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The Application of Succession-Based Management Techniques to Invasive-Dominated Prairie Areas

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The Application of Succession-Based Management Techniques to Invasive-Dominated Prairie Areas

by

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Abstract

Invasive species have become a serious economic and ecological issue. Historical treatments of these area infested by terrestrial exotic species consisted of single treatment herbicide application, and frequently, areas treated were reinvaded. This observation has led to integrated management that features several different techniques. In this experiment the effects of four seedbed preparations, the use of a cover crop, and distribution method of native seeds will be evaluated, alone and in combination with a selective broadleaf herbicide, Milestone®. Targeted species include spotted knapweed (*Centaurea stobe* L. ssp *micranthos* (Gugler) Hayek) and common tansy (*Tanacetum vulgare* L.); both species have infested and degraded area on Camp Ripley, Minnesota. Site manipulation began in 2010 and was completed during the 2011 growing season. Data collection includes senescent, emergent, and peak flower percent cover of targeted invasive species, and vegetative species counts. Analyses determined significant impacts on percent cover and plot diversity when considering selective herbicide treatments. No significant impact on percent cover or diversity was detected when comparing either of the seeding variables; the use of a cover crop or the method of native seed dispersal. Future experimental trials should focus on complete removal of targeted specie prior to the effects of reseeding can be significantly determined.

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Introduction

Invasive Species are non-native species whose introduction does or is likely to cause harm. This harm can be in the form of economic loss, ecosystem degradation, or direct impact(s) to humans (Executive Order 13112 1999). Examples of ecological damages caused by invasive species include: alteration of native succession pathways, competitive exclusion, niche displacement, predation, and in some cases, extinction of native biota (Mooney and Cleland 2001). Invasive species arrive via intentional and unintentional introductions. Intentional introductions include medicinal and agricultural use of foreign species; Unintentional introductions include contaminated traded goods such as within packing material or ballast water of ocean vessels; additional unintentional introductions can result from global tourism (Costello and McAusland 2003). Invasive species impact 42% of threatened and endangered species primarily by competition and predation. Individuals and agencies across the United States spend nearly $120 billion annually in losses, damages, and control costs due to alien-invasive species (Pimental et al. 2004). This genuine threat of damages associated with invasive species led to Executive Order 13112. This order was passed by former president Bill Clinton; it mandates that all federal agencies take the following actions:

> …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; and…not authorize, fund, or carry out actions that it believes are likely to cause or promote the introduction or spread of invasive species in the United States or elsewhere…(Executive Order 13112)

The Minnesota Department of Military Affairs (MNDMA) is a federal agency required to be in compliance with Executive Order 13112. MNDMA maintains two different locations within the state of Minnesota; Arden Hills Military Training Site, located in the southern portion of the state in Ramsey County, and Camp Ripley, located in Morrison County. Camp Ripley was the study site. Historical evaluations, completed by graduate student Jill Babski (2004) on Camp Ripley have identified eighteen established terrestrial invasive plant species within Camp boundaries, of which two will be addressed for experimental work: spotted knapweed (*Centaurea stobe*), and common tansy (*Tanacetum vulgare*) (SCSU 2008). These species are of particular concern due to their wide distributions and ability to alter existing ecosystems.

Spotted knapweed (*C. stoebe)* is a member of the aster family and is native to areas in Europe and Asia; it was introduced around 1900 (Lacey et al. 1989). Spotted knapweed flowers from early June through October, each plant produces over 1,000 seeds per plant which can remain viable in the soil for up to seven years (Davis et al. 1993). Heavily infested sites can have up to 50,000 seeds in the soil per meter squared (Jacobs and Sheley, 1998). New shoots develop from existing taproot structures annually, a single established taproot can develop up 20 new shoots, though shoots do not develop from fragmented root structures (Sheley et al. 1998).

Spotted knapweed rapidly invades pastures, rangeland, dry meadows, flood plains and roadsides. It thrives in soils that are lightly textured and well-drained (WIDNR, 2004). The tall erect stems are easily swayed by wind and wildlife, which can disperse viable seeds up to one meter away from parent plant (Renz, 2007). Studies have shown spotted knapweed invasions in established and non-disturbed sites (Sheley et al. 1998). Wildlife consumption does account for some dispersal (Story, 2002), though long-distance movement is understood to be the result of human interactions (Renz, 2007). Spotted knapweed densities have been found to have an inverse relationship with species richness, indicating that spotted knapweed has the ability to alter ecosystem structure (Key 1988).

Mechanical control options for controlling spotted knapweed include hand-removal, mowing, and prescribed burning. Small populations can be controlled as long as special caution is taken to remove entire root structure or to sever the caudex (WIDNR 2004). This treatment will need to be repeated annually for several years to ensure depletion of the seed bank. One study found significant reduction in adult densities when a single fall mowing was implemented (Rinella et al. 2001). Other studies indicate that mowing reduced overall biomass, though plants were able to develop viable flowers regardless of the time of year of mowing (WIDNR 2004). Some studies examining integrated management techniques indicate control efforts may be successful when herbicides and fertilizers are used in combination (Jacobs and Sheley, 1999).

Common tansy (*T. vulgare*) is a member of the aster family, native to Europe. It was introduced intentionally as a medicinal herb, and has escaped cultivation and invaded native prairie ecosystems across the United States and Canada (McClay 2009). This species of terrestrial invader produces alkaloids that are toxic if eaten; as a result it is not consumed by native wildlife. Common tansy produces many flowers in an umbel-shaped cluster; the primary method of reproduction is sexual, with pollination occurring though a variety of insects (LeCain and Sheley 2006). A single plant can produce up to 50,000 seeds annually.

New shoots will develop from existing root structures, so thorough removal is required for effective mechanical control (Royer and Dickinson 1999).

Common tansy quickly infests disturbed sites and displaces native vegetation. It is tolerant of a variety of soil types, and thrives in areas of full sunlight. Roadsides, riverbanks, pastures and frequently disturbed sites are susceptible to invasion. Common tansy can out-compete native grass species and reduces forage for wildlife and livestock alike (LeCain and Sheley 2006). Existing management recommendations include mowing existing infested sites multiple times per season in order to prevent the production of new seeds. This method will not prevent annual re-growth (Lane 2008). Hand-pulling of small stands can be effective if root structures are completely removed or destroyed. Proper safety equipment should be used and skin should be covered during control efforts as some reports of poisoning though contact have been reported (LeCain and Sheley 2006). Mechanical control with a follow-up herbicide has been effective (Jacobs 2008); this was supported by experimental trials performed by St. Cloud State University graduate Joe Carlyon (2010).

These two terrestrial invasive plant species have invaded and degraded large areas on Camp Ripley and are thoroughly established. These established populations can serve as source populations, allowing for the dispersal of seeds to adjacent locations within camp boundaries. In order for Camp Ripley to be in compliance with Executive Order 13112, management strategies and techniques need to be in place to control and reduce these invasive populations. A partnership between Camp Ripley and Saint Cloud State University was been established to develop effective control efforts. Participant groups include Minnesota Department of Military Affairs, The Nature Conservancy, Camp Ripley Environmental

Office, Camp Ripley Range Control, Camp Ripley Department of Public works and Saint Cloud State University. Research completed since the beginning of this partnership has produced distribution maps and predictions of future spread rates (Babski 2004), established various herbicide controls for multiple target species (Eisterhold 2008), evaluated integrated management on common tansy infestations including herbicide and prescribed fire in-combination (Carylon, 2010). Recommendations have been made on how to control common tansy and spotted knapweed, though previous work does not address how to effectively restore invaded and degraded areas to healthy native communities.

Historical management of invasive species has focused on control and removal of the targeted species exclusively; studies suggest that management plans should be based on ecological processes and components of native communities in addition to the control of a specific targeted species (Pickett et al. 1987, Sheley et al. 1996, LeCain and Sheley 2006). Long-term management is dependent on the ecological principal of succession as defined by Pickett et al. (1987). Succession is the directional change in the types of vegetative species that occupy a location; it involves the processes of colonization, establishment and extinction.

