	P-Olsen ppm	K-ppm
0+F	8 ± 0.09	16.2 ± 2.9	0.9 ± 0.2	10 ± 1.9	23 ± 4.7	196 ± 42
0+0	8 ± 0.09	16.6 ± 3.2	0.7 ± 0.1	9.5 ± 1.8	17.7 ± 3.1	164 ± 12

Table 3.5. Means and standard errors for the % sand, silt and clay from both the Ashcraft and Novich sites. Samples were taken at split-plot level (0+0 or 0+F) on 29 May 2013. Soils were sent to AGVISE Laboratories for analysis (AGVISE Laboratories, Northwood, ND).

	Ashcraft			**Novich**		
Split-plot	**Sand**	**Silt**	**Clay**	**Sand**	**Silt**	**Clay**
0+F	50 ± 2	26 ±1	25 ± 0.8	58 ± 2.5	25 ± 2	17 ± 1
0+0	53 ± 3	24 ± 1	22 ± 2.1	57 ± 3	22 ± 3	20 ± 2

Discussion

Herbicides have been the primary method of invasive plant control and can effectively manage plant invasions; however, they often do not provide long-term control when used alone. When herbicides are used as part of an integrated management system, they can become increasingly effective over the long term (DiTomaso 2000). Successful integrated weed management strategies are designed to both control the target invasive plants while encouraging the growth and fitness of the desired species (Jacobs et al. 2006). Integrated management programs often include a combination of mechanical, biological and chemical control (DiTomaso 2000). The integrated management methods utilized in my experiment included herbicides applied alone and in combination with either fertilization, mowing or both, and were designed to not only control tall buttercup, but to encourage the growth of desirable forage species including perennial grasses and clovers. Tall buttercup control was achieved, but the effect varied among years and between both sites.

The three herbicides used in my experiment (AMCP+CHL, AMP and DIC) are growth-regulator or auxin herbicides used primarily to control broadleaf weeds in grass crops, pastures and turfs (Gibson 2001). Auxin herbicides are absorbed through the roots and foliage of the plant (Grossmann 2007). These herbicides have been shown to provide control for annual, biennial, and perennial invasive forbs (Gibson 2001). When examining the overall effectiveness of these herbicides in controlling tall buttercup across 2012 and 2013 and between the two sites, both AMCP+CHL and AMP reduced tall buttercup biomass and cover to the greatest extent. DIC also reduced biomass and cover,

but plots with this treatment typically had higher amounts of tall buttercup relative to the AMCP+CHL treatments. AMCP+CHL can remain active in the soil for at least two years, depending on the soil environment and climactic conditions (Westra et al. 2008) and has a half-life ranging from 22 to 126 days (Finkelstein et al. 2008). AMP has a shorter half-life of 30 days; however, it is more persistent in the soil when compared to DIC. Moreover, it is relatively immobile in the soil, so it typically stays within the upper 30 cm of the soil profile (Hartzler 2006). The long half-life of AMCP+CHL and increased persistence in the soil of AMP likely resulted in better tall buttercup control when compared to DIC.

While DIC still reduced tall buttercup biomass and cover, it was not as effective as the other two herbicide treatments. This may be attributed to the fact that DIC has a half-life in the soil of only 30 to 45 days (Krueger et al. 1991). The degradation of DIC was studied in a grassland type soil in Oklahoma where the half-life was 17 days (Altom and Stritzke 1973). It is possible that the half-life of DIC in these flood and sub-irrigated hayfield meadows dominated by perennial grasses may be less than 20 days as was observed in the grassland in Oklahoma, thus decreasing its likelihood of increased tall buttercup control.

When comparing herbicide effects across years, varying results were seen between sites. At the Novich site, AMCP+CHL, AMP, and DIC effectively controlled tall buttercup in both 2012 and 2013. However, none of the three herbicides decreased tall buttercup in 2012 at the Ashcraft site. Possibly better control in 2012 at the Ashcraft site would have been achieved if herbicides had been applied prior to tall buttercup

flowering. The Milestone^{TM} (AMP) label suggests applying it when a species is actively growing (Dow AgroSciences), which would have been prior to flowering. In addition, weed seedlings and younger plants are typically killed more easily than large or more mature vegetation (Radosevich et al. 2007). In 2013, a year after treatment application, all three herbicides successfully reduced tall buttercup. Timing of herbicide application in June 2012 may have attributed to the year lag in tall buttercup control.

Moreover, in 2012 at the Ashcraft site there was minimal tall buttercup biomass when compared to the other vegetation including perennial grasses and grass-like species. Tall buttercup biomass in the non-sprayed treatment was only 11.7 g m^{-2}, so perhaps the amount of tall buttercup at the site was so small that any effect of herbicides was difficult to discern. The population of tall buttercup in 2012 may have been lower due to normal variability as plant populations often vary from year to year due to environmental conditions which can influence population growth rates and species abundance and distribution (Buckley et al. 2010, Horvitz et al. 1998, Warton and Wardle 2003). Further, variation in population growth rates often depends on the species life-form (Dalgleish et al. 2010). Forbs, herbs and grasses are more likely to experience variable population growth rates than trees and shrubs (Buckley et al. 2010). A species like tall buttercup is more likely to experience variation in its growth rate due to its life-form and the dynamic environmental conditions that occur in the habitats it invades. For example, from 2012 to 2013 tall buttercup increased by 3 g m^{-2} in the non-sprayed treatment, an increase which is likely within the natural variation in the abundance of the species from year to year. All three herbicides may have been effective at the Novich site across both years because the

biomass and cover of tall buttercup was much greater compared to the Ashcraft site.

