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Managing Spotted Knapweed (Centaurea stoebe) Using Restoration Methods

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Managing Spotted Knapweed *(Centaurea stoebe)* Using Restoration Methods

By

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Abstract

This thesis project addressed the effectiveness of integrating ecological restoration into traditional mechanical and chemical methods of invasive species control. Spotted knapweed, an abundant invasive plant species at Camp Ripley Military Training Site, is capable of prolific reproduction, and therefore, causes great ecological distress to the native community it invades. The purpose of this research was to determine if spotted knapweed can be controlled by re-introducing native prairie grasses to the disturbed sites at Camp Ripley, and ideally, apply these findings to the methods of invasive species control in native prairies across central Minnesota. Furthermore, the sequence of the application of selective, broadleaf herbicide (Milestone) and native grass seeding was varied in order to determine the sequence of treatments most likely to decrease the density of spotted knapweed, increase the density of target native grass species, and decrease the percentage of bare soil visible. Three research plots were used in the experiment: two of which received the native grass seeding in conjunction with the selective, broadleaf herbicide in varied order, one of which received only broadleaf herbicide. Data analysis, at the conclusion of the experiment in October 2016, showed that ecological restoration as an integrated method of control did not effect the spotted knapweed density, nor did the varied sequence of treatment applications. The broadleaf herbicide, Milestone, was solely responsible for the decrease in spotted knapweed density. A negative consequence of using Milestone was a decrease in species richness, including a negligible amount of target native grass species and increase in nonnative grasses and forbs. Finally, bare soil visible was not decreased in the experimental plots receiving both native grass seed and herbicide application. A supplemental greenhouse experiment was conducted January through March 2017 in order to determine if Milestone was responsible for lack of native grass growth at the end of the field experiment. Similar experimental methods were used, with the addition of an experimental group that lengthened the amount of time between herbicide and grass seed application to four weeks. Data analysis after ten weeks of growth showed that Milestone negatively affected native grass seedlings, regardless of treatment sequence or length of time between applications. Due to the nature of native prairie restoration, it is recommended that the site continue to be monitored over subsequent years for potential target grass population growth. Also, further research is recommended to determine a more appropriate chemical to integrate into a restorative method of control. Ecologists and land managers play a critical, cooperative role in determining control methods that allow native prairies to remain healthy and intact in order to resist invasive species known to degrade them.

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INTRODUCTION

Invasive Species

Invasive species are formally described as "alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" (Executive Office of the President, 1999). Alien species are also known as weeds, nonnative, exotic, or nonindigenous. Recognizing the ambiguity of the definition as well as the correct terminology, the National Invasive Species Council (NISC) provided further clarification of the term invasive species as "a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health" (NISC, 2016). Animals, plants, fungi, and bacteria all have the capabilities to become invasive if it is nonnative to an ecosystem and causes harm. For the purposes of this research, however, invasive plants will be the focus of study.

Within these definitions, there are key understandings that must come through before designating a species as invasive. First, it is important to note that, by definition, problem-causing native organisms cannot be deemed invasive, nor can feral populations that are domesticated or under the control of humans. Second, an organism that is a designated invasive species in one geographical location may not be controlled or legally managed in another. Third, some non-native organisms are not labeled as invasive species for services they may provide to humans; human values play a large role in determining if a species is invasive (Invasive Species Advisory Committee, 2006).

Humans rely on nonnative organisms for many aspects of survival: food, shelter, medicine, ecosystem services, aesthetic enjoyment and cultural identity (Ewel et al., 1999). In particular, nonnative plants were introduced wherever humans colonized for purposes such as ornamentals, erosion control, wildlife foods, forestry and agriculture (White and Schwarz, 1998). Today, those introduced plants account for seventy percent of the world's food source (Ewel et al., 1999), and therefore, it is essential that they are grown outside of their natural ranges.

Nonnative plants and plant parts are moved to and from varying ecosystems by means of natural and anthropogenic pathways. Atmospheric, oceanic, and river currents have always formed pathways for plant dispersal (Ruiz and Carlton, 2003). In a predictable manner, propagules, or any plant part that aids in reproduction, travel from one geographic range to another using water and air currents. These infrequent, natural forces of plant dispersal are small in their global impact, compared to pathways developed by humans (Ruiz and Carlton, 2003). Advances in ship navigation, construction of canals and railways, and the expansion of air travel have all influenced the intentional and unintentional spread of species further than what was once naturally possible (Ruiz and Carlton, 2003; Ricciardi, 2016). Remote geographical locations that were, at one time, not subject to the arrival of new plant species, are now finding a substantial increase in nonnative species from this manmade, worldwide transportation system (Carlton and Geller, 1993). At any given moment, thousands of species are being carried throughout this system in the ballasts of ships, on their hulls, as contaminants of seed cargo, or in packaging made of plant material (Kaufmann and Kaufmann, 2012; Ricciardi, 2016).

In general, the number of species that will become invasive after dispersal, by either natural or manmade means, is explained by the Tens Rule (Williamson and Brown, 1986; Richardson and Pysek, 2006). The Tens Rule predicts that ten percent of imported species will escape after transport to flourish in their new habitat. Those that survive will enter a lag phase that can last up to 100 years (Hobbs and Humphries, 1995). During this time, plant species may maintain a stable, low population, becoming naturalized to their new environment. This means that the new habitat is colonized by self-replacing populations without the assistance of continual, human-influenced introductions (Richardson and Pysek, 2006). Of these species, only ten percent will be able to proliferate in large enough numbers to spread over a large area and become problematic to the structure, composition, and functioning of the native ecosystem (Richardson and Pysek, 2006). Genetic modifications, changing environmental conditions, and lack of human awareness have all been attributed to species reaching this final stage (Hobbs and Humphries, 1995).

The Convention on Biological Diversity has adopted and confirmed a management approach to reduce the likelihood that introduced plant species will reach the final stage of invasion. In this approach, the optimal management strategy evolves with time since species introduction, since management efficiency decreases and management costs increase as the time since introduction lengthens (Simberloff et al., 2013). Therefore, as often as possible, preventing the spread of potentially invasive plants should be implemented. Prevention strategies such as constricting pathways, intercepting movements at borders, and assessing risk for intentional imports have all proven effective in deterring the spread of nonnative invasive species (Simberloff et al., 2013). White and Schwarz (1998) determined a risk assessment that includes five criteria for researchers to use when assessing an introduced species: “(1) history of invasive behavior elsewhere, (2) relatedness to species that show invasive behavior, (3) climatic match between original range and proposed introduction area, (4) noxious and undesirable traits, and (5) biological attributes of the plant itself.”

The second step to the management approach is early detection and rapid response. Early detection strategies include interception of undesirable plants or plant parts, monitoring and surveillance, public awareness and education, and removal. Early detection allows for cost-effective removal as long as it is done in a timely manner; management costs at this level are, on average, forty times less than attempting to remove larger, more established populations (Simberloff et al., 2013). Responding to an invasive population promptly will also lessen the likelihood of that population establishing strong interspecific relations within the invaded community (Simberloff et al., 2013). The last option for land managers is long term management. At this stage, the invasion is so widespread that management of the population becomes complicated, costly, and sometimes ineffective.

Most nonnative species display phenotypic advantages, or biological attributes that help them outcompete native plants (Sutherland, 2004). Plants that are capable of vegetative reproduction, are monoecious, or have perfect flowers have an advantage over those that reproduce sexually or have unisexual flowers (Baker, 1962). Monoecious plants and plants with perfect flowers have gametes belonging to both sexes on a single plant or flower, meaning they are capable of fertilization and reproduction without requiring another plant. Those plants that reproduce sexually, are dioecious, or have unisexual flowers rely on other plants being present to provide the opposite sex’s gametes. Nonnatives that are pollinated by the wind are more competitive than natives that require specialized pollinators. If a nonnative is tolerant of high light levels and low moisture levels, this gives them a selective advantaged when invading new sites (Baker, 1967). Finally, nonnative plants that chemically armed against herbivory and may also initiate an allelopathic response to reduce competition with nearby native plants (Baker, 1965).

There are a number of hypotheses to explain this pattern of the selective advantages of nonnative plant species over native plant species. The most common, and most tested, include length of residence time (Richardson and Pysek, 2006), release from competition (Crawley, 1987; Wolfe, 2002), release from predation (Crawley, 1987; Wolfe, 2002), and evolution of increased competitive ability (EICA) (Blossey and Notzold, 1995).

Due to unknown introductions, variation in species lag times, and potentially secluded or undiscovered populations, the number of invasive plant species in the United States may vary. As of 2012, the University of Georgia's Center for Invasive Species and Ecosystem Health reported 1,231 grasses, forbs, shrubs, trees, and vines that are causing harm to humans and to the environment (Swearingen and Bargeron, 2016). Invasive species cause harm to humans by affecting economies and businesses as well as affecting human health. Economic harm includes two components: losses and costs. Losses include reductions in production, quality, efficiency, or functionality while costs reflect the investment made to control an invasive population (Bridges, 1994). Across the United States, invasive plants are encroaching croplands, pastures, forests, recreational areas and rights-of-way. These plants are causing the owners, both private and public, to lose money that they would have made had the invasive species not been present as well as spend money managing the invasive populations to prevent further spread. Invasive plants outcompete crops, reduce land values, and effect plantation proficiencies, all of which harm industries that rely on the land and plants to earn their income (Westbrooks, 1998). According to Pimentel et al. (2005), the United States spends about $120 billion a year on invasive species damage and prevention. Of that total, about $27 billion is spent on the management of introduced, invasive plants.

Another way that invasive species cause harm to humans is by directly affecting their health. Individuals who have unknowingly come in contact with certain species (e.g. giant hogweed, wild parsnip, and poison ivy) display a number of dangerous symptoms: skin irritation, rash, or skin photosensitization from irritating plant compounds, internal poisoning from consuming unknown plants' fruits, and airborne induced allergic reactions from pollen (Westbrooks, 1998).

Environmental harm includes three components: biologically significant decreases in native species populations, alterations to plant and animal communities, alterations to ecological processes (NISC, 2016). Nonnative, invasive species are now considered by some experts to be the second most important threat to biodiversity, after habitat destruction (Westbrooks, 1998). Many of these plants are outcompeting native species through rapid resource acquisition, which in turn, leads to nonnative plant populations capable of altering the key ecosystem parameters necessary to maintain the native populations (White and Schwarz, 1998). Ecosystem functions such as fluvial geomorphology (Graf, 1978), nutrient cycling (Vitousek and Walker, 1989), fire regime (Hughes et al., 1991), erosion rates, and soil pH are often changed, depending on the species in question, so much that native species can no longer tolerate their habitat (NISC, 2016).

In response to the imminent threat to native communities and rising cost of managing invasive species in the United States, President William Clinton signed Executive Order 13112 on February 3, 1999. This executive order mandated federal government agencies "to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause" (Executive Office of the President, 1999). The National Invasive Species Council was established within the executive order, initiating the cooperation and action of eight federal agencies to "prevent the introduction of invasive species, detect and respond rapidly to and control populations of such species in a cost-effective and environmentally sound manner, monitor invasive species populations accurately and reliably, provide for restoration of native species and habitat conditions in ecosystems that have been invaded, conduct research on invasive species and develop technologies to prevent introduction and provide for environmentally sound control of invasive species, and promote public education on invasive species and the means to address them" (Executive Office of the President, 1999).

The Minnesota Department of Military Affairs is one agency that is required to comply with Executive Order 13112. Camp Ripley Military Training Site (hereafter Camp Ripley) is a 53,000-acre military base managed by the Minnesota Army National Guard (MNARNG) under the jurisdiction of the Minnesota Department of Military Affairs (MNDMA). According to the 2002 MNDMA Environment Protection and Enhancement Regulation, all MNARNG operations are responsible for "preserving, protecting, restoring, and enhancing the quality of the environment" during and after military training operations around base (Minnesota Department of Military Affairs, 2002). Through a partnership with St. Cloud State University, students involved in the Camp Ripley Invasive Species Program have found and identified twenty-five invasive species at Camp Ripley (e.g. common tansy, leafy spurge, baby's breath, and buckthorn), including spotted knapweed (Minnesota Department of Natural Resources and Minnesota Army National Guard, 2016). Spotted knapweed is the focus of this research project due to its aggressive invasability, ability to rapidly change key ecosystem features, and widespread distribution at Camp Ripley.