Ecological Succession

Ecological Succession is the observed change in vegetation composition and community structure over time after some type of disturbance (Connell and Slatyer 1977). Primary succession occurs after a geological event occurs resulting in bare rock; it has been studied after de-glaciation events (Chapin et al. 1994), or after volcanic eruptions (Andersen and Machmahon 1985). Secondary succession occurs on sites that are disturbed (Finegan

1984), resulting in open sites and availability of resources in previously colonized soils, and may be the result of natural or anthropogenic causes.

Connell and Slatyer (1977) examined species composition changes over time and proposed a model that could be used to determine the potential causes of the observed change in species post-disturbance. This model begins with a disturbance resulting in an availability of space and/or resources, and includes three observed patterns of vegetation change over time: facilitation, tolerance, and inhibition. The facilitation mechanism is dependent on the arrival of early successional species which modify the environment to make the site more suitable for later colonizing species. The tolerance mechanism (the order of vegetation change) is determined based on the life-history traits of species present at the site exclusively. The inhibition mechanism is dependent on the arrival of early successional species which utilize available resources, preventing the establishment of later successional species until additional disturbances or removal of the inhibiting species occurs.

Several sequential steps were defined by Connell and Slayter (1977) along each of the succession pathways. The facilitation pattern is dependent on site suitability post-disturbance. Species which first colonize available sites must modify the environment in such a way that makes the site more suitable for later successional species; late successional species which arrive on-site after a disturbance will not establish until after site conditions have been modified. This pattern of vegetation change is commonly observed in locations with harsh environmental climates or after geological events that result in primary succession (Zanini et al. 2006). A study of primary succession after a de-glaciation event was conducted (Chapinet al. 1994), which found that late successional species were not able to establish on the study site until after large amounts of nutrients, specifically nitrogen and phosphorous, had been deposited onto exposed surfaces by earlier successional species. Facilitation may be driven by many different factors include community composition, protection from predation, increased pollination, increased dispersal, or be impacted by the mycorrhizal, microbial and vegetative use of nutrients within the community (Baumeister and Callaway 2006). Facilitation is observable pattern of change in vegetation that occurs when early successional species modify the disturbed site resulting in the establishment of later successional species.

An additional interaction, described as tolerance, has been observed in vegetation changes over time and includes the arrival of species post-disturbance that do not directly impact the structure of the community, except due to life history traits (Connell and Slayter 1977). Early arrival species are observed post-disturbance due to quick growth rates and high reproduction; over time, different species are observed due to longer time to maturation, and slower rates of reproduction. Late successional species are able to grow in the presence of populations of early successional species without consequence, whereas early succession species cannot survive on sites with later successional species, usually due to competition for limiting resources. One strong example of tolerance is observed in forest ecosystems in which disturbance opens space in both the canopy and the forest floor. Finegan (1984) explains that the species which establish quickly post-disturbance tend to be quick growing species and cannot develop in the shade. Their development and growth casts shade onto the forest floor, in which new individuals of the species cannot survive. Once the available sunlight has been utilized, shade intolerant species will not be able to grow whereas shade-tolerant species are able to continue to develop and mature. Over additional time post-disturbance, the vegetation community will move from shade-intolerant to shade-tolerant species in structure.

The inhibition mechanism of succession includes the arrival and establishment of a species which modify the environment so that it is less suitable for all species, both early and late successional species (Connell and Slayter 1977). Only removal of the established species or additional disturbances may result in future vegetation community change. One example of species inhibition occurs when propagules from invasive species are present on a site at the time of disturbance. Spotted knapweed produces allelopathic chemicals that prevent the germination and development of other species. Over time, the increase in allelopathic chemicals in the soil can result in a monoculture of spotted knapweed. In this case, the continual presence and growth of spotted knapweed will halt successional change until additional disturbances occur or until the species which is causing the inhibiting effects are removed (Sheley et al. 1996).

Each of the mechanisms above have generally been accepted and adapted into many forms of successional research from temperate rangeland (Sheley et al. 1996) to subtropical fields (Zanini et al. 2006). These three patterns of succession describe observable changes between variable species and the environment, but offer little predictive value as they are based on the presence or absence of species post-disturbance, the life history trails of the species and the possible interactions of a species with the site. It does not include any information regarding the degree of disturbance, causality or the influence of intra-species interactions (Pickett et al. 1987).

These patterns of vegetation change provide information to the ultimate cause of vegetation changes, but do not offer much value in determining proximate causation. The three patterns of observed vegetation change are not mutually exclusive, nor do they provide much predictive information, making them difficult to incorporate into testable hypothesis (Pickett et al. 1987). Some of the specific critiques for the Connell and Slayter model by Pickett (1987) include the observation that a larger variety of patterns of vegetation change have occurred in successional studies than are accounted for in the succession model, as well as the original presentation of these patterns as mutually exclusive within a system. In the Connell and Slayter model, each pattern of vegetation change is reinforced by the vegetation change that follows in the sequence. In natural systems, a large number of each of the described patterns of vegetation change may be occurring between all the species present in an area post disturbance, resulting in infinite possible interactions, all which can impact successional change. The representation of these changes in the patterns of vegetation as community-wide and mutually exclusive has led to over-simplification of species interactions which does not account for all possible succession causes (Pickett 1987).

Pickett (1987) describes situations in which the Connell and Slayter models of succession are not complete. For example, a succession may exhibit several mechanisms more than a single pattern of vegetation change, different mechanisms of replacement may act over time, or a single species may participate in several mechanisms depending on the scale and scope of the succession being studied. As a result of the wide array of possible issues with the terms facilitation, tolerance and inhibition have when used to explain vegetation change over time, Pickett recommends preserving the use of those vocabulary terms when describing particular interactions and species-based replacements.

Due to some of the confusions associated with succession observations and research, Pickett (1987) proposed a new framework for studying succession which allows for the incorporation of historically held concepts from Clements (1916) and Connell and Slayter (1977) into testable hypothesis. In order to begin building a framework for studying succession in a way that is scientific, testable, and applicable to many different ecological situations, Pickett recommends working from a top-down hierarchy of causation, which allows for clarification across the entire discipline.

At the large-scale, widely applicable level, Pickett posed the question, “What causes succession?” Succession, regardless of the specifics of a site, is driven by the availability of open sites, the species which are available at the site, and how the species perform in relation to additional species on a site. These answers provide categories to build off of when attempting to examine site-specific interactions. The next level of hierarchal consideration examines the interactions, processes, or conditions which contribute to the core causes of succession; examples of the second level of hierarchy include ecological occurrences which illuminate the range of potential processes that effect vegetation dynamics. The third and final level of hierarchal considerations includes mechanistic level and site specific interactions.

This framework of successional studies defines a level of impact that each of the three primary causes of successional change, as they are not mutually exclusive. For example, the size and intensity of an initial disturbance can impact the survival of propagates, deter regeneration, and impact the availability of open sites. In this example, the disturbance has an effect on the availability of sites, the availability of species as well as the performance of species within the site.

Changes in species composition are dependent on three interacting factors: availability of open sites, availability of species, and the performance of the species within an area. Disturbances are recognized as a major influence on site availability as they affect communities and typically result in native species compositions similar to an earlier phase of succession (Larson 2003). Disturbances are defined as any event or action that results in heterogeneity in the ecosystem; heterogeneity may exist in the form of population dynamics, resource availability, community structure, or physical space (Pickett et al. 1987, Larson 2003, Sheley et al. 1996, Sheley et al. 2006). Colonization post-disturbance is dependent on species availability (seed dispersal from existing plants) and species performance. Species performance refers to the overall growth and reproduction of a species, and is dependent on the site, climate growth rates, allopathic tendencies, competition, and predation (Sheley and Krueger-Mangold, 2003).

In 2006, Sheley et al. conducted a four-year study testing successional theory and its ability to successfully restore invaded areas. Treatments included combinations of seeding methods, seeding rates, cover crop application and herbicide application. The results indicated 1) any combination of treatments is more likely to reduce targeted species percent cover more than any singular treatment, 2) drill seeded species experienced higher establishment, but only during the first two growing seasons, 3) disturbances that minimize nutrient release, while providing a site for seed germination would favor native species, and 4) all herbicide treatments decreased native forb density while increasing exotic-grass components. This work was done focusing on exotic invaders spotted knapweed (*C. stobe*) and sulpher cinquefoil (*Potentilla recta* L.).