Across both years, mowing decreased tall buttercup at the Ashcraft site. Mowing has reduced tall buttercup in red fescue-dominated meadows in Russia (Saurina 1972, Andreev and Gamidov 1985), Scotland (Forb 1996), Austria (Buchgraber and Sobotick 1995). Harper (1957) noted that frequent cutting reduced both the flowering and vigor of the plant. Mowing likely provided tall buttercup control for several reasons. Mowing occurred during the flowering period of tall buttercup, likely reducing tall buttercup seed set in 2012. The success of mowing often depends on timing, and it is recommended during the flowering stage before seed development occurs (DiTomaso 2000). A study conducted by Rinella and others (2001) that used mowing for spotted knapweed (*Centaurea stoebe*) control found season of mowing was more important than mowing frequency. In addition, mowing can suppress perennial invasive plants through carbohydrate starvation via stimulating new shoot development, which can deplete the plants carbohydrate reserves if conducted often enough (Radosevich et al. 2007). Management practices in hayfield meadows infested with tall buttercup could be altered, even if only for a period of several years, to include several cutting events during the growing season to potentially suppress the species.

Another possible explanation as to why mowing reduced tall buttercup is that it removed dense vegetation that was growing in conjunction with tall buttercup. Although I did not see an interaction between herbicide and mowing, mowing can result in increased herbicide contact, which may have occurred at the Ashcraft site especially for first or second year basal rosettes. Renz and DiTomasso (2004) found that mowing

followed by an application of glyphosate reduced perennial pepperweed (*Lepidium latifolium*). They proposed that this occurred because mowing not only changed the canopy structure, but it also increased the deposition of the herbicide on the basal leaves. This is promising for both forage production and tall buttercup control in irrigated hayfield meadows in Montana. In tall buttercup infested areas, grazing could be used in the spring (in lieu of cutting), followed by a herbicide application; harvest events could still occur in late July or early August after important forage species have had adequate time to re-grow.

Fertilization reduced tall buttercup at the Ashcraft site averaged across both years. Tall buttercup control in addition to increases in the abundance of desirable species following fertilization has been observed in several studies. In Greece, the application of N fertilizer reduced the relative abundance of tall buttercup (Tzialla et al. 2002); the application of a N-P-K fertilizer to a tall buttercup infested pasture resulted in an increase in desired palatable grass species (Melnikov et al. 1975). The success of fertilizer in controlling tall buttercup at the Ashcraft site may be attributed to its low density at the site. DiTomaso (1995) found that when weed densities were low, adding N fertilizer increased crop yield over the invasive forb and made the crop a more vigorous competitor. However, when invasive plant densities are high, fertilizer may favor the invasive plant over the desired vegetation (Ahuja and Yaduraju 1989, Carlson and Hill 1986, and Liebman 1989). If tall buttercup densities had been higher, fertilizer addition may have favored tall buttercup over perennial grasses (DiTomaso 1995). In contrast, both the mow and fertilize treatments had no effect on tall buttercup biomass or cover at

the Novich site. Because the abundance of tall buttercup was higher at the Novich site, fertilization may have had no effect on perennial grasses, and instead may have favored tall buttercup (DiTomaso 1995).

Both of my study sites had a history of annual applications of high rates of N fertilizer prior to the study. The rate of N fertilizer utilized in the study may not have been high enough to shift the competitive relationships between tall buttercup and associated vegetation at the Novich site considering the amount of fertilizer applied to the site annually. These hayfield meadows are highly productive and dynamic systems, a direct result of the land management practices that take place there (grazing, fertilizer applications, irrigation, and harvesting). The highly productive nature of hayfield meadows may explain why no effect of mowing or fertilizer was observed at the Novich site in addition to the higher overall abundance of tall buttercup at the site.

Overall, because AMCP+CHL, AMP, and DIC provided adequate control of tall buttercup at both sites, it is promising that these herbicides would yield similar results in other hayfield meadows throughout North America. Moreover, since the fertilizer and mowing applications at the Ashcraft site provided some tall buttercup control, they may also be integrated into tall buttercup management in other hayfield meadows in Montana. Because fertilization and mowing or harvesting are already a part of the management practices for these systems, the timings and rates of these practices could be altered to specifically target tall buttercup. However, further research is necessary to better understand the potential of fertilization and mowing in for controlling tall buttercup.

Even though individual control methods provided control of tall buttercup, my

results do not support the integration of methods to improve tall buttercup control. The combination of herbicides and mowing or herbicides and fertilizer did not increase tall buttercup control; rather herbicides, mowing, and fertilizer used individually were effective. Despite this, invasive plant control has a greater chance of success when several methods of management are used in combination with each other (DiTomaso 2000). Integrated management has been successful in controlling invasive forbs in many studies. For example, integrated management on spotted knapweed infestations that utilized a combination of herbicide and fertilizer decreased spotted knapweed and increased desired vegetation (Jacobs and Sheley 1999). Moreover, grazing in combination with biological control was successful in reducing the invasive forb leafy spurge (*Euphorbia esula*) (Jacobs et al. 2006). Other successful studies using an integrated approach to manage invasive forbs included a combination of herbicide, revegetation and biocontrol to control yellow starthistle (*Centaurea solstitialis*) (Enloe and DiTomaso 1999), and mowing in combination with herbicide application controlled infestations of perennial pepperweed (Renz and DiTomaso 1999). Further, it is important to recognize that tall buttercup has a history of evolved resistance to herbicides (Lamoureaux and Bourdôt 2007). Utilizing an integrated approach instead of relying solely on herbicides lessens the risk of tall buttercup developing herbicide resistance in the state of Montana and elsewhere in North America. Future research in tall buttercup control should examine the timings and intensity of mowing and fertilization and how to best integrate these methods with herbicides.

Encouraging the growth and fitness of desired vegetation is one of the goals of

integrated weed management (Jacobs et al. 2006). In the irrigated meadows where tall buttercup invades, perennial grasses are the most important species for hay forage. The integrated management strategies implemented in my experiment were designed to not only control tall buttercup, but also to encourage the growth of perennial grasses. In previous tall buttercup control studies, fertilization and/or mowing promoted the competitiveness and growth of perennial grasses over tall buttercup (Melnikov et al. 1975, Saurina 1972, Forb 1996, Buchgraber and Sobotick 1995 Andreev and Gamidov 1985). However, there was no effect of the mow or fertilize treatments on perennial grasses at either site. Again, this may be attributed to the highly dynamic and productive nature of these hayfield systems. The rate of fertilizer applied in the study may not have been enough to influence any change in grass biomass or cover due to the high rates of N application the sites received annually prior to the study. The results from the soil nutrient analyses indicated that split-plots that had the fertilizer application did not always increase the amount of N in the soil relative to the control split-plots.