Spotted Knapweed

Centaurea stoebe L. ssp. *micranthos* (Gugler) Hayek, commonly referred to as spotted knapweed, is one of these nonnative plants that are an ever-increasing economic and environmental detriment in the United States. *Centaurea* is a group of forbs that occupy at least five million acres of United States pastures, rangelands, and forests. Spotted knapweed has invaded more land within the country than any other knapweed species (Wilson and Randall, 2005). Everything about spotted knapweed, from its morphological characteristics to its genome, is made to reproduce quickly and outcompete native species. As a result, spotted knapweed is responsible for the reduction of variety of species in native and agronomic habitats by reducing the availability of quality livestock forage, degrading wildlife habitats, and hindering reforestation and landscape restoration projects (Jacobs and Sheley, 1998).

Spotted knapweed's native range is central Europe and eastward to central Russia, Caucasia, and western Siberia. It was first seen in North America in the 1880's and is believed to have been brought across the oceans in the contaminated soil used as ship ballasts as well as in contaminated seed mix used for livestock forage (alfalfa and clover) (Watson and Renney, 1974). Since arriving, spotted knapweed has continued to spread by agricultural means, traveling in transported alfalfa seed and contaminated hay, and by other human means including recreational vehicles and the disturbance of established seed banks. By 2012, spotted knapweed had spread across the continent of North America, distributing itself throughout Canada and the United States. It has been documented in 46 states within the U.S., including Alaska (Figure 1.1), and deemed invasive by 26 of those states (United States Department of Agriculture, 2017).

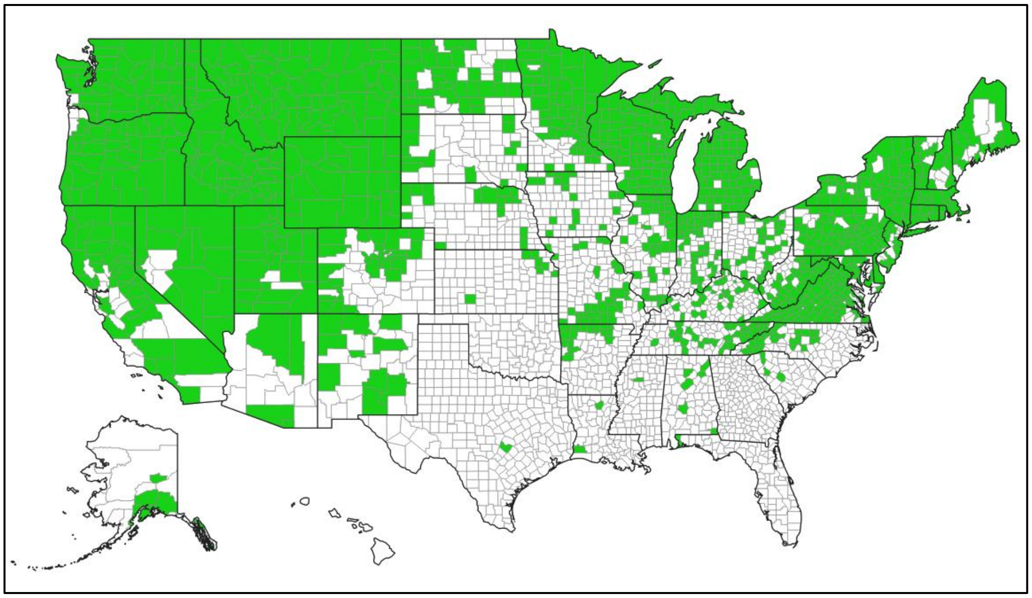

Figure 1.1. Spotted knapweed's nonnative range within the United States as of 2015. Map created by EDDMaps (2015). Spotted knapweed was unintentionally introduced to the western United States in the 1800s and has since infested states throughout the country, even Hawaii.

Spotted knapweed (Figure 1.2), is an herbaceous, short-lived perennial. It can range in height from two to four feet tall and anchors itself to the soil with a sturdy, elongated taproot system (Watson and Renney, 1974). In its first year, spotted knapweed usually occurs as a basal rosette of leaves (Figure 1.3B). Each grayish-green leaf is deeply lobed, about eight inches long and two inches wide (Figure 1.2B). This rosette usually lasts throughout the winter, and in the early spring, the plant will reach its bolting stage. Around early April through May, one to 10 stems ranging from eight to 50 inches tall grow from the center of the rosette (King County Noxious Weed Control Board, 2010). Stem leaves are smaller than the rosette leaves and alternate along the stem, decreasing in size as they go up the stem. Most large spotted knapweed plants have branched stems supporting a larger number of flowers (Figures 1.2A and 1.3E).

Flowering, which occurs from May-October, produces pinkish-purple flower heads (Figure 1.3C). Each flower head has 10 to 15 ray flowers (Figure 1.2D) which are surrounded at their base by rigid bracts that have dark vertical markings and dark, comb-like fringes (Figures 1.2C and 1.3C) (King County Noxious Weed Control Board, 2010).

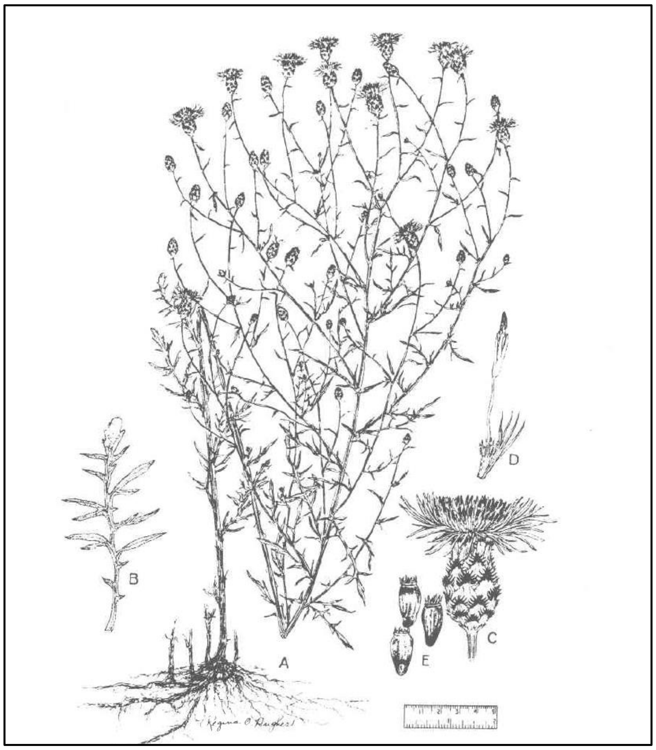

Figure 1.2. Spotted knapweed illustration. A - growth habit; B – deeply divided leaf; C – flower head with multiple flowers and dark bracts; D – disk flower; E – seeds (Hughes, 1970).

Figure 1.3. Spotted knapweed photographs. A – Spotted knapweed seeds; 3mm long (United States Department of Agriculture, 2017). B – Spotted knapweed rosette displaying many deeply divided leaves (Montana Weed Control Association, 2017). C – Spotted knapweed flower head; 6 mm diameter, 16-20 mm high; many radial flowers, bracts with black-fringed tips 1-2 mm long (Montana Weed Control Association, 2017) D – Spotted knapweed taproot and root crown of mature plant (Hess, 2017). E – Spotted knapweed mature plant displaying many stems and flower heads (Montana Weed Control Association, 2017).

Most spotted knapweed plants reproduce by cross-pollination and fertilization. Once fertilized by a pollinator, spotted knapweed is capable of producing between 350-20,000 seeds per plant, per year (Figure 1.3A) (Watson and Renney, 1974). The seeds have hard outer coatings and can be viable in the soil for up to 5-8 years (NPS, 2005), creating an extensive seed bank allowing the population to extend largely through peripheral enlargement of existing stands (Watson and Renney, 1974). After maturity, spotted knapweed is capable of independently dispersing seeds about a meter from the parent plant with a flicking motion (Watson and Renney, 1974). Seeds are dispersed long-distance by becoming attached to passing animals and birds, the undercarriage of vehicles or the bottom of shoes in mud, by waterways, or in crop seed and hay (Sheley et al., 1998). Even though spotted knapweed is most successful through sexual reproduction, many plants are capable of self-replication. Individual plants can grow a number of lateral shoots, just under the surface of the soil, to grow from the parent plant's root crown (Figure 1.3D) or form new rosettes next to the parent plant (Watson and Renney, 1974). By these means of reproduction, spotted knapweed can form stands of over 400 plants per square meter (Watson and Renney, 1974).

In its' native range, taxonomists have identified two genetic forms of spotted knapweed. The diploid form, *Centaurea stoebe L. ssp. stoebe* (formerly *C. maculosa L. spp maculosa*) has eighteen chromosomes in each cell's nucleus whereas the tetraploid, *Centaurea stoebe L. spp. micranthos*, contains thirty-six. These two forms are similar in morphological structure and reproduction methods, however, the tetraploid has a higher fecundity (Broz et al., 2009) and is capable of producing multiple flowering stems, withstanding drier environments, and surviving in dense vegetation making it more competitive and efficient at invading non-native rangeland in North America (Broz and Vivanco, 2009). Genetic studies have indicated that spotted knapweed may have had multiple introductions to North America and that, in the time it has been here, spotted knapweed most likely has hybridized with diffuse knapweed (another invasive *Centaurea* species) (Henery et al., 2010). This data suggests that when designing management strategies, land managers must take into account the genetic variation of the spotted knapweed species and its ability to evolve and adapt to the selection pressures it faces.

Spotted knapweed has adapted to a wide variety of natural and disturbed habitats. It is especially suited to mesic habitats that receive a moderate amount of rainfall and are well drained. Although it can survive in differing soil types, spotted knapweed prefers sandy, dry soils

(Watson and Renney, 1974). It prefers open habitats and quickly invade disturbed sites; the greater the disturbance, the higher the plant density of spotted knapweed (Atkinson and Brink, 1953; Watson and Renney, 1974). It most easily establishes itself into disturbed, unmaintained areas including forest and field margins, mining areas, non-maintained gravel pits, and is commonly found growing along roads, railways, and trails. From there, it will spread well into adjacent rangelands, meadows, and other open habitats (Figure 1.4). It is capable of living at a wide range of altitudes (30m-1,200m) as well as latitudes (19°N − 62°N) within North America (Watson and Renney, 1974).

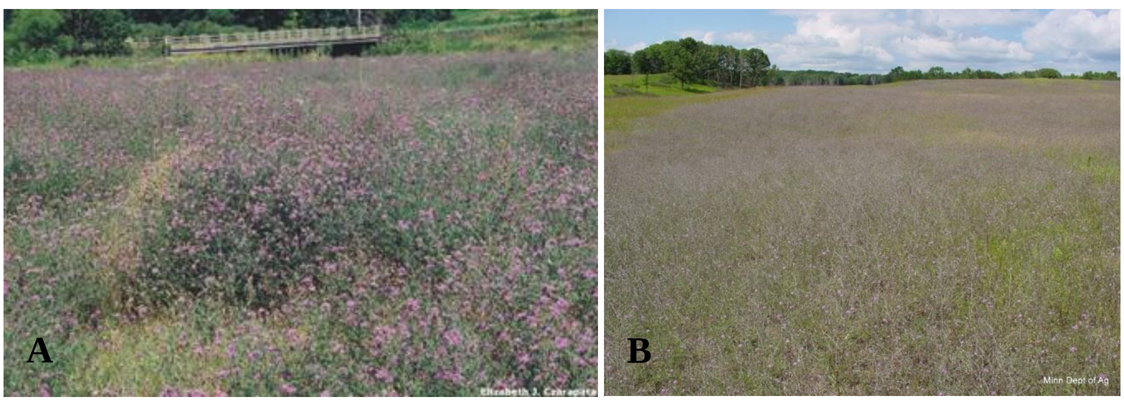

Figure 1.4. Spotted knapweed infestation. A – Infestation spreading from roadway (King County Noxious Weed Control Board, 2010). B – Infested field (Minnesota Department of Agriculture, 2017).