This project will be evaluating the effectiveness of several combinations of terrestrial invasive species management techniques for reducing the percent cover of spotted knapweed and common tansy, as well as determining treatment influences on the diversity of vegetative species. This study will determine what combination of management techniques results in the most effective decrease of percent cover in targeted species post-treatment, while monitoring vegetation compositional changes. The experimental design includes manipulation of site availability, species availability, and species performance. Techniques altering site availability are experimentally referred to as 'seedbed preparations', which include a combination of mowing, herbicide application and tilling. Species availability is manipulated by the introduction or absence of a cover crop, as well as seeding strategy of native grass species. Species performance was manipulated with the application of a selective broad-leaf herbicide. The project was implemented during the 2010 and 2011 growing seasons. Initial results indicated the only significant difference between treatments was the application of a selective broad-leaf herbicide (Hanson 2011).

Continued data collection and analysis on the experimental plots will uncover relationships which can help assess the effectiveness of any specific successional management technique. Hypothesis for this study predict 1) seedbed preparation utilizing mowing and chemical application will reduce target invasive percent cover to a higher degree than other seedbed preparations, 2) the introduction of a competitive cover crop immediately post-disturbance, followed by a native seeding will reduce invasive percent cover more dramatically than without the introduction of a cover crop, and 3) plots which received a selective herbicide treatment will have a lower percent cover of targeted invasive species than plots that did not receive the treatment.

Methods

Study Site

Camp Ripley is located in Morrison County in central Minnesota. It is composed of 21,448 hectares and falls within the transition zone between Eastern Broadleaf Forest and Laurentian Mixed Forest (lat. 46.2N, long. 94.3W). Camp Ripley is bordered on two sides by river systems; Mississippi River to the east, and Crow Wing River to the north. According to the Minnesota Department of Natural Resources (2011) three subsections are represented within Camp Ripley borders; Hardwood Hills, Anoka Sand Plain, and Pine Moraines and Outwash Plains. Grasslands at Camp Ripley are characterized by well-drained, sandy, slightly acidic soils. Historical data indicate that the native plant community was that of an Upland Prairie System Southern Dry Prairie and is described as grass-dominated community occurring on level to sloped sites, with moderate precipitation deficits, severe droughts are infrequent with occasional fires (MNDNR 2011). Camp Ripley has a large variety of species present due to the ecosystem variability within its borders; it is home to than 560 species of plants, and 50 species of mammals (MNDoD 2008). Targeted invasive species currently infest areas on Camp Ripley that are composed of open grasslands, remnant prairies, and oak-savannah habitats.

Targeted Species

Spotted knapweed (*Centaurea stobe*)

Family: Asteraceae

Spotted knapweed is a biennial or short lived perennial herb, growing from 20 to 120 centimeters tall. Stems are erect and rough, with one or more shoots from a central tap root;

individuals develop many slender branches smooth to scabrous (Story 2002). Leaves develop alternately, ranging from 2.5 to 7.6 cm long, pale green to grey in color. The lower portion of leaves possess a rough surface, and deeply divided margins on both sides of central vein. Leaves present on the upper portion of the plant can be thin, with linear venation (CDFA 2009). Flowers are thistle-like, solitary heads located at the tips of branches and are light pink to purple in color; petals are surrounded by dark, stiff bracts with thin light streaks and dark fringe on apex edge (Renz 2007). The bract structure and color gives the appearance of spots, thus explaining the name ‘spotted knapweed’ (CDFA 2009).

Spotted knapweed tends to flower from June through October. The primary reproductive strategy is seed dispersal with each plant producing over an average of 1,000 seeds per plant. The fruits produced are achenes, ranging from 2 to 3 millimeters in diameter (Sheley et al. 1998). Seeds remain viable in the soil for more than seven years (Davis et al. 1993). New shoots develop from existing taproot annually.

Seed germination occurs in early spring through fall. Over wintered rosettes begin growth in early May and produce one to twenty stems. A large portion of stem development occurs in June. Flowering continues throughout summer and into fall (defined as late June through August) (Renz, 2007; CDFA 2009; Sheley et al. 1998).

Spotted knapweed is an aggressive plant that rapidly invades pastures, rangeland, dry meadows, flood plains, roadsides and any other dry, gravely or sandy locations. This species tends to grow in soils that are lightly textured, well drained, and receive summer precipitation, though spotted knapweed can tolerate a range of moisture regimes (Sheley et al. 1998).

At maturation, the central herbaceous stem becomes hardened which allows for a large dispersal area. Wildlife and wind can cause the stems to sway. After seeds are fully developed this swaying action causes the seeds to drop out of the head and onto the ground (Renz 2007). Small mammal and bird consumption does allow for some seed dispersal (Story 2002); Long distance dispersal is likely the result of human movement as seeds become attached to vehicles or clothing when individuals travel through infested sites (Renz 2007).

Common Tansy (*Tanacetum vulgare*)

Family: Asteraceae

Common tansy is a perennial herb, erect shape ranging from .5–1.5 meters tall. Stems grow singularly with several alternate leaves that are pinnately compound ranging from 10 to 20 centimeters long by 5 to 10 centimeters wide. The leaves are green, irregularly lobed, finely toothed, and fern-like. Button-like rayless flowers are arranged in tight umbels at the apex of each stem. Individuals produce 20-200 flower heads per plant, each .1.25 cm wide and bright yellow in color.

Common tansy reproduces via viable seeds and rhizomatic regrowth. Each plant can produce up to 50,000 seeds per year (Royer and Dickinson 1999; Whitson *et al.*, 2000). New shoots will develop from existing root structures and fragments. Stem growth occurs in mid-spring resulting in meter-tall plants by June. Flowers bloom in summer and seeds are developed by later summer through fall (July through October). Seeds depend on a period of stratification in order to germinate (Jacobs 2008).

Common tansy quickly infests disturbed sites and displaces native vegetation. It is tolerant of most soil types and prefers areas that are exposed to full sun. Roadsides, stream banks, pastures, and open disturbed areas are likely areas of infestation. Common tansy is an ecological threat due to its ability to out-compete native grass species and reduce forage for wildlife and some livestock (LeCain and Sheley 2006).

Experimental Design

A randomized complete block design was used containing 32 unique treatments, creating a 4x2x2x2 design (Figure 1). Four replicates have been established for both targeted species, resulting in eight blocks. Each individual plot received one of four seedbed preparations, one of two cover crop treatments, one of two seeding methods, and one of two herbicide treatments. The first three factors were used to assign each treatment a unique number 1-16. These numbers were assigned to a plot via a random number generator (See Figure 2 for an example of final research block design). The northwest portion of each of the 16 treatment areas were treated with a fourth factor, a selective broad-leaf herbicide, Milestone™. Each unique observation is 10x10 meters with a three meter buffer zone between treatments to prevent treatments from influencing closely located sites. The final size of each block is 107 meters by 49 meters. Blocks were delineated in March 2010, and plot locations were recorded with a hand held GPS. Individual plots were marked with rebar stakes to preserved boundary locations. Colored duct tape was applied to each rebar based on the seedbed preparation method applied. This was done to prevent confusion during data collections. All blocks are located within a relatively small area in order to limit the impact land use and soldier training may have on experimental trials. Only sites with an initial percent cover above fifty percent invasive species cover were used for study locations.