Further, the herbicides applied in my study were designed to decrease tall buttercup, which in turn should have either increased or maintained the density of perennial grasses. An increase in grasses following control of an invasive forb have been documented in a study by Jacobs and Sheley (1999) where the application of 2,4-D to a site dominated by spotted knapweed resulted in an increase in grass density and biomass. At the Ashcraft site, an application of AMP or DIC increased perennial grasses relative to the non-sprayed treatment by about 70 g m^{-2} the year following application. The increase in perennial grass biomass from both the AMP and DIC treatments may be attributed to a

decrease in other species, including tall buttercup and other exotic forbs like dandelion and red and white clover, which allowed the perennial grasses to utilize essential plant resources (i.e. light, nutrients, water) that would have otherwise been used by exotic forbs. At the Novich site, perennial grass biomass in AMP and DIC treated plots was similar to the non-sprayed plots. While an increase was not observed, maintained forage production following herbicide treatments is still a desired outcome.

The herbicide AMCP+CHL reduced perennial grass biomass and cover and increased grass-like species at the Ashcraft and Novich site across both years. Any decrease in forage production following herbicide application is not desirable for hay production. The decrease in perennial grasses following the application of AMCP+CHL was not surprising as grass injury (curling and twisting of leaves) was observed at both sites in 2012 and 2013. In fact, it was apparent that species composition had changed in this treatment from 2012 to 2013. This herbicide was applied as Perspective™ because it contains aminocyclopyrachlor (AMCP), the target active ingredient to control tall buttercup. However, Perspective™ also contains chlorsulfuron (CHL), which is a chemical occasionally used to control certain grass species like Kentucky bluegrass (*Poa pratensis*), ryegrass (*Lolium* spp.) and fescue grasses (*Festuca* spp.) (Derr 2012), and has been documented to cause perennial grass injury (Wallace and Prather 2006). Further, grass-like biomass increased in these treatments relative to non-sprayed, AMP, and DIC treatments. Because perennial grasses decreased in AMCP+CHL treatments at the Ashcraft site, grass-like species filled the open space created by the herbicide.

At the Novich site, applying AMCP+CHL increased cover of the perennial grass

red top from 2012 to 2013. While the overall cover of perennial grasses decreased due to AMCP+CHL, red top filled the empty niche previously occupied by other perennial grass species. Interestingly, AMCP+CHL only increased grass-like cover but had no effect on biomass which was the opposite of what occurred at the Ashcraft site. The Novich site had higher species richness than the Ashcraft site (see Chapter Two). While the cover of grass-like species increased, it may not have equated to an increase in biomass because there were more species of perennial grasses.

It is important for land managers and landowners to take into consideration the potential for grass injury following the use of AMCP+CHL. While it provided adequate control of tall buttercup, the herbicide also reduced dominant perennial grass species while it increased red top and grass-like species. The increase in red top would not be desirable for producers as it has a somewhat prostrate growth form and lower palatability when compared to other seeded perennial grass species (Hannaway and Larson 2004). Further, grass-like species including sedges and rushes are morphologically different than perennial grasses. Their surface area is smaller, and they are often found in bunches unlike rhizomatous perennial grasses. Rushes in particular are thick and wiry, do not produce as much biomass as perennial grasses, and are not considered desirable forage for hay (Novich and Ashcraft, Personal Communication 2012).

Exotic forbs responded in a similar manner as tall buttercup following treatments. Exotic forb biomass and cover were reduced following herbicide treatments at the Novich site across both years. At the Ashcraft site, all three herbicides in addition to the fertilize, mow, and combination of fertilize and mow treatments reduced exotic forb cover. There

was no effect of the herbicide treatments on native forbs at the Novich site, likely because there were low amounts of native forb biomass across both years and because irrigated hayfield meadows are not managed to have a high diversity of native forbs.

From a management perspective, the non-target effects on exotic forbs other than tall buttercup may be both beneficial and detrimental to forage production. Reductions to some exotic forbs present at the sites like dandelion and sowthistle are advantageous, however, decreases in red and white clover following herbicide treatments is not desirable for forage production. It is important for land owners to take into account whether the benefits of reducing the prevalence of tall buttercup outweigh the non-target effects of the herbicide treatments on desirable clover species (Crone et al. 2009).

There was no effect of the fertilizer treatments on the soil nutrient levels across both sites. The absence of an effect of the fertilizer treatments may be due to a variety of factors including the fact that both sites were fertilized annually with high rates of N prior to this study. Furthermore, these samples were taken a year after fertilizer was applied so recorded levels in 2013 may not have been indicative of what nitrogen levels were in 2012. Moreover, soils were only sampled once during the growing season, and nutrient levels can fluctuate greatly across the growing season, so levels of nitrogen may have been different if samples were taken again at a different time over the summer. These systems are highly dynamic; however, nutrient addition is still a valuable management tool that may be used to improve the growth of desired forage species which in turn could provide better control of tall buttercup.