Due to its phenotypic and morphological characteristics, spotted knapweed is capable of causing great ecological and economic distress. First, spotted knapweed infestations have been shown to reduce the biodiversity of native species (Tyser and Key, 1988) by means of vigorous resource competition and acquisition (Herron et al., 2001), allelopathy (Fletcher and Renney, 1963), and surface runoff and sedimentation (Lacey et al., 1989). Spotted knapweed is capable of exuding biochemicals into the soil that have both antimicrobial and growth inhibiting properties (Alford et al., 2009), preventing the necessary soil conditions and microbiota needed for native plants to grow. Areas infested with spotted knapweed show runoff and sedimentation rates 56% and 192% higher, respectively, than areas dominated by grasses, thus risking the protection of soil and nearby water sources (Lacey et al. 1989).

Economically, areas of land infested with spotted knapweed have decreased in value, farmers and ranchers have seen a significant reduction in the amount of forage production (Watson and Renney, 1974), and the amount of money spent attempting to manage the ever-growing populations is on the rise.

Land managers across the United States have deployed several methods for the control and management of spotted knapweed. Each method relies on a number of criteria in order to be successful: plant type, soil type, population size, time of year, weather conditions, and proximity to bodies of water. One method alone has not proven to successfully control spotted knapweed populations, rather, they are most successfully controlled when an integrated approach is applied (King County Noxious Weed Control Board, 2010). Every land manager must evaluate their unique situation to make a control plan using a variety of methods including biological, chemical, cultural, manual, and mechanical control.

Biological methods of control use the natural enemies of spotted knapweed to decrease the size of the population or infestation. In Minnesota, herbivorous insects such as flies, moths, and weevils have been released to cause stress to the spotted knapweed populations and lower their rate of reproduction. After hatching, the root-boring weevil larvae, *Cyphocleonus achates,* consume plant resources as well as the plant itself, causing physical damage which can weaken or kill the plant (Figure 1.5A). Seedhead weevils, *Larinus minutus* and *Larinus obtusus*, reduce the future spread and plant reproduction by laying eggs that will eventually hatch, consuming developing seeds (Figure 1.5B) (Chandler, 2015). These forms of biological control have proven to be effective over long periods of time—taking up to a decade for heavily infested sites (Chandler, 2015).

Figure 1.5. Spotted knapweed biological control. A - Seedhead weevils lay their eggs in the flower head. B – Root-boring weevils weaken or kill plants by damaging root tissues. (Minnesota Department of Agriculture, 2017).

Currently, several different herbicides are used to control spotted knapweed. Selective, broadleaf herbicides are used to control knapweed populations while limiting the effects on the native grass and forb populations surrounding them. The most common herbicides used on spotted knapweed include Picloram, Dicamba, Clopyralid, Aminopyralid, and 2, 4-D. All of these broadleaf herbicides are Group 4 herbicides, meaning they effect plant growth by disrupting meristematic cells in new leaves and stems (Lym and Zollinger, 1992). The use of these chemicals varies in application rates and number of applications for adequate results, with each having unique characteristic residual soil effects, animal and plant toxicity, and chemical mechanism for control.

Methods of cultural control include introducing grazing livestock to pastures or grasslands where spotted knapweed has colonized. Severe defoliation will reduce root, crown, and aboveground growth (Kennet et al., 1992), however, after the plants have matured, cultural control is not a successful method of suppressing spotted knapweed growth and seed dispersal (Panke et al., 2012). Mature spotted knapweed plants' rough flowering stems are fibrous, coarse, and spiny, which are unpalatable and can irritate the animals (Sheley et al., 1998). Farmers and ranchers who own livestock and horses are encouraged to control spotted knapweed by being mindful of the rate at which native grasses are being removed from their pastures, as not to allow too much disturbance for knapweed plants to colonize.

Manual methods of control include hand pulling and small scale digging. Mechanical methods of control include mowing, discing, and prescribed burning. Small populations of spotted knapweed can be managed using these methods. When hand-pulling or digging, managers need to be sure that they extract as much of the crown (Sheley et al., 1998) and taproot as possible, which is easiest in wet, sandier soils (Panke et al., 2012). Cutting or mowing needs to be performed repeatedly throughout the growing season before plants reach the seed production or flowering stages. It has been proven successful in some populations of spotted knapweed, however, it is also capable of causing the plants to flower at shorter heights (Panke et al, 2012).

Prescribed burns on spotted knapweed infestations have inconsistent results. Most low intensity fires are not capable of damaging the taproot, and the mature, fallen seeds are not affected by fall or springtime burning (Ditomaso et al., 2006). However, most native grasses benefit from burning, making them more competitive in a landscape infested with spotted knapweed (McDonald et al., 2007). Prescribed burn plans, however, must consider the type and number of desirable species within the site, as fires may also create the type of disturbance that promotes the colonization of spotted knapweed (Sheley et al., 1998).

Ecological Restoration

"Ecological restoration is the process of restoring one or more valued processes or attributes of a landscape" (Davis and Slobodkin, 2004). The concept of ecological restoration merges together the science of ecology and societal or cultural values to achieve a wide range of outcomes meant to restore natural areas that have been degraded, damaged, or destroyed (Society for Ecological Restoration, 2002). Outcomes such as "restoring high levels of diversity and/or productivity, restoring a habitat so that it is again suitable for one or more target species, restoring desired aesthetic qualities or recreational opportunities of an environment as well as restoring a historic ecosystem" (Davis and Slobodkin, 2004) all have the potential to (re)create an environment that is capable of long-term productivity, natural succession, and withstanding a wide range of climatic, biotic, and anthropogenic changes (Chapin et al., 1992).

Due to the nature of military operations at Camp Ripley, grassland and prairie habitats throughout the base have been repeatedly disturbed by means of tank maneuvering operations and training area maintenance procedures. According to Watson and Renney (1974), spotted knapweed density is correlated with the degree of soil disturbance: the greater the disturbance, the higher the density. It is in these disturbed grasslands at Camp Ripley that spotted knapweed has taken advantage of the disturbance to the soil bed, established itself within the now-available niches (Sheley and Larson, 1996), and has become the dominant forb in the habitat. Over time, spotted knapweed has degraded the habitat, changing key ecosystem functions vital to the native plants that live there. Therefore, rather than simply eliminate the undesirable species as is common in most traditional management plants, it is essential to incorporate the concept of ecological restoration into the integrative invasive species management plan at Camp Ripley.

Prairie restoration may enhance key ecosystem services such as nutrient retention, pollution mitigation, productivity, soil sustainability, hydrological services and pollination (Benayas et al., 2009). The most desirable species to revive these services in an infested, degraded habitat at Camp Ripley are native grass species (Reetz, 1998). Compared to spotted knapweed's characteristic taproot, native grass communities are known for their extensive, fibrous root systems, some of which are capable of growing sixteen feet in depth. These roots provide soil holding capabilities and improve impurity and nutrient uptake, decreasing the amount of sedimentation and polluted run-off to nearby bodies of water (Reetz, 1998). In addition, thriving native grass communities accumulate more aboveground biomass creating sustainable food sources and habitat for prairie wildlife and foraging grounds for pollinators (United States Fish and Wildlife Service, 2016).

Invasion biology and research grew rapidly as a field after leading ecologist Charles Elton published the first book on invasion biology, The Ecology of Invasions by Animals and Plants, in 1958. Elton's diversity-invasibility hypothesis suggested "that species diversity enhances invasion resistance by increasing the diversity of functional traits, by filling resource niche space and by enhancing resource-use complementarity among species" (1958). This early, resource-based hypothesis has led to many studies on the efficacy of restoration for invasive species management (Foster, 2015). While there have been significant gains in understanding and implementing control methods and native species establishment techniques, rates of successful transition from an invaded system to a native community has had mixed results (Kettenring and Adams, 2011).

There are several examples within the literature of ecological restoration successfully managing invasive species. Through these studies, it has been identified that the key to restorative method success is held in two main ideas. First, ecological restoration and invasive species management are most successful when active revegetation takes place, rather than using methods that rely on native species natural seeding cycles. Blumenthal et al. (2003) determined that the propagule pressure of prairie species may sometimes be sufficient enough to control undesirable weeds. Petrov and Marrs (2000), Wilson and Partel (2003), and Foster (2015), all similarly concluded that actively reintroducing native species into a community where previously successful integrated invasive plant control has left open niches, catalyzed the development of the native plant community to serve as a natural barrier to colonization and the expansion of undesirable species.

Second, ecological restoration and invasive species management are most successful when revegetation efforts include seeding diverse native species. Masters and Sheley (2001), and Fargione and Tilman (2005) concluded that the more diverse the reintroduced population, the faster that the native assemblages can capture resources and space, creating considerable resistance to invasive species regrowth, further colonization, and further spread. Bakker and Wilson (2004) and Pokorny et al. (2005) added to those conclusions, stating that, not only does diversity play a role in invasion resistance, species identity, or functional group, may have an impact on how successful a community of native plants is at resisting invasion. Since plants in similar functional groups have similar phenology and means of acquiring resources, diverse communities of plants that include an assortment of functional groups will be better occupied and more likely to resist the variety of type of invaders threatening their community. Both of these main ideas support Elton’s diversity-invasibilty hypothesis.

There are, on the other hand, several examples in the literature that have shown complications in the research of integrating ecological restoration into traditional control plans.

Martin and Wilsey (2014) conducted an experiment in which native seeding did not successfully restore a native community. They concluded that native reseeding alone cannot shift a community from infested to native and that integrative methods of control as well as community assembly evaluations must be used in order to be successful.

In particular, integrative management strategies including herbicide have produced mixed results. Sheley et al. (2000) experimented on herbicide efficacy in relation to the plant growth stage that the chemical is applied. They suggested that in the case of spotted knapweed, applying chemical treatments at the spring rosette/bolt stage is best, while other stage applications do little to prevent seed bank expansion. Thompson et al. (2001) concluded that when herbicides are chosen to be a part of a management strategy, often times, reinvasion is more likely due to the rapid resource release and decreased competition caused by the chemical treatment. Sheley et al. (2001) conducted research that showed that active ingredients from different herbicides have varying effects on the native species involved. They found that particular chemicals were not selective in their modes of action, causing seed limitation to native species, and an increase in non-native grasses and forbs over time.

Despite the available research, both successful in restoring native communities and not, invasive species interventions must be specifically tailored to the situation at hand. The most useful research is done in consideration of logistics and resources needed to complete full-scale management. Sometimes, the cheapest methods are the least successful (for example, burning; Musil et al., 2005) and the most effective methods are impractical for large scale infestations (for example, hand-pulling; Martin et al., 2014).

Using the body of literature from the field as well as logistical and resource considerations at Camp Ripley, this thesis project has been designed to assist Camp Ripley in its

Sustainable Range Program and Native Grass Plan. It will use an integrative method of invasive species control specifically targeting spotted knapweed. This plan was made in consideration for the cost of materials, amount of time and manpower needed, and applicability to large scale infestations on Camp Ripley.

Objectives

The primary objective of this thesis project was to use ecological restoration to restore an invasive-species-dominated prairie into a prairie dominated by warm-season grasses native to central Minnesota plant communities. This method incorporated traditional, successful invasive species management techniques, including discing and chemical treatments, with the unused method of ecological restoration to specifically control spotted knapweed and reestablish a native prairie at Camp Ripley Army Training Site. With this method, there were three distinct secondary objectives. First, to reduce spotted knapweed density so as to reduce the established seed bank and therefore further spread of the species to other areas at Camp Ripley as well as adjacent areas beyond the Camp Ripley border. Second, to reduce the amount of bare soil to add soil stabilization to the most disturbed areas at Camp Ripley and lessen the amount of soil erosion and sedimentation of runoff and surface water. Third, to determine the effect of the sequence of broadleaf herbicide treatment and implementation of native grass seed mix on the plant density of spotted knapweed, plant density of four, dominant native grasses, and percent cover of bare soil. The first experimental hypothesis stated there will be fewer living spotted knapweed plants in the area treated with broadleaf herbicide followed by native grass mix application compared to the area treated in the reverse order. The reasoning for this hypothesis was that by weakening or killing the spotted knapweed plants before laying grass seed, the eight species of warm-season grasses will be allowed to germinate and grow, occupying space, consuming resources, and creating propagule pressure that would restrict spotted knapweed regrowth. The second experimental hypothesis stated that the broadleaf herbicide application followed by the native grass mix application would result in a higher native grass species density than if the order of those applications are reversed. The reasoning for this hypothesis was that the early application of the broadleaf herbicide will damage any young spotted knapweed plants that have over-wintered, evaded the discing treatment, or begun to grow due to the exposed seed bed. Those eliminated plants would open niches throughout the plant community for the native grasses to fill, without being subjected to resource competition or the later chemical application.