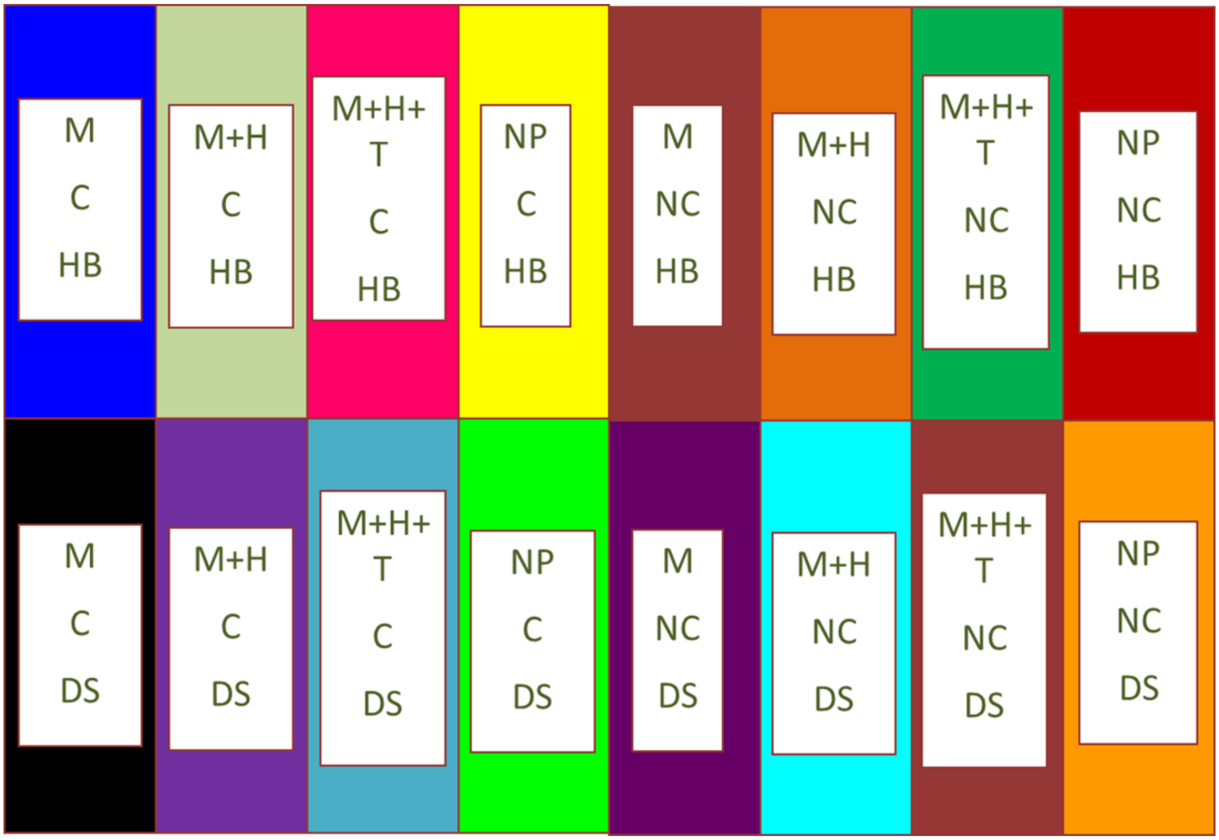

Figure 1. A randomized complete block design (4x2x2). Each individual treatment will have one of four seedbed preparations (Mow, Mow/Herbicide, Mow/Herbicide/Till, or no treatment), one of two cover crop types (Canada wild rye or no cover crop), and one of two distribution methods (drill seed or hand broadcast). M=Mow. H=Herbicide. T=Tilling, NP= No preparations. C=Cover Crop. NC=No cover crop. HB = Hand broadcast. DS=Drill Seeded. Original graphic by Jamie Hanson (2011).

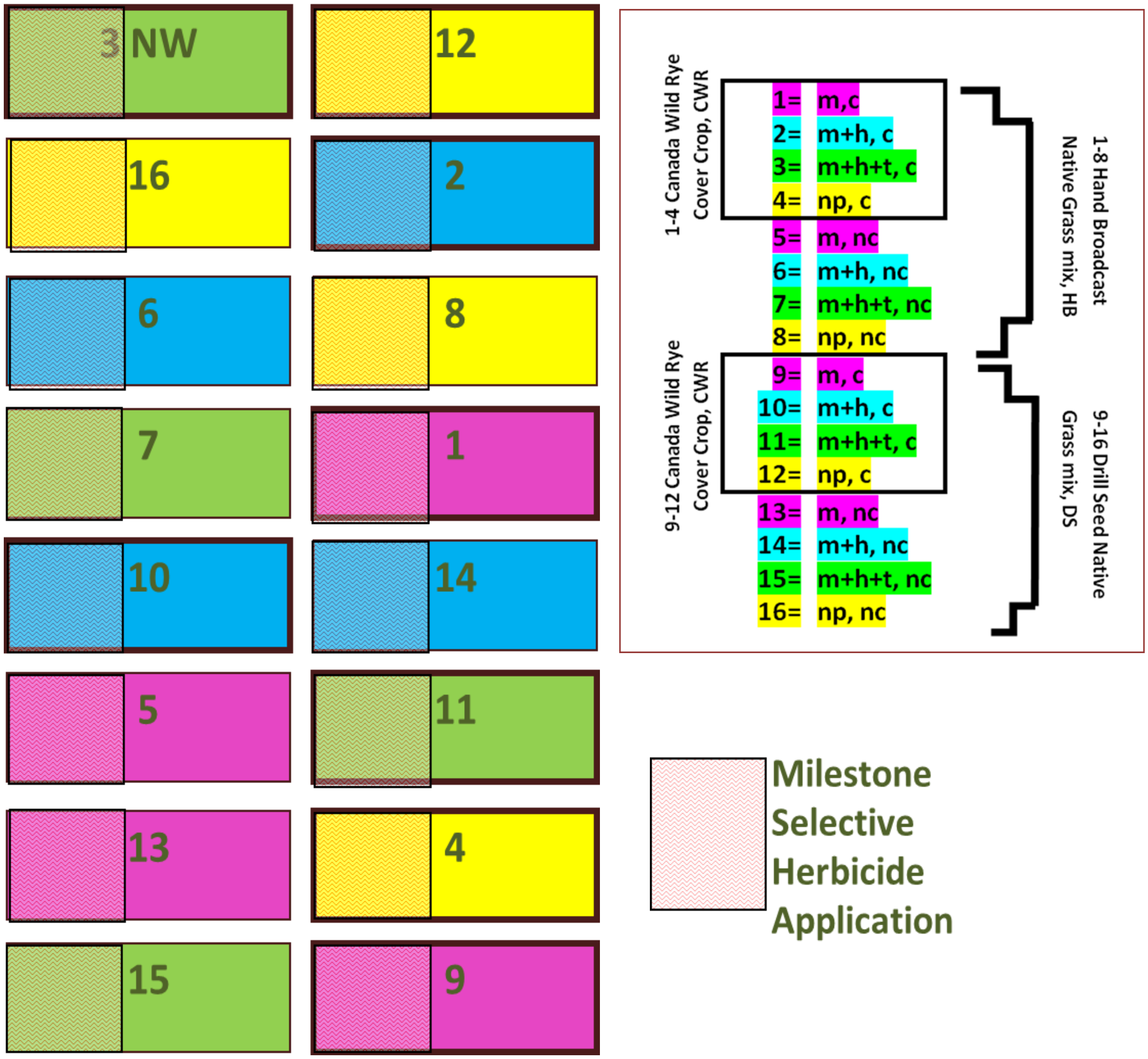

Figure 2: Each unique treatment combination was assigned a number and randomized before plots were established. Plot colors refer to seedbed preparation. Each unique number refers to a treatment combination pertaining to seedbed preparation, cover crop treatment and native grass dispersal method. The fourth factor, Milestone, was applied on the Northwest portion of each treatment plot. This is represented with pink shading. Original graphic from Jamie Hanson (2011).

Treatment and Controls

All thirty-two treatment plots received a unique treatment combination. Each plot was treated with one of four seedbed preparations: 1) mowing, 2) mowing and herbicide application, 3) mowing, herbicide application and tilling, or 4) no seedbed preparation. All equipment was provided by the Camp Ripley Department of Public Works. Herbicide application used active chemical glyphosate (commercial name RoundUp Pro) at 1.5% solution, applied with a mounted John Deere Gator sprayer tank with a one meter boom unit. Tilling was done with a rear-mount roto-tiller. Seedbed preparations were conducted by Jamie Hanson and Kayla Malone from May 17 to June 11, 2010.