Management Implications

Results from my study indicate that the use AMCP+CHL, AMP, and DIC may be effective in controlling tall buttercup infestations in hayfield meadows. However, it is important to consider the timing of these herbicide applications. Control across both years at the Ashcraft site may have been achieved if herbicides were applied prior to peak tall buttercup flowering. Moreover, the use of mowing and fertilizer as individual treatments also reduced tall buttercup at the Ashcraft site. As fertilizing and mowing/harvesting are already a part of the management strategies used in these systems, applying these treatments in tandem with other tall buttercup control methods is an option that may encourage the growth of desirable forage species while decreasing the prevalence of tall buttercup. While the combination of integrated management techniques did not improve control tall buttercup in this study, it is still important to utilize an integrated approach when managing the species. The use of herbicides in combination with other integrated methods can increase the effectiveness of invasive species control over the long term (DiTomaso 2000). Further, because tall buttercup has evolved herbicide resistance in New Zealand (Lamoureaux and Bourdôt 2007), it is even more imperative to manage the species using integrated methods to prevent resistance from occurring in Montana and North America.

Given that this is the first and only known study of tall buttercup control methods in the state of Montana and North America, further research is warranted. Different rates and timings of herbicide applications should be tested. There is potential for tall buttercup control following a late summer/early fall application of herbicides in addition to

herbicide application prior to tall buttercup flowering (Duncan 2014, Personal Communication). Different rates of AMCP+CHL, AMP and DIC should be tested in other tall buttercup infestations throughout Montana. While AMCP+CHL reduced desired forage and increased unpalatable species, applying a lower rate of this herbicide may be effective in tall buttercup control while maintaining or increasing forage production. Moreover, different rates of fertilizer and timing and frequency of mowing could also be incorporated into future studies. Because these sites already had high rates of N fertilization, the rate applied in my study may not have been enough to achieve a decrease in tall buttercup. Future research could also incorporate the use of grazing, mowing and herbicides in combination with each other to examine tall buttercup control. Finally, because desired forage species like clovers experienced non-target effects of herbicides, research should explore different ways to promote the growth of desired forage species, including forbs, following broad-leaf herbicide applications.

CHAPTER FOUR

EMERGENCE AND GROWTH OF TALL BUTTERCUP SEEDLINGS ALONG A SOIL MOISTURE GRADIENT

Introduction

Tall buttercup (*Ranunculus acris* L.) is a perennial, invasive forb that occurs in moist habitats including pastures, river flats, grasslands and flood or sub-irrigated meadows (Lamoureaux and Bourdôt 2007). It is native to central and northern Europe (Coles 1971) where it is a weed of old pastures and hay meadows (Harper and Sagar 1953). The species has spread from its native range in Europe to parts of the Northern Hemisphere including the upper continental states in the United States. and southern territories in Canada (Bourdôt et al. 2013). In the Southern Hemisphere, tall buttercup has naturalized in parts of South Africa, Australia and New Zealand (Webb et al. 1988). In these regions, tall buttercup is found in human-modified environments, typically in irrigated or grazed pastures, roadsides, and drainage ditches (Bourdôt et al. 2013).

Tall buttercup can damage pasturelands both economically and ecologically. It has been a species of concern in New Zealand for many years, especially in dairy farming regions where it competitively displaces pasture grasses and clovers (Conner 1977). Tall buttercup can range in canopy cover from 5 to 50 percent and reduces forage biomass during its peak cover (Bourdôt et al. 2003). It has been considered an economically significant weed in the country's dairying regions, causing national losses in income of up to $NZ 156 million per year (Lamoureaux and Bourdôt 2007). The potential for

further economic and ecological impacts is high in areas where tall buttercup has been spreading rapidly such as in North America (Coles 1971), where it has now been recorded in all but eight states and one Canadian Province (USDA PLANTS Database). There is concern that tall buttercup will become a problematic invader in North America, particularly in Montana where it has invaded over 8300 hectares and was listed as a priority 2A noxious weed in 2003 (Montana Noxious Weed Summit Advisory Council 2008). Noxious weeds in Montana are prioritized based upon how widespread and abundant they are across the state. Priority 2A species are common in isolated areas and management practices are focused on eradication and containment (Montana Noxious Weed Summit Advisory Council 2008).

Tall buttercup has been predominantly troublesome in western Montana in flood and sub-irrigated hayfield meadows. In fact, the species is tolerant of flooded conditions and has been found in areas with approximately 30 days of flooding per year (He et al. 1999). Irrigation has created habitat conditions suitable for the species to thrive and likely has a large influence on the persistence of tall buttercup in many locations where it is invasive. In the absence of irrigation, suitable habitat for the species declines in areas that do not receive adequate rainfall (Bourdôt et al. 2013). A survey sent to landowners in Madison County, Montana, in June 2012 indicated that landowners also have noticed a decrease in the prevalence of the species following a reduction in irrigation. One landowner stated; "We have had tall buttercup for years. We have converted from flood to sprinkler [*irrigation*]. The reduction of irrigation in wetter areas allows the other plants to out compete the buttercup. Where we still flood irrigate, there is a little more

buttercup" (Appendix A).

Bourdôt and others (2013) hypothesized that tall buttercup has expanded into more arid regions like Montana due to irrigation. While it is believed that tall buttercup requires high soil moisture conditions on its global distribution, the amount of moisture required for optimal seedling emergence and growth has not been explored. Understanding the importance of soil moisture on seedling recruitment will be essential for future management, especially in light of climate change, as the suitable habitat for tall buttercup is predicted to increase due to a probable rise in irrigation (Bourdôt et al. 2013). A greenhouse experiment was conducted to assess seedling emergence and growth along a gradient of soil moisture. I predicted that tall buttercup emergence and growth would be highest at the intermediate soil moisture level (50 percent field capacity), while emergence and growth would be lowest at low (25 percent field capacity) or very high (100 percent field capacity) soil moisture levels.