Chapter 2

METHODS

Field Study Site

Camp Ripley (15000 MN-115, Little Falls, MN 56345) occupies approximately 82 square miles in central Minnesota (47.07 N, 94.35 W) (Figure 2.1). It is bordered by the Crow Wing River for 8.5 miles to the north and the Mississippi River for 17 miles to the east. Camp Ripley's landscape and ecosystems were shaped by the last glacial period, the Late Wisconsinan (Minnesota Department of Natural Resources and Minnesota Army National Guard, 2016). It is situated along the divide between the Eastern Broadleaf Forest Province and the Laurentian Mixed Forest Province (Minnesota Department of Natural Resources, 2017). Three ecological subsections converge on Camp Ripley: Anoka Sand Plain, Hardwood Hills, and Pine Moraines and Outwash Plains (Minnesota Department of Natural Resources, 2017). Fifty-five percent of Camp Ripley is dominated by dryland forest while the remaining forty-five percent is divided equally between wetlands, dry open grasslands, and brush lands (Minnesota Department of Natural Resources and Minnesota Army National Guard, 2016). The variety of habitat types situated on Camp Ripley results in a wide variety of wildlife. There have been over six-hundred plant species, two-hundred migratory and resident bird species, fifty mammal species, and twenty reptile and amphibian species documented at Camp Ripley (Minnesota Department of Natural Resources and Minnesota Army National Guard, 2016).

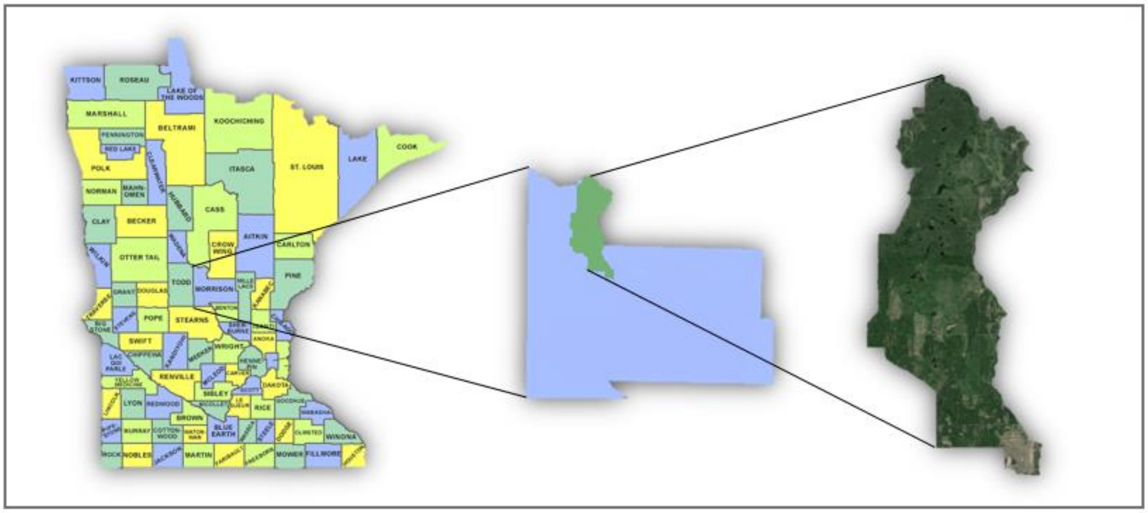

Figure 2.1. Camp Ripley location. Camp Ripley is located in Morrison County in central Minnesota.

Spotted knapweed is most significantly present in oak sand savannah and open, dry sand to mesic grassland ecosystems on Camp Ripley. Research will be completed on the disturbed, knapweed-infested grasslands in Training Area 18 (Figure 2.2). These grasslands are situated over excessively drained, sandy, or sandy loamed soils (Minnesota Department of Natural Resources, 2017). The grassland ecosystems located on Camp Ripley belong to the ecosystem classification Upland Prairie System, Southern Dry Prairie. According to the Minnesota Department of Natural Resources (2017), an Upland Prairie System is a “grass-dominated herbaceous community on level to steeply sloping sites with droughty soils. Moderate growing-season moisture deficits occur most years, and severe moisture deficits are frequent, especially during periodic regional droughts. Historically, fires probably occurred every few years.” Upland Prairie Systems contain fifty to one-hundred percent grass species, five to fifty percent forb species, less than five percent shrub species, and occasional tree species (Minnesota Department of Natural Resources, 2017) A specific list of vegetation found in a Southern Dry Prairie can be found in Table 2.1.

The total precipitation from May 12 to October 10, 2016 was 49.25 centimeters. The average rainfall from May to October over a thirty-year span is 49.48 centimeters.

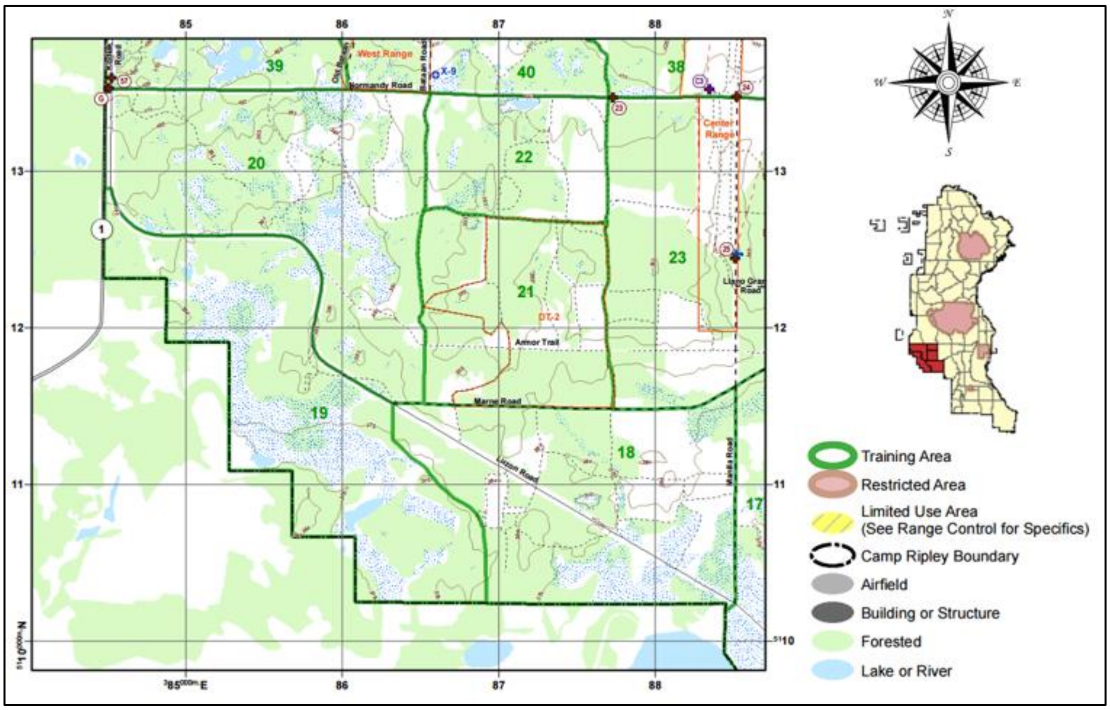

Figure 2.2. Training area 18 can be found on the southwestern portion of Camp Ripley (see locator map on right). Map created by Minnesota Army National Guard (2011).

Table 2.1. Upland Prairie System Southern Dry Prairie native plant community. Defined by the Minnesota Department of Natural Resources' Ecological Classification System (2005).

	Common Name	***Scientific Name***
Forbs, Ferns, and Fern Allies	*Purple prairie clover*	*Dalea purpurea*
	Gray goldenrod	*Solidago nemoralis*
	Silky aster	*Aster sericeus*
	Heath aster	*Aster ericoides*
	Stiff goldenrod	*Solidago rigida*
	Long-headed thimbleweed	*Anemone cylindrica*
	Bearded birdfoot violet	*Viola pedatifida*
	Rough blazing star	*Liatris aspera*
	Daisy fleabane	*Erigeron strigosus*
	Pasque-flower	*Anemone patens*
	Stiff sunflower	*Helianthus pauciflorus*
	Narrow-leaved purple coneflower	*Echinacea angustifolia*
	Tall cinquefoil	*Potentilla argute*
	Bastard toad-flax	*Comandra umbellata*
	Prairie turnip	*Pediomelum esculentum*
	Prairie wild onion	*Allium stellatum*
	Dotted blazing star	*Liatris punctata*
	Hoary puccoon	*Lithospermum canescens*
	Aromatic aster	*Aster oblongifolius*
	Virginia ground cherry	*Physalis virginiana*
	Flodman's thistle	*Cirsium flodmanii*
	Bird's food coreopsis	*Coreopsis palmata*
	Grooved yellow flax	*Linum sulcatum*
	Western ragweed	*Ambrosia psilostachya*
	Canada goldenrod	*Solidago canadensis*
	Heart-leaved alexanders	*Zizia aptera*
	Wild bergamot	*Monarda fitulosa*
	Harebell	*Campanula rotundifolia*
	Toothed evening primrose	*Calylophus serrulatus*
	Missouri goldenrod	*Solidago missouriensis*
	Skyblue aster	*Aster oolentangiensis*
	Mock pennyroyal	*Hedeoma hispida*
	Prairie sagewort	*Artemisia frigida*
	Hoary vervain	*Verbena stricta*
	Flowering spurge	*Euphorbia corollata*
	White sage	*Artemisia ludoviciana*
	Whorled milkweed	*Asclepias verticillata*
	Field blue-eyed grass	*Sisyrinchium campestre*
	Tall wormwood	*Artemisia dracunculus*
	Hairy golden aster	*Chrysopsis villosa*
	Prairie ragwort	*Senecio plattensis*
	False boneset	*Kuhnia eupatorioides*
	False gromwell	*Onosmodium molle*
	Green milkweed	*Asclepias viridiflora*
	Narrow-leaved puccoon	*Lithospermum incisum*
	Plantain-leaved pussytoes	*Antennaria plantaginifolia*
	Hairy puccoon	*Lithospermum caroliniense*
	Silky praire clover	*Dalea villosa*
Grasses and Sedges	*Little bluestem*	*Schizachyrium scoparium*
	Sideoats grama	*Bouteloua curtipendula*
	Big bluestem	*Andropogon gerardii*
	Prairie dropseed	*Sporobolus heterolepis*

Grasses and Sedges (cont.)	*Porcupine grass*	*Stipa spartea*
	Plains muhly	*Muhlenbergia cuspidata*
	Indian grass	*Sorghastrum nutans*
	Junegrass	*Koeleria pyramidata*
	Hairy grama	*Bouteloua hirsuta*
	Scribner's panic grass	*Panicum oligosanthes*
	Wilcox's panic grass	*Panicum wilcoxianum*
	Blue grama	*Bouteloua gracilis*
	Sand reed-grass	*Calamovilfa longifolia*
	Needle-and-thread grass	*Stipa comata*
Shrubs and Semi-Shrubs	*Smooth sumac*	*Rhus glabra*
	Wolfberry	*Symphoricarpos occidentalis*
	Leadplant	*Amorpha canescens*
	Prairie rose	*Rosa arkansana*

Field Experimental Design and Procedures

Preceding this project, the entire research location in Training Area 18 received prescribed burning for weed management during the summer of 2014 as well as discing for seedbed preparation during the fall of 2015. These tasks were completed by the Camp Ripley Environmental Department per their Vegetation Management Plan using equipment provided by the Environmental Department and Department of Public Works at Camp Ripley. In the spring of 2016, one control plot and two experiment plots were placed in the northeast quadrant of Training Area 18 (Figure 2.2). All of the plots are 400 square meters in size with at least three meters of buffer in between each research plot and at least three meters of buffer around the outside perimeter of the research area (Figure 2.3). On May 12th, 2015, the margins of the entire research area were marked with T-posts while the corners and midpoints of the plots were marked with rebar posts, both of which were provided by the Environmental Department at Camp Ripley. On May 23, 2015, even though the ground remained mostly bare soil from the previous discing treatment, a plant cover survey was conducted (Table 2.2).