The application of a cover-crop immediately seeded post-disturbance is also being evaluated. Plots received one of two treatment options; 1) cover crop of Canada Wild Rye (*Elymus canadenis* L.), drill seeded at a rate of 11.2 kg per hectare (10 pounds per acre) or 2) no cover crop. Cover crop implementation occurred June 16-22, 2010. Equipment used was a Tru-ax drill seeder, which deposited seeds at a soil depth of six centimeters. Treatments that did not receive the cover crop will serve as the control for this variable. The cover crop was implemented in late June 16-22, 2010.

Two dispersal methods of native grass species are being evaluated. Plots received one of two treatments; 1) hand broadcast, or, 2) no-till drill seeding. Drill seeding was done with a2.44 meter-wide, three-point mount Tru-axe drill seeder at a depth of 1.3 centimeters. Native seed mix contained 8 recommended prairie species; big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), Indiangrass (*Sorghastrum nutans* (L.) Nash), side oats grama (*Bouteloua curtipendula* (Michx.)Torr.), switch grass (*Panicum virgatum* L.), kalm’s brome (*Bromus kalmia* A. Gray), june grass (*Koeleria macrantha* (Ledeb.) Schult.), and sand drop seed (*Sporobolus cryptandrus* (Torr.) A. Gray). Hand broadcast seeding was done at the rate of 14.6 kg/ hectare, and drill seeding at a rate of 11.2 kg/ hectare. The species, seed mix proportions, and rate of distribution were based on recommendations by Prairie Restoration Inc. catalog. The native seeding was implemented October 13-14, 2010.

A chemical application one year post-treatment was al also tested to determine effects of selective herbicide. Plots received one of two treatment options 1) application of selective broad-leaf herbicide Milestone or 2) no Milestone treatment. Milestone was applied at maximum label rate of 551.54 ml/hectare via gator-mounted one meter, three-point fan boom spray. Milestone was applied to all treatment areas on May 25-26, 2011.

Research plots were closed to all training activities and included the establishment of a research area buffer that was not utilized by military personnel. Annual maintenance procedures were implemented to preserve boundaries between treatment combinations; maintenance included mowing of buffer zones twice, as well as the straightening of the corner rebar stakes as necessary. The colored duct tape indicating treatment combination was also replaced annually due to fading caused by continuous exposure to the weather.

Data Collection

Data collected during the 2010, 2011, 2012, and 2013 growing seasons included; senescent stand percent cover, emergent percent cover, and grass and forb species diversity counts. Data collected are consistent each year to ensure observations are comparable.

Senescent stand percent cover estimates were taken in early spring immediately after snow-melt; senescent percent covers are representative of the previous year’s growth, as the remaining above-ground biomass is being measured. Estimates were taken in two one-meter square quadrant locations placed in the northwest and southeast corners of treatment plots.

Visual estimates of senescent target invasive percent cover were recorded; native vegetation and bare ground cover were not recorded.

Emergent stand percent cover estimates were taken using two one-meter quadrant locations in the northwest and southeast corners of each of the plots. Data collections for emergent percent cover occurred thirty days after senescent stand evaluations. These locations are consistent with the locations of the previous year’s emergent percent cover. This was done to ensure the ability to compare an individual treatment to an earlier stage and will be used to analyze change over time.

Peak flower percent cover estimates were taken using two one-meter quadrant locations in the northwest and southeast corners of each of the plots. Data collections for peak flower percent cover occurred thirty days after senescent stand evaluations. These locations are consistent with the locations of the previous year’s percent covers.

Species diversity data were recorded by randomly placing one one-meter quadrant within each of the plots. All species within the quadrant were identified, counted and recorded. Species identification is crucial to be able to compare differences between forb and grass reactions to treatment effects. This was completed annually during the first week of August.

Analysis

All research plots received one of each of four variables; seedbed preparation (4 different methods), cover crop (2), native seed dispersal method (2), and the application of milestone post-treatment (2). The measurements of percent cover, collected in the northwest and southeast corners of research plots were averaged to produce a single value for each record. Biodiversity data from 2013 were analyzed to determine significant effects. Analysis were conducted to determine the significant effects of any single treatment, as well as to determine the effect of interactions occurring within the research plots.

A Repeated Measures Analysis of Variance was run for each variable separately over time to determine the effect on targeted species percent cover. This analysis was run to determine the effects seedbed preparation methods, cover crop, native seeding dispersal method, and application of selective herbicide Milestone® on targeted species percent.

One Way Analysis of Variance were run for each variable separately using 2013 species count data to determine the effect of seedbed preparation method, cover crop, milestone application, and native seeding method on total number of vegetative species recorded in a sample.

This analysis was completed separately for each target invasive species, spotted knapweed and common tansy, to determine differences between species responses, as well as on all 2013 biodiversity data. Previous statistical analysis, conducted by Jamie Hanson included Univariate Analysis of Variance.

Results

Analysis conducted by Jamie Hanson (2011) included Univariate Analysis of Variance (ANOVA) on data collected during the 2010 and 2011 growing seasons, which demonstrated only one significant treatment effect; Milestone application. This effect was significant for targeted species spotted knapweed (F (1,129) = 56.258, $p<.05$). All other treatment combinations did not have a significant effect one year post-implementation.

Spotted Knapweed

Percent cover. In order to determine the effects of each seedbed preparation method on percent cover of spotted knapweed, independent of other factors, repeated measures ANOVA was used on data collected from 2012 and 2013. Mauchlys Test indicated that the assumption of sphericity had been violated $X2(14) = 170.00$, $p <.05$, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .58). Using the Test of Within-Subjects Effects, a significant relationships between percent cover over time were detected F (2.88, 8.65) = 34.248, $p > .05$. There is no significant effect of seedbed preparation method on percent cover of spotted knapweed over time.

In order to determine if the application of a cover crop had a significant impact on spotted knapweed percent cover, repeated measures ANOVA was used on data collected in 2012 & 2013. Mauchlys Test indicated that the assumption of sphericity had been violated $X2$ (14) = 171.2, $p <.05$, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .578). Using Greenhouse-Geisser test of within subjects indicates a change in percent cover over time, F (2.89, 2.89) = 34.01, $p > .05$. No significant difference in percent cover of spotted knapweed was found between plots that received a cover crop and plots that did not receive a cover crop *F* (1,126) = .25, p>.05.

A significant difference in percent cover of spotted knapweed was found between plots that did receive a Milestone treatment and plots that did not. Mauchlys Test indicated that the assumption of sphericity had been violated $X2$ (14) = 110.38, p <.05, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .70). Using Greenhouse-Geisser test of within subjects indicates a change in percent cover over time, *F* (3.48, 3.48) = 46.79, *p* > .05. Between subject effects indicate a significant reduction in percent cover of spotted knapweed in plots that did receive a Milestone® treatment when compared to plots that did not *F* (1,126) =215.51, p>.05.

No significant difference in the percent cover of spotted knapweed was found between plots that were hand broadcast and plots that were drill seeded with native grass species. Mauchlys Test indicated that the assumption of sphericity had been violated $X2$ (14) = 169.57, p <.05, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .58). Using Greenhouse-Geisser test of within subjects indicates a change in percent cover over time, *F* (2.90, 2.90) = 33.82, *p* > .05. No significant difference in percent cover of spotted knapweed was found between plots that and plots that did not receive a cover crop *F* (1,126) = .13, ns.

Statistical analysis of percent cover of targeted species data collected from 2012 and 2013 indicate a significant effect when considering the use of selective herbicide Milestone®. The seedbed preparation method did not have a significant impact on the percent cover of spotted knapweed. The use of a cover crop of Canada wild rye did not have a significant influence on targeted species percent cover for spotted knapweed research blocks. The method of native seed dispersal did not have a significant main effect on the percent cover of targeted species spotted knapweed.

Biodiversity. In order to determine the effects of each seedbed preparation on biodiversity of the spotted knapweed research blocks, independent of other factors, a Univariate ANOVA was used on data collected from 2013. A relationship between seedbed preparations and biodiversity was found, $F(3, 128) = 8.11$, $p = 0005$. Tukey's HSD post hoc tests revealed that plots with treatments including mowing, non-selective herbicide and tilling had a significantly higher biodiversity when compared to plots with no seedbed preparation ($p = .0005$) and seedbed preparations including mowing only ($p = .001$) See Table 1 for means and standard deviations.