Materials and Methods

Experimental Design

The study was conducted in the Plant Growth Center (PGC) at Montana State University in Bozeman, Montana, and followed a randomized block design with 12 replications of three soil moisture treatments. Soil moisture treatments included 25, 50, and 100 percent field capacity. The study was conducted between 14 October and 17 December 2013. Seeds were collected in July 2013 from naturally occurring tall buttercup populations approximately 16 km southwest of Twin Bridges, Montana. Seeds

were stored at room temperature (23.5ºC) for three months prior to the study. Soil in each pot contained equal parts of the ‘Sunshine Mix #1’ (Canadian Sphagnum Peat Moss, perlite, vermiculite, starter nutrient charge, wetting agent and Dolomitic lime) and the ‘PGC Soil Mix’(equal parts by volume of loam soil, washed concrete sand, Canadian Sphagnum Peat Moss and AquaGro 2000 G wetting agent). Soil was placed into 2 liter pots. Prior to initial watering, 10 seeds were planted in each pot. Seeds were placed approximately 2.5 cm beneath the soil surface and were planted at least 1.3 cm apart from one another.

Target field capacities were determined by averaging the amount of water needed in milliliters to reach 100 percent field capacity or saturation across 10 pots. One hundred percent field capacity was defined as the point at which the soil could no longer hold any additional moisture and water began to drip from holes at the bottom of the pot. The average amount of water in milliliters needed for the soil to reach 100 percent field capacity was used to determine the amount of water needed for the 25 and 50 percent field capacity treatments. Pots were watered with the calculated amount and then weighed to the nearest gram. Weights were recorded as the desired weight at which to maintain each of the pots throughout the duration of the study. Target field capacities were maintained throughout the duration of the study. Greenhouse conditions included supplemental light to maintain 12-hour days and the temperature was held at 22˚C during the day and 18˚C at night. Following each watering event, pots were randomly rearranged within a block to prevent unintended effects on tall buttercup seedling emergence and growth due to any micro-site conditions that may have been present in the greenhouse.

Data Collection

Tall buttercup seedlings were harvested on 17 December 2013, approximately two months after planting. Height to the nearest cm and number of leaves on each seedling were recorded in addition to the number of seedlings that emerged per pot. Aboveground biomass was clipped at the surface of the soil, placed in the PGC plant dryer at 65°C for a minimum of 72 hours, and weighed to the nearest 0.01 gram. Seedling biomass, height, and number of leaves were averaged per individual pot to normalize values across the different seedling densities.

Statistical Analysis

Analysis of variance (ANOVA) was conducted using RStudio 0.98.501 statistical software to assess the effect of the soil moisture treatments (25, 50 and 100% field capacity) on the density of emerged seedlings, seedling biomass, height and number of leaves. Response variables were ln transformed to meet assumptions of normality and constant variance. When significant main effects or interactions were found ($P \leq 0.05$), means separations were performed using the post hoc Tukey's Honest Significant Test ($\alpha \leq 0.05$). Non-transformed means are presented for ease of interpretation.

Results

Seedling Density

There was an effect of soil moisture treatments on tall buttercup seedling density (Table 4.1). The lowest seedling density was found in the 25% field capacity treatment

where 18% of the seeds emerged (1.8 ± 0.3 seedlings pot^{-1}). The 50% and 100% field capacity treatments resulted in similar seedling density with 40% and 36% of the seeds emerging as seedlings (4.0 ± 0.6 and 3.6 ± 0.5 seedlings pot^{-1}).

Table 4.1. *P*-values for effects of soil moisture on tall buttercup seedling biomass, density, leaf number and height. Significant *P*-values (<0.05) are indicated in bold.

Parameter	Df	density (seedlings pot^{-1})	biomass (g seedling^{-1})	height (cm seedling^{-1})	leaf number (seedling^{-1})
Treatment	2	**0.005**	**<0.001**	**<0.001**	**<0.001**
Replication	11	0.135	0.063	0.131	0.707

Seedling Biomass

Tall buttercup seedling biomass was affected by the soil moisture treatments (Table 4.1). All three field capacity treatments resulted in different tall buttercup seedling biomass. The 50% field capacity treatment had the highest seedling biomass with 0.2 ± 0.03 g seedling^{-1}. The 100% field capacity treatment had a seedling biomass of 0.1 ± 0.02 g seedling^{-1}, while the 25% field capacity treatment had the lowest biomass with 0.009 ± 0.001 g seedling^{-1}.

Seedling Height

Tall buttercup seedling height was affected by the soil moisture treatments (Table 4.1). All three soil moisture treatments resulted in different seedling heights. The 50% field capacity treatment had the tallest seedlings with a height of 6 ± 0.3 cm seedling^{-1}. The 100% field capacity treatment had seedlings with a height of 4 ± 0.5 cm seedling^{-1}, while the 25% field capacity treatment had the smallest seedlings with a height of 0.8 ± 0.2 cm seedling^{-1}.

Number of Leaves Per Seedling

There was a treatment effect on the number of leaves on tall buttercup seedlings (Table 4.1). The lowest number of seedling leaves was from the 25% field capacity that had 1.2 ± 0.3 leaves seedling^{-1}. The 50% and 100% field capacity treatments resulted in similar tall buttercup seedling leaf numbers with 5.7 ± 0.4 leaves seedling^{-1} and 4 ± 0.6 leaves seedling^{-1}, respectively.

Discussion

Suitable habitat for tall buttercup encompasses areas of high soil moisture content including flood and sub-irrigated hayfield meadows, grasslands, and pastures (Lamoureaux and Bourdôt 2007). While prior to this study there was no known data on the influence of soil moisture on tall buttercup seedling recruitment, Bourdôt et al. (2013) reported that without irrigation, habitat for the species declines in areas that do not receive sufficient rainfall. The flood and sub-irrigation management practices in western Montana with problematic tall buttercup infestations are likely playing a role in the species' ability to persist on the landscape. Similarly to Bourdôt and others (2013), Montana landowners have also made the connection between soil moisture and prevalence of tall buttercup, observing that there is more tall buttercup in areas where they flood irrigate (Appendix A). Landowners with tall buttercup infestations can use the results from this experiment to guide and modify their own irrigation to discourage the recruitment of tall buttercup seedlings.