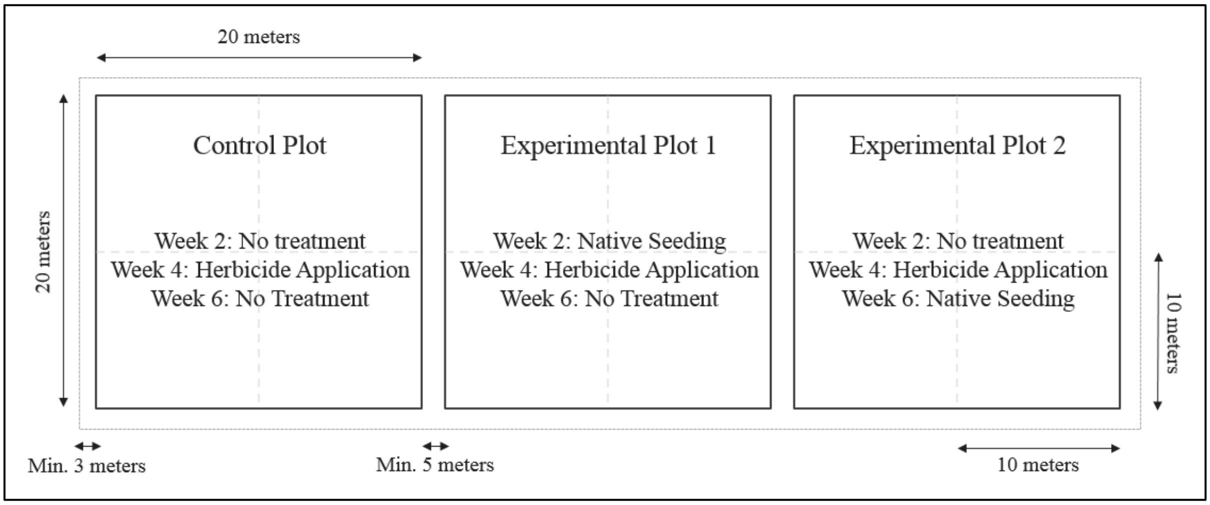

Figure 2.3. An illustration of the experimental plot design (not to scale). A different treatment procedure was applied in each of the plots. A T-post perimeter was set up at least three meters from the experimental plots. The minimum five-meter gap between subplots allowed for ATV and tractor clearance when applying the herbicide treatment and seedbed preparation. For data collection purposes, each plot was divided into four quadrants.

Table 2.2. Experimental plots initial plant survey. A significant portion of the research plots were exposed, bare soil due to the discing treatment given during the fall prior to this research project. Plants are listed in order of most dominant to least dominant.

Common name	Scientific name	Classification
Spotted knapweed	*Centaurea stoebe* L. *ssp. micranthos* (Gugler) Hayek	forb
Quackgrass	*Elymus repens*	grass
Crabgrass	*Digitaria* Haller	grass
Yarrow	*Achillea millefolium*	forb
Common mullein	*Verbascum thapsus*	forb
Common cinquefoil	*Potentilla simplex*	forb
Common tansy	*Tanacetum vulgare*	forb
Common dandilion	*Taraxacum officinale*	forb
Prairie clover	*Petalostemum*	forb
White clover	*Trifolium rapens*	forb
Common strawberry	*Fragaria virginiana*	forb
Hoary allyssum	*Berteroa incana*	forb
Field pussytoes	*Antennaria neglecta*	forb
American Elm	*Ulmus americana*	tree

Native grassland restoration and a control is being investigated in this experiment. Both experimental plots received a mixed height, mesic grass mix at a rate of one pound of pure live seed (one-and-a-half net weight pounds) per 400 m^2 plot. This premade grass mix was purchased from Prairie Restorations Inc. and consists of 33% *Andropogon gerardii* (big bluestem), 23% *Schizachyrium scoparium* (little bluestem), 22% *Sorghastrum nutans* (indiangrass), 13% *Bouteloua curtipendula* (sideoats grama), 5% *Elymus canadensis* (Canada wild rye), 2% *Koeleria macrantha* (junegrass), 1% *Panicum virgatum* (switch grass), and 1% *Sporobolus heterolepis* (prairie dropseed). All of the species within this grass mix are native to central Minnesota dry prairies. Before the native grass seed mix application, a tractor-mounted Brillion© soil packer was driven over all three research plots to loosen and prepare the soil. Then, one pound of pure live seed was hand broadcasted to cover the entirety of the 400 m^2 experimental plots. Finally, the Brillion© soil packer was driven over all three plots once again to ensure seed to soil contact in the experimental plots. One experimental plot received this method of treatment on May 24, 2015, the other experimental plot received this method of treatment on June 23, 2015. This difference is due to the second investigation of the experiment. The equipment needed for this investigation was provided by the Department of Public Works at Camp Ripley.

The sequence of management methods is also being investigated in this experiment. Experimental plots, chosen at random, received a combination of treatments including native grass seeding as well as a selective broadleaf herbicide application; chemical treatment followed by native grass seeding or native grass seeding followed by chemical treatment. Milestone, by Dow AgroSciences©, has been proven to be effective at damaging and/or eliminating populations of spotted knapweed at Camp Ripley. The active ingredient, aminopyralid (40.6%), is absorbed through the leaves and roots, moves throughout the plant, and deregulates meristematic cells affecting the growth process of the plant. For this experiment, a mixture of 3.5 fluid ounces Milestone with 50 gallons of water was added to a 50-gallon tank. Using an all-terrain vehicle, the tank was pulled evenly over all three plots spraying chemical out of the rear fanning nozzles at a rate of 7 fluid ounces per acre as recommended by Dow AgroSciences©. All three research plots were chemically treated on June 8th, 2015. The equipment and chemical needed for this portion of the investigation was provided by the Environmental Department at Camp Ripley.

For the remainder of the growing season, research plots were observed. Data collection took place on October 3rd and 10th, 2015. First, a random number generator was used to determine ten random sample locations from each quadrant in each plot. Each random sample was one square meter in size and outlined using a PVC frame. Next, percent of bare soil visible was estimated and grass and forb surveys were conducted. For the target plant species (spotted knapweed, big bluestem, little bluestem, indiangrass, and sideoats gramma), plant density was calculated by counting the number of stems per square meter. For non-target plant species, presence was recorded. For a timeline of field study procedures, see Table 2.3.

Due to the nature of the data collected, descriptive statistics were used to analyze the data.

Table 2.3. Timeline of events during field study that took place at Camp Ripley during May through October, 2016.

Date	Description
May 12	Determined experimental plots; pounded corner posts and placed reflective post tops around research area perimeter
May 13	Pounded rebar posts for measured 20 m x 20 m research plots. Flagged the corner and midpoint posts defining 10 m x 10 m quadrants for data collection
May 24	Brillion© packed experimental plot 1, hand-broadcast 1.5 lbs. of seed in experimental plot, and Brillion© packed experimental plot 1
June 8	Applied Milestone to all three plots
June 23	Brillion© packed experimental plot 2 and control plot. Hand seeded 1.5 lbs. of seed in experimental plot 2. Brillion© packed experimental plot 2 and control plot
July-August	Observation
October 3, 10	Collected Data: 1. Used random number generator to pick 10 random samples from each of the 4 quadrants in each plot. 2. Placed PVC quadrant, took photograph from above (eye height), estimated bare ground, counted stems of target grasses, counted stems of spotted knapweed (dead, flowering, rosettes), identified other grass/herb species present

Greenhouse Study Site

A supplemental greenhouse experiment was conducted in Robert H. Wick Science Building on the campus of St. Cloud State University (825 1st Ave S St. Cloud, MN 56301). The greenhouse is south-facing and maintains a controlled growing environment.

Greenhouse Experimental Design and Procedures

This greenhouse experiment was set up to supplement the data gathered from the previous field study. A similar experimental design and procedure was executed to determine if the selective broadleaf herbicide used in the field experiment had a direct impact on the native grass seed's germination and growth. One difference between the field study and this greenhouse study was the amount of time allowed for the grass seeds to germinate before or after the herbicide is applied. With this study, not only was a time interval of two weeks tested between herbicide and grass seed application (as seen in the field study), a four-week interval of time between treatments was tested as well. Figure 2.4 shows a simplified diagram of the greenhouse set-up used in this study.

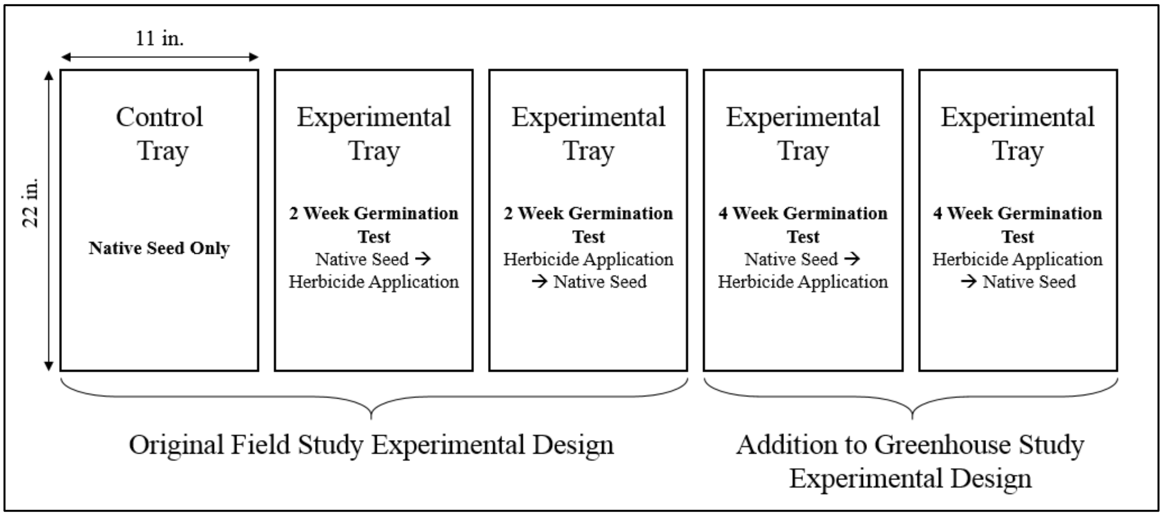

Figure 2.4. Greenhouse study experimental design.

On January 9, 2017, six planting trays were prepared by filling six, 11-inch by 22-inch black Jiffy© trays with drainage holes with a three-to-one all-purpose soil to sand ratio. The planting trays were then placed in drip trays and placed on greenhouse tables with clear, Jiffy© GroDome© covers. Next, two control trays and two experimental trays were hand-seeded with a locally-collected native grass seed mixture consisting of 40% big bluestem, 20% little bluestem, 20% indiangrass, 15% sideoats grama, and 5% switchgrass and lightly pressed to ensure seed-to-soil contact. The experimental trays that were hand-seeded were those that were scheduled to receive native seed before the herbicide application.

On January 23, 2017, two experimental trays (those testing the two-week treatment interval) received a Milestone application. To do this, a chemical mixture was made using a micropipette to measure and distribute 2.070 milliliters of Milestone into a one-gallon water sprayer. The one-gallon container was agitated for two minutes. Each planting tray was placed in a large container to control overspray and drift, sprayed with the chemical mixture evenly until the soil was visibly moist, and returned to the greenhouse table. For this greenhouse study, the same concentration and spray rate were used as was used in the field study.

On February 6, 2017, the final experimental tray testing the two-week treatment interval was seeded by repeating the hand-broadcasting method described above. On this same day, the two experimental trays testing the four-week treatment interval were treated with Milestone as described above. Four weeks later, on March 6th, 2017, the second four-week treatment interval experimental tray was seeded using the same procedure as described above.