Table 1. Mean spotted knapweed species counts and standard deviation including plots that received a Milestone treatment.

Seedbed preparation	Mean	Standard Deviation
No Preparation	6.41	2.31
Mow	6.78	2.49
Mow, Herbicide	7.97	2.99
Mow, Herbicide, Till	9.59	3.47

No significant difference in total species number was found between plots that received a cover crop ($M = 7.7031$, $SD = 3.24003$) and plots that did not receive a cover crop ($M = 7.67$, $SD = 2.94$), $t(126) = .06$, $p = .96$.

No significant difference in biodiversity was found between plots that received hand broadcast ($M=7.6094$, $SD = 3.19035$) and plots that received drill seeded native seed dispersal method ($M = 7.7656$, $SD = 2.99068$) $t(126) =-.29$, $p = .76$.

A larger number of species were recorded in plots that did not receive a Milestone® treatment (M = 8.45, SD = 3.29) when compared to plots that did (M = 6.92, SD = 2.66) $t(126) = 2.89$, $p = .005$.

Statistical analysis of species count data collected from 2013 indicate a significant effect when considering the seedbed preparation method, or the use of selective herbicide Milestone® one year post-treatment. Seedbed preparation methods including mow, non-selective herbicide, and till had significantly higher species compared to any other seedbed preparation method. Milestone® significantly reduced species in spotted knapweed blocks. The use of a cover crop of Canada wild rye did not have a significant influence on diversity for spotted knapweed research plots. The method of native seed dispersal also did not have a significant main effect on the diversity of spotted knapweed plots.

Common Tansy

Percent cover. In order to determine the effects of each seedbed preparation on percent cover of common tansy, independent of other factors, a Repeated Measures ANOVA was used on data collected from 2012 and 2013. Mauchlys Test indicated that the assumption of sphericity had been violated $X2(14 = 117.89, p <.05)$, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .68). Using the Greenhouse-Geisser Test of Within-Subject Effects, the effect of seedbed preparations was found for percent cover of common tansy over time, $F(3.411, 10.234) =53.77$, $p = .000$. In a test of Between-Subjects Effects a significant difference between seedbed preparation methods $F(3)=5.71$, $p <.05$. Tukeys HSD post-hoc test reveals that sites receiving a combination of mow and non-selective herbicide demonstrated a significantly higher percent cover from sites with no prep; mow only; and sites with mow, herbicide, and tilling.

No significant difference in percent cover of common tansy was found between plots that included a cover crop and plots that did not include a cover crop. Mauchlys Test indicated that the assumption of sphericity had been violated $X2$ (14) = 132.86, p <.05, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .66). Using Greenhouse-Geisser test of within subjects indicates a change in percent cover over time, F (3.28, 3.28) = 51.39, p > .05. No significant difference in percent cover of common tansy was found between plots that included a cover crop and plots that did not include a cover crop F (1,126) = .02, *ns*.

No significant difference in the percent cover of common tansy was found between plots received hand broadcast and plots that received drill seeded native seed dispersal. Mauchlys Test indicated that the assumption of sphericity had been violated $X2$ (14) = 137.19, p <.05, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .65). Using Greenhouse-Geisser test of within subjects indicates a change in percent cover over time, F (3.25, 3.25) = 51.69, p > .05. No significant difference in percent cover of common tansy was found between plots that were drill seeded and plots that were hand broadcast native species F (1,126) = .68, ns.

A significant difference in percent cover of common tansy was found between plots that did not receive a Milestone® treatment and plots that did. Mauchlys Test indicated that the assumption of sphericity had been violated $X2$ (14) = 128.11, p <.05, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (e = .65). Using

Greenhouse-Geisser test of within subjects indicates a change in percent cover over time, *F* (3.27, 3.27) = 54.02, *p* > .05. Between subject effects indicates a significant decrease in percent cover of common tansy was found in plots that did receive a Milestone® treatment compared to plots that did not receive a Milestone® treatment *F* (1,126) =52.32, p>.05.

Biodiversity. In order to determine the effects of each seedbed preparation on biodiversity of the research plots (independent of other factors) a Univariate ANOVA was used on data collected from 2013. The main effect of seedbed preparations was found for biodiversity F(3, 128) = 22.10, p = 000. Tukey's HSD post hoc tests revealed that plots with seedbed preparation treatments including mowing, non-selective herbicide and tilling had a significantly higher biodiversity, (p<.001) when compared to any other seedbed preparation method tested. Sites with no preparation demonstrated significantly lower biodiversity than plots that received mow and non-selective herbicide seed bed preparations (p=.003), and plots that received mow, non-selective herbicide, and tilling seedbed preparations (p=.000). See Table 2 for mean and standard deviations.

Table 2. Mean common tansy species counts and standard deviations including plots that received a Milestone treatment.

Seedbed preparation	Mean	Standard Deviation
No Preparation	4.59	2.03
Mow	5.06	1.95
Mow, Herbicide	6.53	2.34
Mow, Herbicide, Till	8.59	2.31

No significant difference in total species number was found between plots that received a cover crop (M = 6.1563, SD = 2.79579) and plots that did not receive a cover crop (M = 6.23, SD =2.52), t(126) = -.17, p = .868 in common tansy research blocks.

No significant difference in biodiversity of targeted species was found between plots received hand broadcast (M=6.08, SD = 2.59) and plots that received drill seeded native seed (M = 6.31, SD = 2.72) t (126) = -.50, p = .62.

No significant difference in total species was found between plots that did not receive a Milestone® treatment (M = 6.52, SD = 2.42) and plots that did (M = 5.88, SD = 2.85 t (126) = 1.37, p = .17.

Statistical analysis of species count data collected from 2012 and 2013 indicate a significant effect when considering the seedbed preparation method. The use of a cover crop of Canada wild rye, the method of native seed dispersal, and the application of Milestone® did not have a significant influence on diversity for common tansy research blocks.

Common tansy plots demonstrated a significant increase in total number of species present in a sample as the total amount of seedbed preparation increases. Sites with no seedbed preparation had the lowest mean diversity; this was significantly lower than plots with a combination of seedbed preparations including mow and non-selective herbicide or mow, non-selective herbicide, and till. Plots that received a seedbed preparation method that combined mowing, non-selective herbicide application and tilling significantly increased total species compared to any other method of seedbed preparation.

Discussion

The primary objective of this study was to determine the impact combinations of commonly used land management techniques have on the percent cover of two targeted species, and the influence these treatments may have on vegetative diversity.

Seedbed preparation includes one of four possibilities; no preparation; seedbed preparation of a single early season; seedbed preparation including early season mow and non-selective herbicide treatment; or a seedbed preparation including early season mow, non-selective herbicide, and a mid-season till. An additional variable, the use of a cover crop, was assessed and plots received one of two possible treatments; the application of native grass Canada wild rye post seedbed preparation; or no application of Canada wild rye post seedbed application. A third variable was tested which included one of two methods for native seed dispersal; hand-broadcast or drill seeded. After observing unexpectedly high success of targeted species one year after initial experimental manipulation, an additional variable was tested with all previously completed experimental plots; the application of selective herbicide Milestone.

Milestone®

Treatments including selective herbicide Milestone® effectively reduced the percent cover of spotted knapweed when comparing the means of plots that receive an application of Milestone® to plots that did not receive a Milestone® application. On plots that received a Milestone® treatment an average percent cover of .5% was reported, with numerous plots that had no measured spotted knapweed. See Figure 3 for a comparison of mean percent cover of plots that were treated with selective herbicide Milestone® and plots that were not treated with selective herbicide Milestone®.