Seedling emergence and growth are critical stages of plant development that

result in the recruitment of new individuals in plant communities (Fay and Schultz 2009). Both are sensitive to environmental variability and require certain soil moisture conditions (Fay and Schultz 2009). Further, tolerance to varying soil moisture depends on the stage of plant development (Wilson et al. 1974). In my greenhouse study, soil moisture treatment effects were found for all tall buttercup seedling response variables including seedling density (emergence), biomass, height and leaf number. In particular, the density of seedlings was the highest in both the 100% and 50% field capacity treatments and not surprisingly, lowest in the 25% field capacity treatment. My results indicate that tall buttercup emergence is optimal in field capacities of 100% and 50%, and minimal in drier conditions. Similarly, Harper and Sagar (1953) found that tall buttercup seedling recruitment was reduced in well-drained conditions.

Tall buttercup seedling density was generally low relative to the number of seeds planted. Ten seeds were planted in each pot, and only 40% of seedlings emerged in the 50% field capacity treatment while 36% of seedlings emerged in the 100% treatment. It is possible that the viability of seeds used in this study was relatively low. Thompson and Grime (1983) stated that tall buttercup seeds need exposure to warm and moist conditions in order to germinate. In the same study they found that a similar species, *Ranunculus repens*, requires fluctuating temperatures for optimal germination in the light. For this study, seeds were collected in late July 2013 and were stored at room temperature (23.5 $^{\circ}$C) until they were planted in October 2013. Possibly the seed storage prior to the study and greenhouse conditions throughout the study were not optimal to achieve a higher seedling density than what was observed. However, because all seeds were stored in

similar conditions, any reduction in seedling emergence due to low seed viability was equal across all treatments.

Tall buttercup seedling biomass was highest in the 50% field capacity treatment followed by the 100% and 25% field capacity treatments. He and others (1999) found that in river floodplains tall buttercup thrives in the zone with approximately 30 days of flooding per year. Further, they observed that it is more resistant to flooding than other *Ranunculus* species. However, while tall buttercup is tolerant of flooding, perhaps mature tall buttercup plants can withstand long periods of flooding more so than seedlings. While seedling density from the 100% field capacity treatment was similar to the 50% field capacity treatment, seedling biomass was lower, indicating that soil moisture levels at 100% field capacity do not encourage optimal tall buttercup seedling growth. Seedlings from the 100% field capacity treatment also showed signs of stress from overwatering including chlorosis (Figure 4.1). In comparison, seedlings from the 50% field capacity treatment had leaves that were a dark green color and were overall visibly healthier (Figure 4.2). Lower seedling biomass in the 100% field capacity treatment may be attributed to the fact the seeds may have required different conditions to grow than the conditions they required for emergence. For example, in a study of seed germination and growth of various forb and grass seedlings, Lloret and others (2004) found that conditions that the favored the germination of seeds did not always favor the continued growth and survival of the seedlings. Finally, it was not surprising that seedling biomass was lowest at 25% field capacity as previous research has shown tall buttercup to be poorly tolerant of drought (Sarukhan 1974) (Figure 4.3).

Figure 4.1. Tall buttercup seedlings from the 100% field capacity soil moisture treatment.

Figure 4.2. Tall buttercup seedlings from the 50% field capacity soil moisture treatment.

Figure 4.3. Tall buttercup seedlings from the 25% soil moisture treatment.

Similar responses to the moisture treatments were observed for tall buttercup seedling height and leaf number as for seedling density and biomass. The 50% field capacity resulted in the tallest seedlings followed by seedlings in the 100% and 25% treatments. Seedling leaf numbers were similar for both the 100% and 50% treatments and again were lowest in the 25% treatment. Given that the highest seedling biomass came from the 50% treatment, it was not surprising that this treatment also resulted in the tallest seedlings. My results indicate that optimal tall buttercup emergence and growth occurred at 50% field capacity.

While tall buttercup seedling density was highest in both the 50% and 100% field capacity treatments, seedling growth was sensitive to both high moisture conditions (100% field capacity) and low moisture conditions (25% field capacity; Figures 4.1 and 4.3). It is important to understand how both seedling emergence and established seedlings respond to varying soil moisture conditions to determine how soil moisture may

affect recruitment of the species into plant communities (Fay and Schultz 2009). Further research could test tall buttercup seedling emergence and establishment at varying moisture conditions ranging from 30% and 100% field capacity both in the field and under controlled conditions in the greenhouse. Moisture conditions could be altered after the seedlings are established to understand how tall buttercup seedling growth may be positively or negatively influenced by the amount of moisture in the soil. Greenhouse experiments could also explore the differences in tall buttercup emergence and growth in simulated flood or sub-irrigation conditions versus overhead sprinkler irrigation conditions. Field studies could examine tall buttercup infestations in both flood and sub-irrigated hayfields compared to hayfields and meadows that only receive overhead sprinkler irrigation.

In light of climate change it is also important to increase our understanding of the relationship between irrigation and tall buttercup infestations. Bourdôt et al. (2013) developed a CLIMEX model to project potential global tall buttercup distributions under current and future climate scenarios both in the presence and absence of irrigation. CLIMEX models predicted that under the current climate in the absence of irrigation, 34 million km^2 of land is suitable for tall buttercup. The majority of suitable land is confined to the Northern Hemisphere including Asia, North America and Europe. However, when irrigation was included in the current climate model, the area suitable for tall buttercup increased 30 percent to 45 million km^2. When the model was used to examine future climate scenarios, little effect on potential distribution was found, likely because of tall buttercup's wide tolerance to varying temperatures. However, in contrast to changes in

temperatures brought about by climate change, irrigation had a large influence on the potential distribution of tall buttercup because of the extreme sensitivity of tall buttercup to drought stress (Lamoureax and Bourdôt 2007). When irrigation was included with future climate change scenarios in the model, suitable habitat for tall buttercup expanded into central Asia and northern portions of North America, in addition to the southern coastal regions of Australia, high altitude regions in Africa, and central and northern Chile and Patagonia (Bourdôt et al. 2013). It has been predicted that both climate change and increases in global human population will intensify demand for the conversion of land to agriculture in combination with irrigation, thus further increasing suitable habitat for tall buttercup (Bourdôt et al. 2013). A better understanding of the role of soil moisture on the ability of tall buttercup to invade an area is needed, especially if irrigation increases due to climate change.