Every week day, trays were uncovered in the morning and remained uncovered for the duration of daylight hours. At the end of the day, the growing trays were monitored, watered by pouring tap water into the drip trays, and re-covered to ensure minimal moisture loss due to transpiration. Every Monday, data was collected. The total number of seedlings/plants were counted and an average seedling/plant length was measured and calculated. Data was analyzed by combining the two- and four-week treatment interval experiment data points measured on the final day of the experiments. Then, Cohen's f-value was estimated and entered into G-Power to compute the significance levels required to achieve a power of .8 with an ANOVA study having three groups and a sample size of six. After running the ANOVA tests, Dunnett's Method was used to determine the significance between groups. For a timeline of greenhouse study procedures, see Table 2.4.

Table 2.4. Timeline of events during greenhouse study that took place at St. Cloud State University January through March, 2017.

Date	Description
January 9	All trays filled with 3:1 soil-sand 2 control trays, 2-week experimental tray, and one 4-week experimental tray hand-seeded
January 23	Both 2-week experimental trays receive chemical application Data collection: plant count and average height
January 30	Data collection: plant count and average height
February 6	Unseeded 2-week experimental tray hand-seeded. Two 4-week experimental trays receive chemical application
February 13, 20, 27	Data collection: plant count and average height
March 6	Unseeded 4-week experimental tray hand-seeded
March 13	Data collection: plant count and average height

Chapter 3

RESULTS

Field Experiment Results

Compared to the surrounding areas, spotted knapweed density was decreased in all three plots. The control plot was reduced to a density of zero living plants per square meter. Both experimental plots were reduced to an average density of .575 living plants per square meter (Table 3.1). Although living spotted knapweed plants in experimental plot #2 were found in more random samples, the exact same number of living spotted knapweed plants were counted within both of the entire experimental plots. Figures 3.1 and 3.2 show the number of living spotted knapweed plants found in each random sample within the two experimental plots.

Native grass density of all four target species was negligible. Within experimental plot #1, one random sample contained three stems of side oats gramma. Within experimental plot #2, one random sample contained seven stems of big bluestem. All other random samples contained none of the target native grass species planted throughout the experiment for the purposes of ecological restoration.

Bare soil percentage varied between the three plots (Table 3.2). The control plot had the least amount of bare soil visible with an average of 12% (Figure 3.3). Experimental plot #2 had an average of 20% bare soil visible (Figure 3.5). Experimental plot #1 had the highest average of bare soil visible at 26% (Figure 3.4).

At the time of data collection, a grass, forb, and shrub survey was conducted to determine what plants were growing in the research plots at the end of the experiment. Table 3.3 shows the type and abundance of other plants present.

Table 3.1. Live spotted knapweed descriptive statistics. The control plot was not included in these statistics since there were no living spotted knapweed plants counted at the time of data collection.

	Size	**Missing**	**Mean**	**Std Dev**	**Std. Error**	**C.I. of Mean**
Exp. Plot #1	40	0	**0.57500**	2.54082	0.40174	0.81259
Exp. Plot #2	40	0	**0.57500**	1.67772	0.26527	0.53656

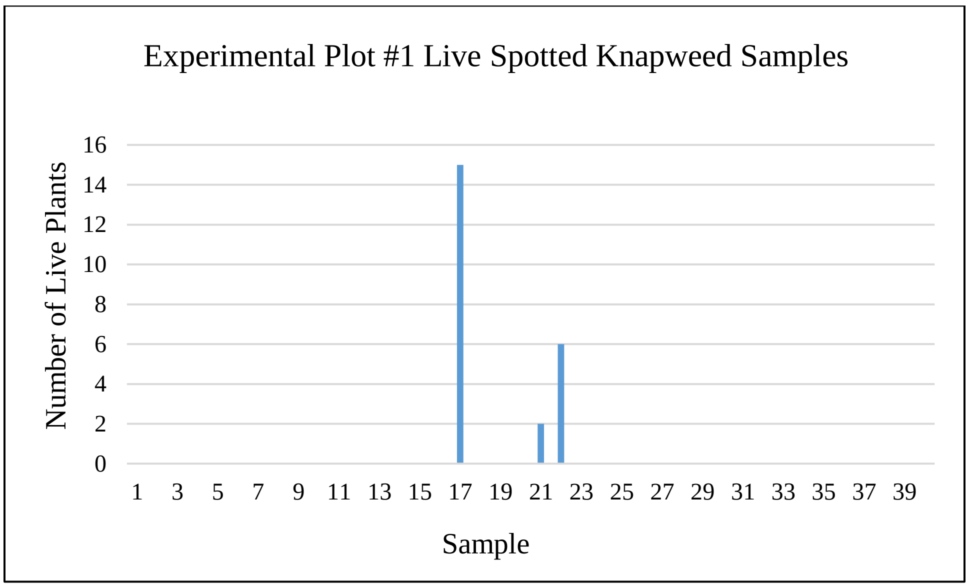

Figure 3.1. Number of live spotted knapweed plants found in each of the forty random samples within experimental plot #1. This plot received native seed treatment two weeks before the broadleaf herbicide treatment.

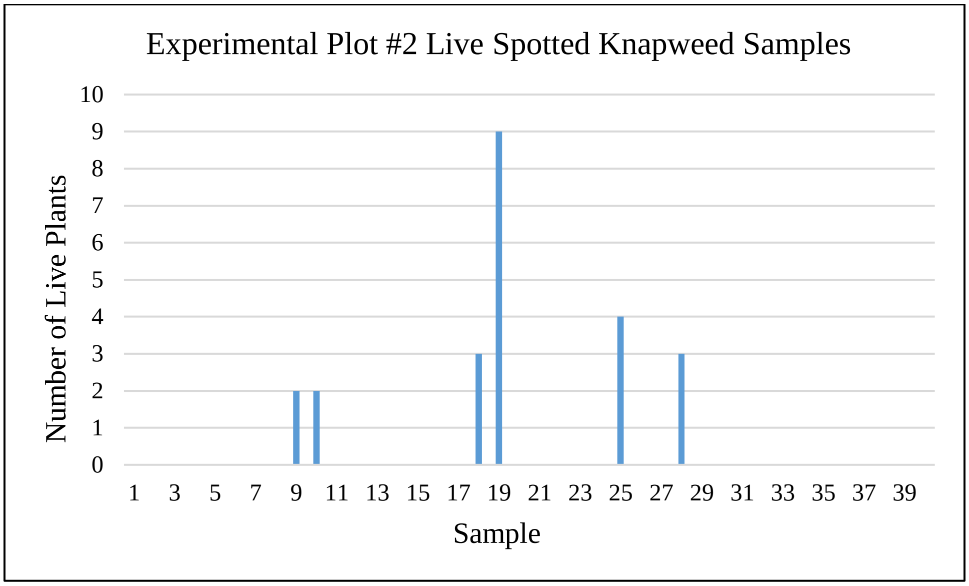

Figure 3.2. Number of live spotted knapweed plants found in each of the forty random samples within experimental plot #2. This plot received broadleaf herbicide treatment two weeks before native seed treatment.

Table 3.2. Bare soil descriptive statistics.

	Size	**Missing**	**Mean**	**Std. Dev**	**Std. Error**	**C.I. of Mean**
Control	40	0	**0.12125**	.12030	.019022	.038475
Exp. Plot #1	40	0	**0.25625**	.255575	.040438	.081793
Exp. Plot #2	40	0	**0.19500**	.18390	.029078	.058815

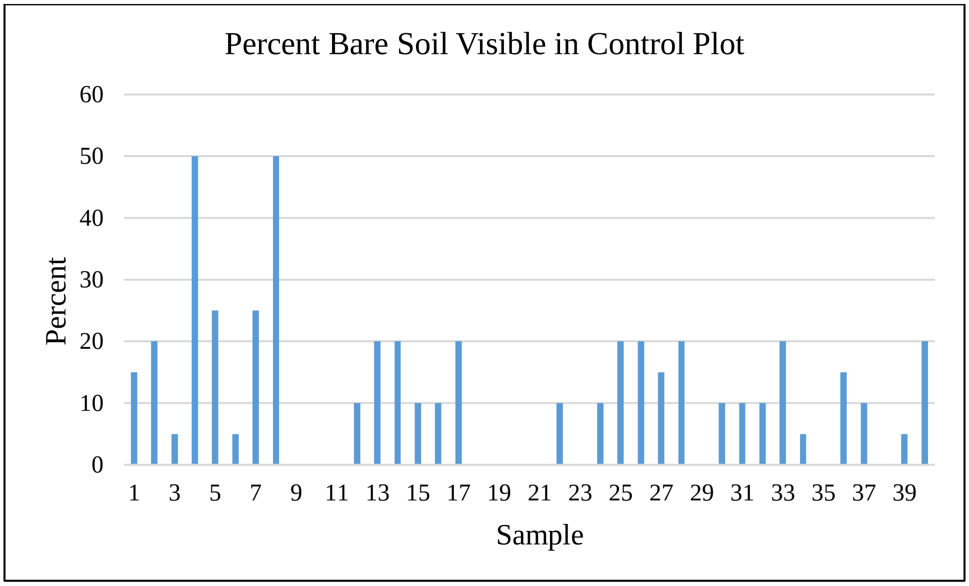

Figure 3.3. Percent bare soil visible in each of the forty random samples within the control plot. The average bare soil visible for the control plot was 12%.

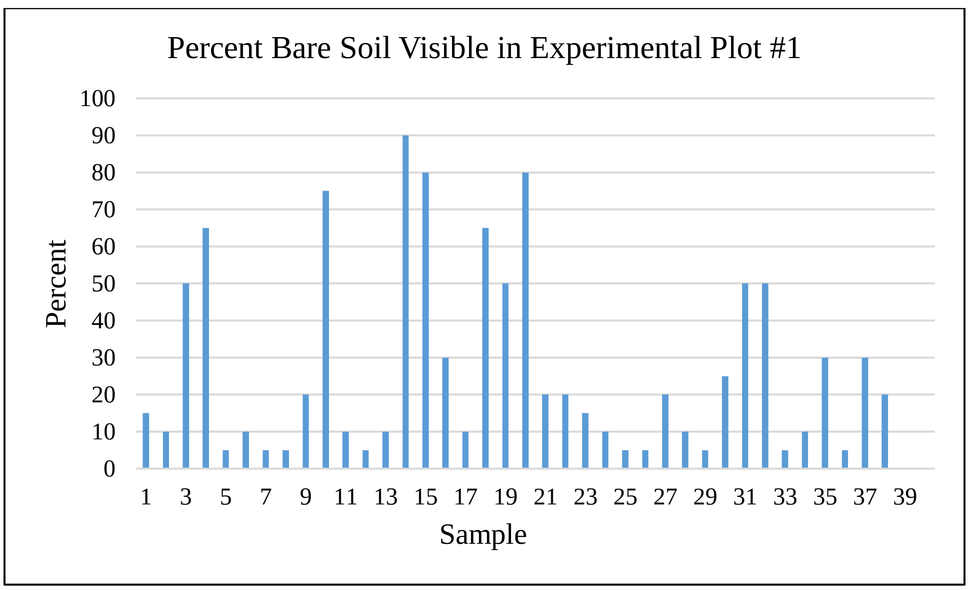

Figure 3.4. Percent bare soil visible in each of the forty random samples within experimental plot #1. The average bare soil visible for this experimental plot was 26%.