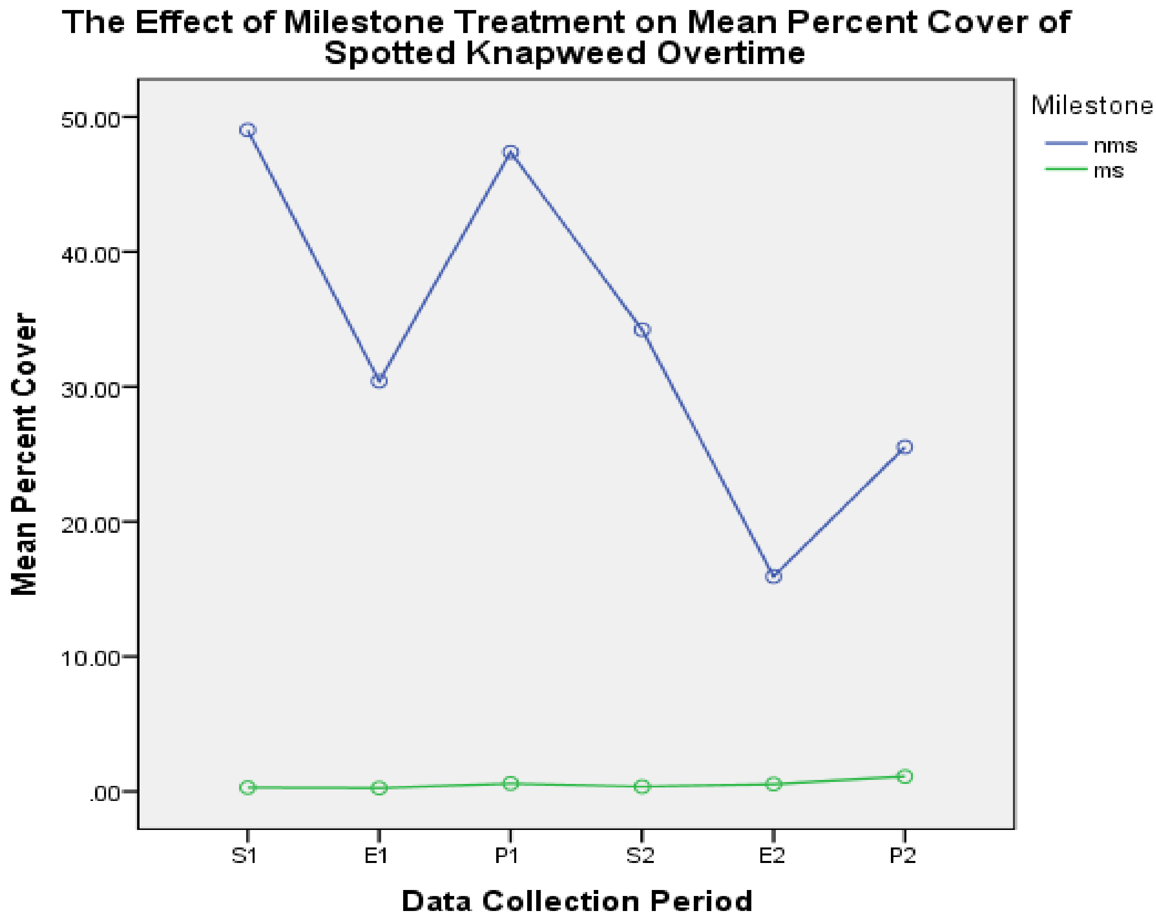

Figure 3: A comparison of spotted knapweed plots which were treated with selective herbicide Milestone® ("ms"= Milestone® treatment) and plots that were not treated with selective herbicide Milestone® ("nms" = No Milestone® treatment) over the data collection time periods (S1=Senescent stand data 2012; E1=Emergent stand data 2012; P1=Peak flower data 2012; S2=Senescent stand 2013; E2=Emergent stand data 2013; P2=Peak flower data 2013)

The use of selective herbicide also significantly reduces the total number of species identified within spotted knapweed plots, resulting in an average difference of two less species counted in Milestone® treated plots. If total species number, regardless of species identification, is desired as part of land management goals, then the use of a Milestone® treatment may be counter-productive. Caution should be used when applying Milestone® to undisturbed or desirable sites where a loss of diversity may not be acceptable. Alterations on the methodology of chemical application may affect the degree of loss of biodiversity. For example, a specific spot application of active chemical to existing individuals may decrease the loss of diversity on a site, as opposed to broadcast application as was used in this experiment. Many studies exist testing the benefit of variable herbicide concentrations and the effects on biodiversity (Laufenberg et al. 2005), though no studies exclusively examining the influence application method has on diversity of treated sites; additional experimental trials testing variable application methods and their impact on total species counts would be needed to confirm this.

Common tansy research plots that received a Milestone® treatment also demonstrated a significant reduction in the percent cover of common tansy with a mean of 9.52 percent. See Figure 4 for a comparison of the percent cover common tansy differences between plots that did and did not receive a Milestone® treatment.

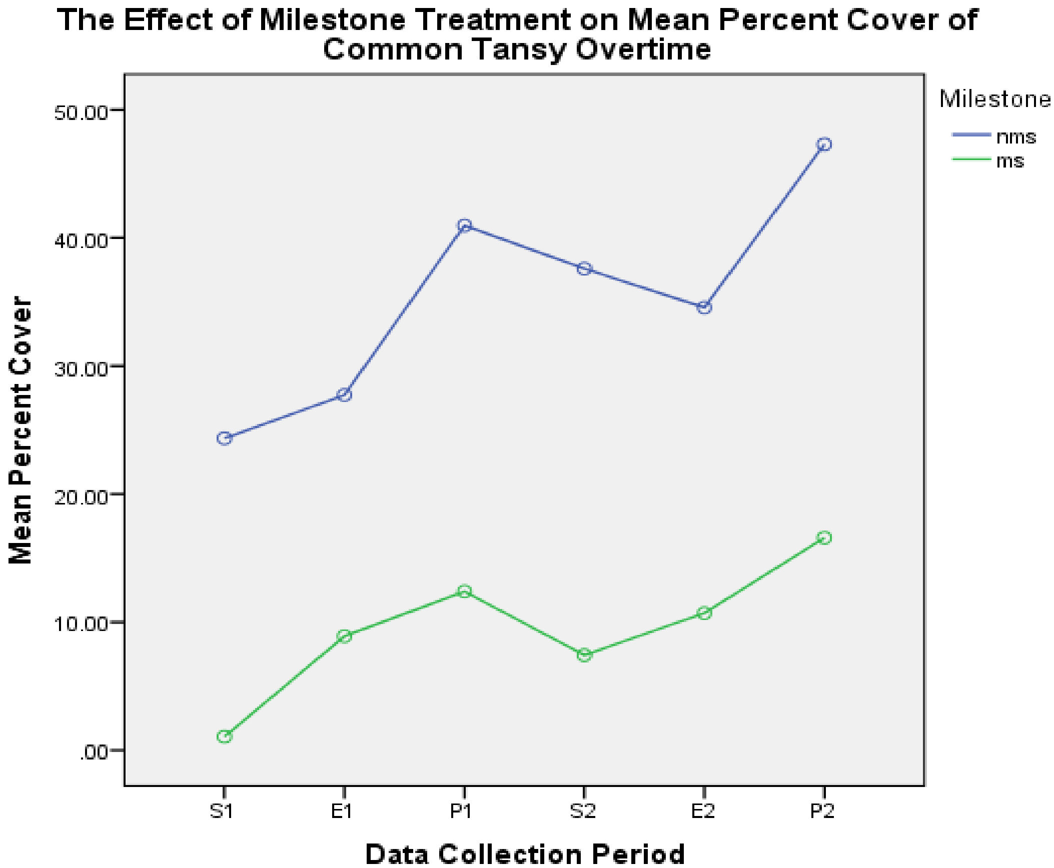

Figure 4: A comparison of common tansy plots which were treated with selective herbicide Milestone® ("ms"= Milestone® treatment) and plots that were not treated with selective herbicide Milestone® ("nms" = No Milestone® treatment) over the data collection time periods (S1=Senescent stand data 2012; E1=Emergent stand data 2012; P1=Peak flower data 2012; S2=Senescent stand 2013; E2=Emergent stand data 2013; P2=Peak flower data 2013)

Milestone's® active ingredient is Aminopyralid, which is a plant growth regulator. It binds at specific receptor sites and causes uneven cell division and growth. This results in death of susceptible species. Due to the specific structure of the chemical, only targeted groupings of plant that contain the specified receptors are highly susceptible to the effects of treatment (Dow AgroSciences 2010). Spotted knapweed cells contain high numbers of these receptors, making this species highly susceptible. Common tansy does not have the same occurrence of the specified receptors, and is not listed as species that is effectively controlled by this herbicide. This is reflected in these experimental trials as a higher rate of emergence was detected for common tansy when compared to the emergence of spotted knapweed. Over time, the effectiveness of Milestone® on common tansy sites is likely to continue to decrease.