Management Implications

Landowners and land managers with tall buttercup infestations can apply the results of this greenhouse experiment to their own irrigation practices. Altering irrigation type or timing could be an efficient management tool to reduce or eliminate tall buttercup infestations. Switching from flood or sub-irrigation practices to overhead sprinkler irrigation in hayfield meadows could reduce tall buttercup abundance. Irrigation results in high soil moisture conditions, providing an optimal habitat for the species and promoting its growth. Because tall buttercup is tolerant of flooding and favors high moisture soils (He et al. 1999, Lamoureaux and Bourdôt 2007), switching to overhead sprinkler irrigation may alter soil moisture conditions enough to discourage both seedling

emergence and growth of tall buttercup while still providing adequate conditions for desirable forage species like perennial grasses and clovers. In fact, one landowner from Madison County, Montana, is planning on altering his irrigation practices in an attempt to control tall buttercup. He has decided to use overhead sprinkler irrigation on some of his fields to see if it will reduce the prevalence of tall buttercup (Dave Ashcraft Personal Communication, 2013). Switching to overhead irrigation or even removing flood or sub-irrigation practices for one to two years may be enough to eliminate tall buttercup infestations. Studies have shown that tall buttercup does not typically accumulate a long-lasting seed bank (Champness and Morris 1948, Harper 1957, Sarukhan 1974). This is promising for landowners because if altering irrigation practices eliminates current infestations of tall buttercup, it is possible that after a few years the tall buttercup seed bank would be depleted. If this is the case, flood or sub-irrigation practices could be reinstated without promoting the growth of any remaining viable seeds in the seedbank.

Continued research on the optimal conditions for tall buttercup created by irrigation practices is necessary, especially in light of climate change. Because climate change is predicted to intensify the demand for irrigated agricultural lands, consequently increasing suitable habitat for tall buttercup (Bourdôt et al. 2013), more information is necessary to understand how irrigation practices influence the prevalence of tall buttercup. These findings would allow landowners to make informed management decisions regarding their own irrigation practices in the context of climate change, thus preventing the spread and occurrence of tall buttercup as demand for irrigation increases.

CHAPTER FIVE

SUMMARY OF FINDINGS AND DIRECTIONS FOR FUTURE RESEARCH

Tall buttercup typically occurs in moist habitats including pastures, grasslands and in flood or sub-irrigated meadows (Lamoureaux and Bourdôt 2007). It has been a problematic invader in New Zealand dairy pastures for many years where it has reduced forage production by excluding palatable pasture grasses (Conner 1977). The species has spread from its native range in Europe to the Northern Hemisphere including the upper continental states in the United States and southern territories in Canada (Bourdôt et al. 2013). The impact of tall buttercup in North America has been of concern, especially in Montana where it infests over 8300 hectares and was listed on the noxious weed list in 2003 (Montana Noxious Weed Summit Advisory Council 2008). Tall buttercup infestations have been problematic in flood and sub-irrigated hayfield meadows in western Montana. Land managers are concerned that the species may be reducing forage productivity in their hayfields. Prior to this study, there was minimal published data on tall buttercup ecology or management in North America. Because tall buttercup is a state listed noxious weed in Montana and has the potential to become an ecologically and economically detrimental invader, I investigated the associations of tall buttercup with the surrounding plant community and tested various integrated management strategies to provide land managers with guidelines to control the species. I also examined the role of soil moisture on tall buttercup seedling emergence and growth to better understand how irrigation practices may be influencing tall buttercup invasion.

My first objective was to assess the association of tall buttercup with species richness, plant diversity and forage production in flood and sub-irrigated hayfield meadows. The plant community was sampled along a gradient of increasing tall buttercup density. My results suggest that tall buttercup is not negatively associated with species richness or plant diversity. In fact tall buttercup was positively related plant diversity and two plant functional groups including grass-like species and exotic forbs. However, at one site tall buttercup was associated with a decrease in perennial grass biomass. While tall buttercup may not be functioning in the plant community as was predicted, control efforts are important and warranted in hayfield meadows as the plant is toxic and hay can serve as a mechanism for seed dispersal for the species (Jacobs et al. 2010). It is also possible that if tall buttercup infestations had been more severe at both study sites that increased negative associations between tall buttercup and the plant functional groups would have been observed.

My second objective tested various integrated management strategies to control tall buttercup, including herbicides, mowing, and fertilization both alone and in combination with each other. Three different herbicides were tested including aminocyclopyrachlor + chlorsulfuron (AMCP+CHL) applied as Perspective™, aminopyralid (AMP) applied as Milestone™ and dicamba (DIC) applied as Vanquish®. AMCP+CHL and AMP provided the greatest control of tall buttercup followed by DIC. All three herbicides provided up to two years of tall buttercup control. However, AMCP+CHL reduced perennial grasses and increased the biomass of grass-like plants. Any decrease in perennial grasses following herbicide application is not desirable for hay

production. Further, mowing and fertilization used individually provided tall buttercup control at one site. While my short-term results did not support the integration of tall buttercup control methods, invasive plant management has a greater chance of success when several methods are used in combination with each other (DiTomaso 2000). Utilizing an integrated approach instead of relying solely on herbicides also lessens the risk of tall buttercup developing herbicide resistance in the state of Montana and elsewhere in North America.

My third objective tested tall buttercup seedling emergence and growth along a soil moisture gradient. Tall buttercup seeds were planted in one of three different moisture treatments including 25, 50 and 100% field capacity and were allowed to grow for a two month time period before they were harvested. The 50% and 100% field capacity treatments had the highest seedling density (emergence). The 50% treatment resulted in the highest seedling biomass and seedling height followed by the 100% treatment. Both the 50 and 100% treatments had similar seedling leaf numbers. The 25% field capacity treatment had the lowest tall buttercup seedling emergence and growth. Without irrigation habitat for tall buttercup will decrease in areas that do not receive enough rainfall (Bourdôt et al. 2013). It is likely that the irrigation practices in western Montana are contributing to the invasion success of tall buttercup. Irrigation management could be a tool used to reduce or eliminate tall buttercup infestations.