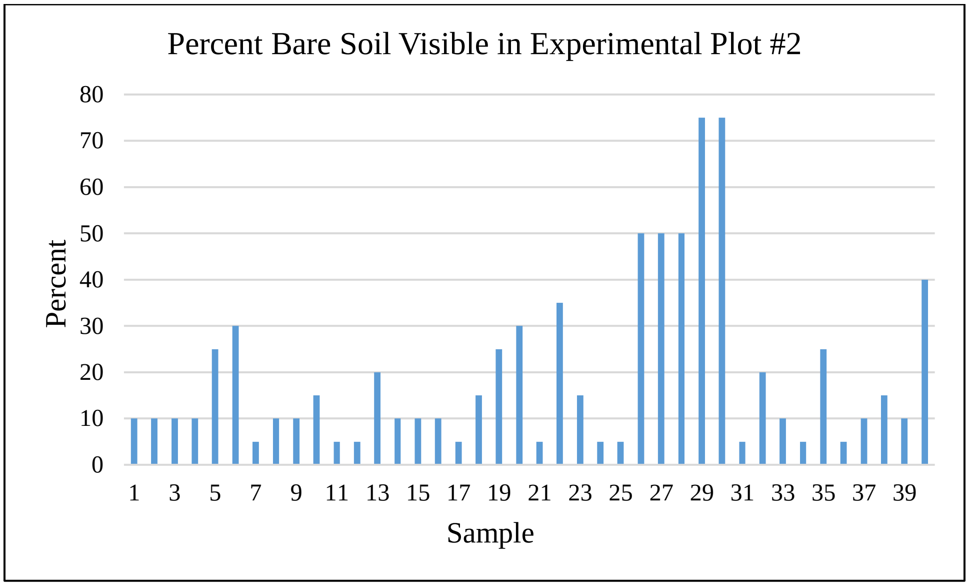

Figure 3.5. Percent bare soil visible in each of the forty random samples within experimental plot #2. The average bare soil visible for this experimental plot was 20%.

Table 3.3. Grass, forb, and shrub survey conducted during data collection, October 2016. Any plant status listed in red indicates species that have been known invaders in other locations or are currently listed on the invasive species control list in Minnesota.

Species	**Scientific Name**	**Classification**	**Percent of Samples Present**	**Status**
Quackgrass	*Elymus repens*	grass	87	introduced
Smooth brome	*Bromus inermis.*	grass	51	introduced
Yellow foxtail	*Setaria pumila*	grass	50	introduced
Crabgrass	*Digitaria sanguinalis*	grass	49	introduced
Red top	*Agrostis gigantea*	grass	41	introduced
Purple lovegrass	*Eragrostis spectabilis*	grass	38	native
Hoary allysum	*Berteroa incana*	forb	33	introduced
New England aster	*Symphyotrichum novae-angliae*	forb	29	native
Prairie clover	*Trifolium repens*	forb	22	introduced
Stinkgrass	*Eragrostis cilianensis*	grass	22	introduced
Witchgrass	*Panicum capillare*	grass	20	native
Kentucky bluegrass	*Poa pratensis*	grass	20	introduced
Common mullein	*Verbascum thapsus*	forb	13	introduced
Sumac	*Toxicodendron vernix*	shrub/tree	6	native
Lance-leafed goldenrod	*Solidago graminifolia*	forb	4	native
Common yarrow	*Achillea millefolium*	forb	3	native
Common milkweed	*Asclepias syriaca*	forb	3	native
Bladder campion	*Silene latifolia*	forb	2	introduced
Intermediate dogbane	*Apocynum medium*	forb	2	native
Common strawberry	*Fragaria virginiana*	forb	1	native
Silky dogwood	*Cornus amomum*	shrub/tree	1	native
Sedge	*Carex sp.*	sedge	1	native
Poison ivy	*Toxicodendron radicans*	forb	1	native
Barnyard grass	*Echinochloa crus-galli*	grass	1	introduced
Common cinquefoil	*Potentilla simplex*	forb	1	native
Bur oak	*Quercus macrocarpa*	shrub/tree	1	native
Crown vetch	*Securigera varia*	forb	1	introduced
Canada thistle	*Cirsium arvense*	forb	1	introduced

<u>Greenhouse Experiment Results</u>

During the two-week treatment interval experiment, both the control and first experimental tray had a large number of seeds germinate, with over 200 and 400 seeds germinate respectively. Without chemical application, the plants in the control tray were able to continually increase in count. After Milestone was applied to the experimental trays in week 2, the grass in the first experimental tray began to show a decrease in count within two to three weeks. The grass in the second experimental tray had a much lower germination rate, about 100 seeds, compared to the seeds grown in chemical-free soil and remained low until the end of the experiment (Figure 3.6).

Measurements for average plant length showed similar results. While the control plants continually increased in length through the duration of the experiment, the grass in first experimental tray began to decrease in length three weeks after herbicide application. The grass in the second experimental tray had half the average length than grass grown in chemical-free soil two weeks after seeding (Figure 3.7).

Observational data for the two-week treatment interval trays described plants grown in chemical-free soil to be green in color, standing upright, having multiple stems and distinguishable blades providing evidence that a variety of species within the mix were able to germinate. After herbicide application, healthy-looking plants began to change from green to yellow to white in color and began to lay down on the soil rather than stand upright. Seeds that germinated in soil that already contained herbicide were described as colorless, thin/weak, laying on the soil (rather than standing upright), or growing in a curved/spiral manor (rather than straight).

Plant count data from the four-week treatment interval experiment showed that grass grown in the control and first experimental trays had a high germination rate. Two weeks after laying the seed, there were 200 and 300 plants in the control and first experimental trays, respectively. Two weeks after laying grass seed in the second experimental tray, 32 plants were counted (Figure 3.8).

Plant length data from the four-week treatment interval experiment showed that while the grass in the control tray continued to grow in length throughout the duration of the test, grass in the first experimental tray showed a decrease in length as soon as the herbicide was applied, changing from four centimeters to two centimeters by the end of the experiment. Grass grown in the second experimental tray, receiving herbicide treatment four weeks earlier, showed 65 percent of the length compared to seeds grown in chemical-free soil at two weeks after germination. Both of the experimental tray's final length measurements were less than 25 percent of the length of grass in the control group (Figure 3.9). Observational data for the four-week treatment interval experiment were similar to those described in the two-week treatment interval experiment.

Based on these final measurements and observations, it was assumed that the length of time between treatments did not have an effect on the count or length of the grass. Therefore, the final plant count and length measurements were compiled for each treatment sequence in order to analyze the data by using an ANOVA. Running Cohen's f value (Table 3.4) through the G-Power program calculated a significance level for each data set: .1236 for plant count and .0310 for plant length. Results of the one-way ANOVAs for both plant count (Table 3.5 and 3.6) and plant length (Table 3.8 and 3.9) showed significance between the control group data and experimental group data. Post hoc analysis for the plant count data (Table 3.7 and Figure 3.10) found significance differences between the control group and both treatment 1 (seed then herbicide) and treatment 2 (herbicide then seed). Post hoc analysis for the plant length data (Table 3.10 and Figure 3.11) found significance differences between the control group and treatment 2 (herbicide then seed), but not between the control group and treatment 1 (seed then herbicide).

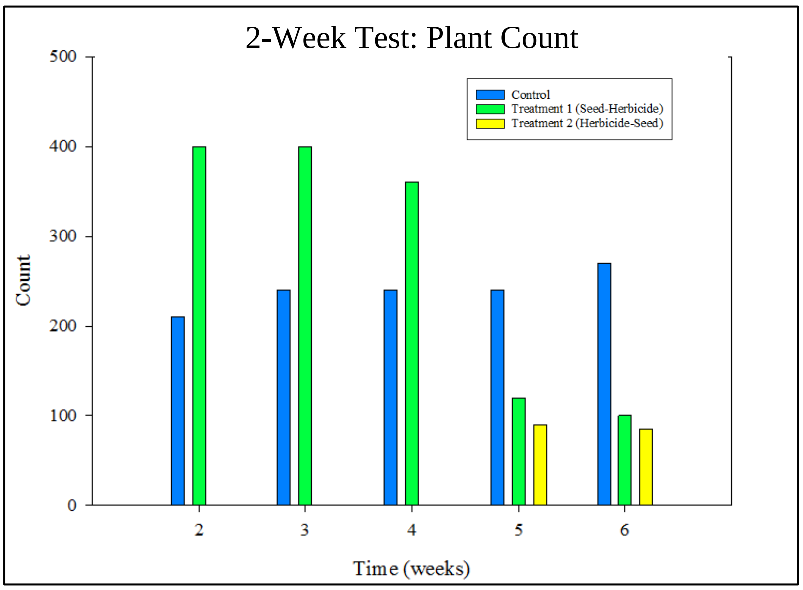

Figure 3.6. Results for number of plants counted throughout the duration of the two-week test. For treatment 1, seed was laid during week 0 and chemical applied week 2. For treatment 2, chemical was applied week 2 and seed was laid week 4.

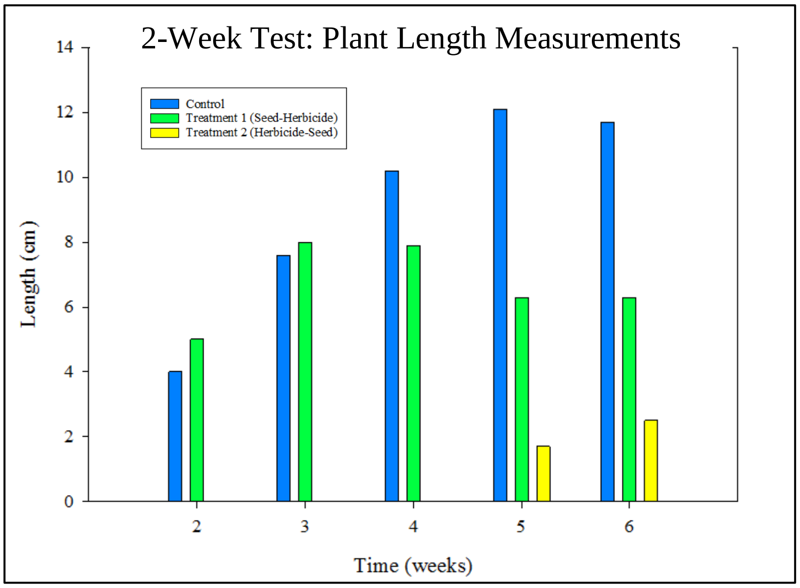

Figure 3.7. Results for average plant length measurements throughout the duration of the two-week test. For treatment 1, seed was laid during week 0 and chemical applied week 2. For treatment 2, chemical was applied week 2 and seed was laid week 4.

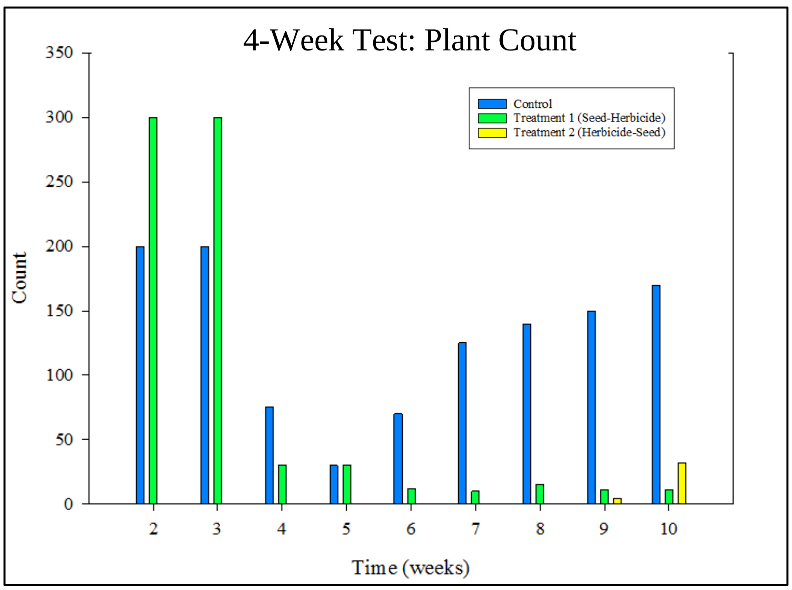

Figure 3.8. Results for number of plants counted throughout the duration of the four-week test. For treatment 1, seed was laid during week 0 and chemical applied week 4. For treatment 2, chemical was applied week 4 and seed was laid week 8.

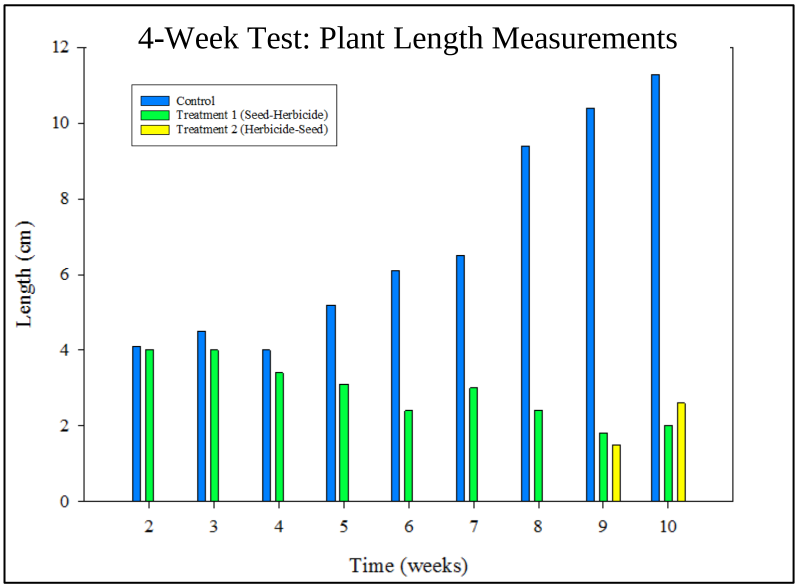

Figure 3.9. Results for average plant length measurements throughout the duration of the four-week test. For treatment 1, seed was laid during week 0 and chemical applied week 4. For treatment 2, chemical was applied week 4 and seed was laid week 8.

Table 3.4. Estimate of Cohen's f-value for the standardized effect size in the study for plant count and length. This value is defined as the ratio of the standard deviation of the set of population means of the groups to the common standard deviation of the group populations. G-Power was used to compute the significance levels for plant count and plant length ANOVA tests.

Statistic	**Count**	**Length (cm)**
Standard Deviation of the Means	94.1200	4.7729
Pooled Standard Deviation of Groups	58.7792	1.7635
Cohen's f	1.6012	2.7065
Significance Level	**0.1236**	**0.0310**

Table 3.5. ANOVA results for plant count data.

Group Name	**N**	**Missing**	**Mean**	**Stddev**	**SEM**	**87.64%**	**Confidence Interval**
Control	2	0	220.00000	70.71068	50.00000	131.680	308.320
Treatment 1	2	0	55.50000	62.93250	44.50000	-32.820	143.820
Treatment 2	2	0	58.50000	37.47666	26.50000	-29.820	146.820

Table 3.6. ANOVA results for plant count data.

Source of Variation	DF	SS	MS	F	P
Between Groups	2	35434.33333	17717.16667	5.12798	**0.10766**
Residual	3	10365.00000	3455.00000		
Total	5	45799.33333			

Table 3.7. Post hoc (Dunnett's Method) results for multiple comparisons to the control group.

Comparison	Diff of Means	q'	P	P<0.124
Control vs. Treatment 1	164.50000	2.79861	**0.10946**	Yes
Control vs. Treatment 2	161.50000	2.74757	**0.11412**	Yes

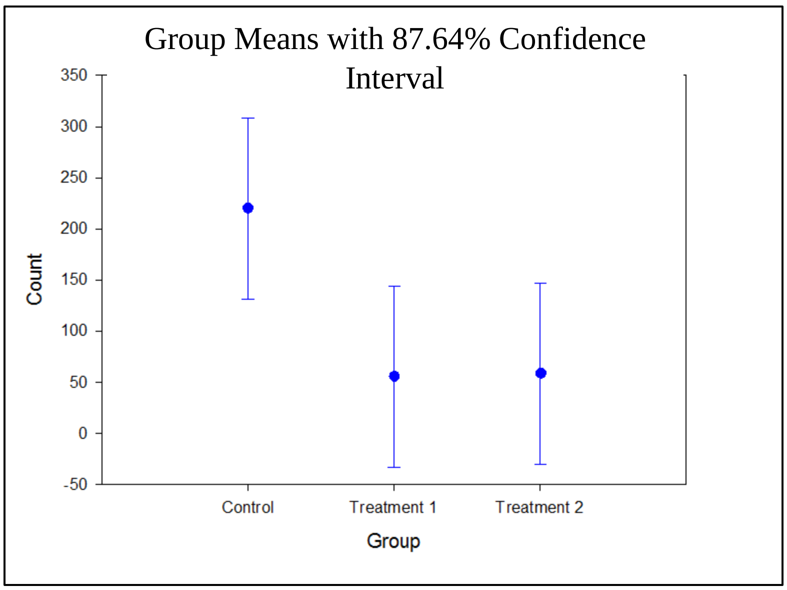

Figure 3.10. Confidence intervals showing group means for final plant count data. When comparing the experimental groups to the control groups, significance is found between both the control group and treatment 1 (seed then herbicide) as well as between the control group and treatment 2 (herbicide then seed).

Table 3.8. ANOVA results for plant length data.

Group Name	N	Missing	Mean	Stddev	SEM	96.9%	Confidence Interval
Control	2	0	11.50000	0.28284	0.20000	6.703	16.297
Treatment 1	2	0	4.15000	3.04056	2.15000	-.647	8.947
Treatment 2	2	0	2.55000	0.070711	0.050000	-2.247	7.347

Table 3.9. ANOVA results for plant length data.

Source of Variation	DF	SS	MS	F	P
Between Groups	2	91.12333	45.56167	14.65005	**0.02831**
Residual	3	9.33000	3.11000		
Total	5	100.45333			

Table 3.10. Post hoc (Dunnett's Method) results for multiple comparisons to the control group.

Comparison	Diff of Means	q'	P	P<0.031
Control vs. Treatment 2	8.95000	5.07508	**0.02227**	Yes
Control vs. Treatment 1	7.35000	4.16780	**0.04024**	No

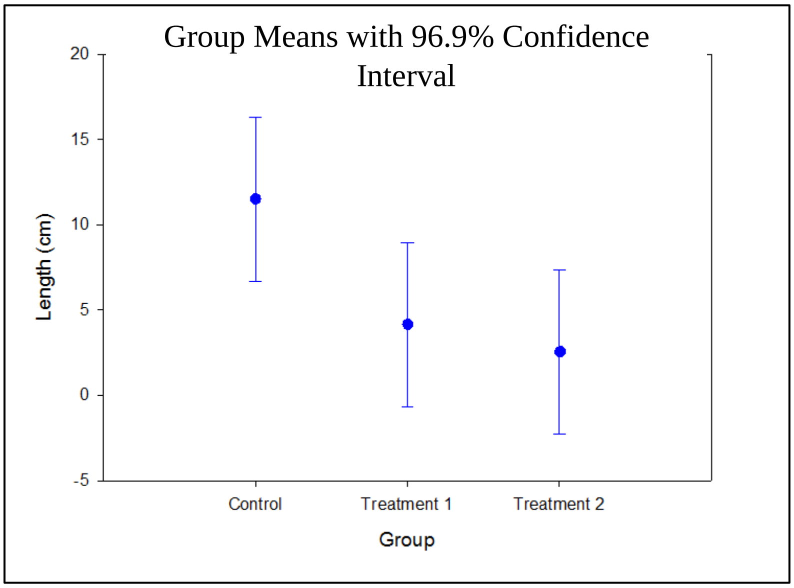

Figure 3.11. Confidence intervals showing group means for final plant length data. When comparing the experimental groups to the control groups, significance is found between both the control group and treatment 2 (herbicide then seed) but not between the control group and treatment 1 (seed then herbicide).

Chapter 4

DISCUSSION

The objective of this thesis project was to use ecological restoration practices to restore an invasive species-dominated prairie into a prairie dominated by warm-season grasses native to central Minnesota plant communities. The experimental method incorporated traditional, successful management techniques, including discing and chemical treatments, with the unused and highly variable method of ecological restoration to specifically control spotted knapweed and reestablish native prairie communities at Camp Ripley Army Training Site. The first experimental hypothesis stated there will be fewer living spotted knapweed plants in the area treated with broadleaf herbicide followed by native grass mix application compared to the area treated in the reverse order. The null hypothesis stated that varying the sequence of treatments would not affect the density of spotted knapweed. Upon reviewing the descriptive statistics that showed no living spotted knapweed plants in the control plot and the exact same density of living spotted knapweed in both experimental plots, the null hypothesis is supported. However, as described in results, restoration efforts were not successful (which made up half of the treatment sequence), therefore, it is believed that Milestone, alone, played a key role in controlling the spotted knapweed plants.

The second experimental hypothesis stated that the broadleaf herbicide application followed by the native grass mix application will result in a higher native grass species density than if the order of those applications is reversed. The null hypothesis stated that varying the sequence of treatments would not affect the density of target grass species within the experimental plots. This hypothesis test was inconclusive, as the number of target grass plants was negligible in both experimental plots.

Even though, as the data showed, spotted knapweed was controlled and target grasses did not grow in the research plots, a variety of grasses, forbs, and shrubs were present at the conclusion of the field study. Many of the species found were nonnative (introduced) plants that have naturalized to the area, meaning that they are not known to cause harm. There were, however, four species found that are known to cause harm and/or are currently on the Minnesota Department of Natural Resources invasive species control list. It is believed that by using Milestone in an effort to control spotted knapweed, species richness decreased and other nonnative grasses and forbs were given the opportunity to occupy niches opened by the removal of spotted knapweed in the research plots. This is not a desired outcome for land management or restoration practices, as native communities, diverse in species, are best at resisting invasion and degradation (Elton, 1958; Masters and Sheley, 2001).

As with all field studies, there were a number of confounding variables or external factors that could have affected the results of this study. For example, after the grass seed was laid, surface runoff or foraging animals could have limited the number of seeds available within the experimental plots to germinate and grow. Also, due to the amount of time that this land has been known to be infested with spotted knapweed, it is possible that the soil conditions themselves needed to be manipulated before attempting restoration.

After reviewing the data collected from the field study, it was decided that a post hoc greenhouse experiment would be conducted in order to determine if Milestone was responsible for the grass growth results. The first experimental hypothesis for the greenhouse study stated that if growing trays are treated with Milestone, then there will be fewer and shorter native grass plants than untreated growing trays. The null hypothesis stated that Milestone would not have an affect on the amount or length of native grass seeds or seedlings. I fail to reject the null hypothesis upon completion of the data analysis. Observational and statistical data show that, regardless of the sequence of treatment, Milestone had a significant, negative effect on the native grass seedling count. In contrast, statistical analysis determined a significant difference for the average length only between the grass in the control tray and the grass that was planted in soil containing the herbicide. Observational data suggests that if the experiment would have been lengthened, a significant difference would have been determined between the grass in the control group and the grass that received herbicide after planting, due to the observed diminishing color, length, and overall health of the grass plants at the end of the project.

The second experimental hypothesis for the greenhouse study stated that growing trays given a longer time interval between seeding and Milestone treatment will produce more native grass plants with longer length compared to the growing trays given a shorter time interval between treatment applications. The null hypothesis stated that the time interval between treatment applications would not have an affect on the amount or length of the native grasses. Due to the lack of replicates in this study, statistical analysis could not be completed for this hypothesis test. Observational and descriptive statistics, however, led to the assumption that the null hypothesis is supported. With this being assumed, data for each treatment sequence could be combined to perform statistical analysis for the first experimental hypothesis.

During the initial experimental design, the time interval between treatment applications was determined by the length of time it took for the target native grass species to germinate. In future research, the time required for chemical degradation should determine the interval between treatment applications. In this case, aminopyralid is known to have a half-life of 45 days. Therefore, it is most likely needed, and suggested, for future research involving ecological restoration and chemical methods of control to span multiple growing seasons to allow for chemical degradation and native grass establishment. Additionally, a review of the cost-benefits of other broadleaf herbicide active ingredients should be completed before designing future experiments, with the possibility of incorporating a variety of herbicides into a future research project, rather than just one single herbicide.

Ecologists and land managers play a critical, cooperative role in determining control methods that allow native prairies to remain rich in species diversity, productive, and intact to resist invasive species known to degrade them. Continuing research focused on incorporating ecological restoration into an integrated invasive species management plan is essential, as manmade disturbances and invasive species will continue to threaten native plant communities indefinitely. The results of this study should be considered when designing site- and species-specific management plans, as well as in future restoration projects targeting invasive species-infested grasslands.

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