Further research is necessary in Montana and throughout North America, and particular emphasis should be placed on infestations found in different habitat types to better understand its impact on the plant community in both hayfield meadows and

natural unmanaged systems. Future research on tall buttercup control should explore different rates and timings of both herbicide and fertilizers and timing and frequency of mowing should be tested. In addition, continued research is necessary to better understand the influence of irrigation on tall buttercup seedling emergence and growth, especially in light of climate change where the suitable habitat for tall buttercup has been predicted to increase (Bourdôt et al. 2013).

The results from my research contribute to the knowledge of tall buttercup ecology and management throughout the regions where it invades. I hope that the findings from my research will provide land managers and landowners with tall buttercup infestations guidelines and knowledge to control the species. Further, because the results indicated that tall buttercup is not forming associations in the plant community as predicted, I hope that both weed managers and researchers will continue to explore the relationship between tall buttercup and the surrounding plant community in its invaded range.

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APPENDICES

APPENDIX A

TALL BUTTERCUP SURVEY

Tall Buttercup Survey questions and summaries of answers; 68 surveys were sent out to landowners in Madison County, Montana in June 2012.18 surveys were returned.

Question	**Answers**
Have you heard of or seen tall buttercup?	12/18 indicate "Yes"
Do you know how to tell the difference between the native and the weedy tall buttercup?	6/18 indicate "Yes"
Do you have tall buttercup on your property?	11/18 indicate "Yes"
If yes, what year to your nearest recollection did you first notice the species on your land?	2005; 2000; 2004; 1995; 1993; 2010; 1980; 1960; 1990; 2012
Where is it typically found?	Irrigated meadow and hayland; grassland; pasture; irrigated meadow; pasture only; a wet area between my house and the pasture; wet soil areas, hay meadows; swales, less in hay fields or cultivated fields
Do you consider it to be a problem?	9/18 indicate "Yes"
If yes, why?	Cattle will not graze the plant or close to the plant; reduces grazing; county and state noxious weed; encroaches on and replaces good grasses; not an overwhelming problem; hard to kill; toxic to cattle, invades hay meadows to the detriment of good grasses; spreads way to easy
How high of a priority is the management of tall buttercup on your land relative to other noxious weeds?	3 indicate "Low Priority;" 3 indicate "Medium Priority;" 6 indicate "High Priority"
Are you currently trying to control tall buttercup?	8/18 indicate "Yes"
If yes, please describe:	Herbicide since 2012; sprayed with Milestone with limited success; county advised weed killer; spray with herbicides that don't kill grass; spray with 2, 4-D no result, spray with Roundup and reseed
What management practices result in an increase in tall buttercup?	Grazing in the spring will allow tall buttercup to over compete with all other grasses; flood irrigation; irrigation carries it into our pastures if those above do not participate in management we end up with their seeds; we have converted from flood to sprinkler, where we flood irrigated there is more buttercup.

APPENDIX B

SOIL NUTRIENT DATA

Results from the soil nutrient analysis at the Ashcraft and Novich sites. Two different samples were taken from each whole plot (#1-4) at the split-plot level. Soils were sent to AGVISE Laboratories for analysis (AGVISE Laboratories, Northwood, ND).

Ashcraft Site

Plot #, Split-plot treatment	pH	% OM	Salts mmhos cm^{-1}	Nitrate-N ppm	P-Olsen ppm	K-ppm	% Sand	% Silt	% Clay	Texture
Plot 1, 0+F*	8.2	9.9	0.76	7.0	24	208	54	23	23	Sandy clay loam
Plot 1, 0+0	7.9	10.9	0.81	9.0	30	82	62	21	17	Sandy clay loam
Plot 2, 0+F	8.1	14.7	0.81	18.0	22	78	46	29	25	Loam
Plot 2, 0+0	8.1	19.6	1.00	20.0	34	141	44	27	29	Clay loam
Plot 3, 0+F	7.7	20.4	1.86	12.0	27	83	46	27	27	Sandy clay loam
Plot 3, 0+0	7.7	35.4	0.72	11.0	81	126	57	21	22	Sandy clay loam
Plot 4, 0+F	8.8	20.0	1.18	19.5	33	97	54	23	23	Sandy clay loam
Plot 4, 0+0	8.1	13.4	0.67	10.5	30	107	52	25	23	Sandy clay loam

*0+F= non-sprayed and fertilized split-plot; 0+0 control split-plot.

Novich Site

Plot #, Split-plot Treatment	pH	% OM	Salts mmhos cm^{-1}	Nitrate-N ppm	P-Olsen ppm	K-ppm	% Sand	% Silt	% Clay	Texture
Plot 1, 0+F*	8.1	25.1	1.00	9.5	16	142	55	29	16	Sandy loam
Plot 1, 0+0	8.1	26.9	0.64	5.5	12	157	59	28	13	Sandy loam
Plot 2, 0+F	8.2	11.7	1.58	14.5	37	310	56	25	19	Sandy loam
Plot 2, 0+0	8.6	11.4	0.96	13.0	28	185	58	21	21	Sandy clay loam
Plot 3, 0+F	8.5	17.7	0.86	12.0	26	239	53	26	21	Sandy clay loam
Plot 3, 0+0	8.5	11.4	0.67	6.0	14	127	64	15	21	Sandy clay loam
Plot 4, 0+F	8.0	10.4	0.54	4.0	13	95	66	19	15	Sandy loam
Plot 4, 0+0	8.5	16.9	0.82	13.5	17	188	48	27	25	Sandy clay loam

*0+F= non-sprayed and fertilized split-plot; 0+0 control split-plot