A chemical application of Milestone® directly affects two of the three factors which influence successional change; the performance of species within a site, the availability of a species within a site. Milestone® has been proven to be effective at controlling only some specific vegetative species for multiple consecutive years, particularly aster species which includes common problem and invasive species such as Canada thistle, ragweed species, and targeted species spotted knapweed (MSDS label). An application of Milestone® selectively inhibits the growth of specific targeted species, and may hinder the performance of additional non-target species within a site. Additional measurements would be necessary to quantify the impact Milestone® has on non-targeted species performance. Some additional measures which could indicate change in non-targeted species performance on a site are time-intensive, difficult, or impossible. Some measures that could be beneficial for quantifying non-target species performance include measurements of water use such as evapotranspiration, hydrology of the site, nutrient use and cycling, or by measurements of biomass changes within species or functional groups over time. By preventing the growth and development of targeted species, Milestone® applications are directly impacting the performance of species and the availability of species within a site. Additional measures would be necessary to quantify changes in the performance of species within the site.

The application of Milestone® also indirectly affects one of the three factors that determine succession change; the availability of open sites. Chemical application of

Milestone® hinders growth and results in the death of susceptible individuals. As these individuals die, the physical space they utilized becomes available to additional species on the site.

Seedbed Preparations

Seedbed preparations were hypothesized to have a negative impact on spotted knapweed percent cover. Repeated measures ANOVA determined that the type of seedbed preparation methodology did not significantly impact percent cover of spotted knapweed. Initial work hypothesized that the application of mowing would remove above-ground viable seeds; a chemical application would kill existing biomass, and reduce successful establishment; and tilling would destroy below-ground root mass. It would be the combination of all three methods that would reduce the percent cover to the greatest degree. In reality, it was observed that each method of seedbed preparation increased the percent cover of spotted knapweed, though no significant differences were detected. See Figure 5 for comparisons of average percent cover of spotted knapweed based on the seedbed preparation method applied.

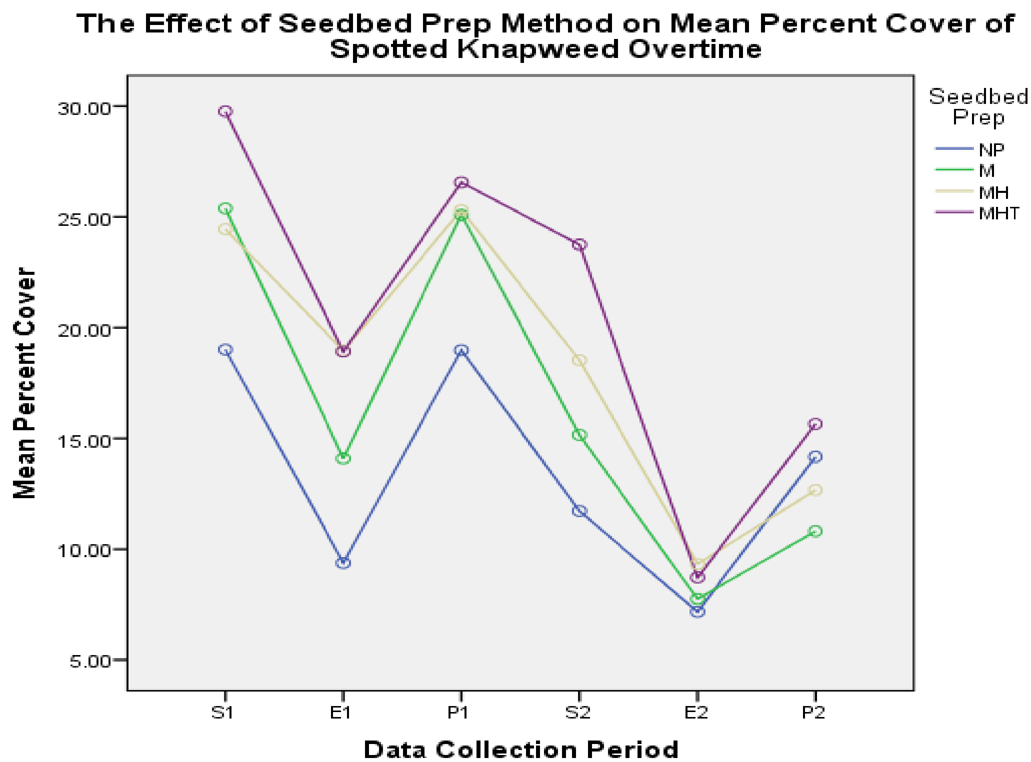

Figure 5: A comparison of spotted knapweed plots which were treated with variable seedbed preparation methods ("NP"= No seedbed preparation; "M"=Mow only; "MH"= Mow + non-selective herbicide; "MHT"=Mow + non-selective herbicide + till) over the data collection time periods (S1=Senescent stand data 2012; E1=Emergent stand data 2012; P1=Peak flower data 2012; S2=Senescent stand 2013; E2=Emergent stand data 2013; P2=Peak flower data 2013)

The use of mowing as a vegetative management tool indirectly manipulates many characteristics of the site that influence the performance of species, including above ground biomass, light availability, heat, and deposits additional materials onto the ground floor. At the time of the initial mowing, stands of spotted knapweed existed on the site prior to initial Camp Ripley invasive species distribution mapping in 2002. This indicates that spotted knapweed had been producing viable seeds on the research site for more than eight years at the initiation of experimental trials. The number of seeds produced by spotted knapweed can reach over 1,000 per plant per year, and seeds remain viable in the soil for over seven years.

These facts lead to the conclusion that a huge volume of viable seeds were present in the soil at the beginning of this experiment. Mowing deposited additional viable seeds, while also providing light and heat to the soil floor. This combination may result in an environment which provides an advantage to spotted knapweed.

The use of a non-selective chemical application of glyphosate was included as part of the seedbed preparation methods. Herbicides containing glyphosate are non-selective in nature and have the capacity to damage or kill any green vegetative materials, including grass species. It was originally hypothesized that the addition of a glyphosate treatment would decrease percent cover of targeted species compared to plots with no preparation. In reality, the opposite effect was seen. This is likely due to the damage caused to additional species on the site. A broadcast application of this chemical damaged all individuals on a site, resulting in a huge increase in the availability of open sites and a reduction in the availability of species. This, in combination with a treatment including mowing (which deposits viable seeds onto the soil), could have resulted in the increase in percent cover observed in spotted knapweed research plots, though this relationship was not significant with current methodology.

The implementation of a seedbed preparation that included tilling was hypothesized to reduce targeted species percent cover when used in combination with all other seedbed preparation methods due to the below-ground disruption of root systems. After experimental testing, this way not supported for spotted knapweed research plots. Tilling dramatically alters many characteristics of the sites, particularly the availability of sites. Tilling damages or destroys all vegetative components to the depth of till, as well as increases exposed soil and reduces above ground leaf litter. Spotted knapweed displayed an increase in percent cover on plots that received a seedbed preparation of mowing, non-selective herbicide, and tilling likely due to the cumulative effects of depositing viable seeds onto the soil body, removing all vegetation including competitors, and creating a huge amount of available sites within research plots. The combination of disturbances resulted in an increase in total percent cover of spotted knapweed, though this was not significantly observed with the established methodology.

It was observed that many of the individuals that emerged immediately produced flowers during the first season after treatment. Spotted knapweed is a biannual plant and requires at least one summer of growth from seed for floral development to occur (Beck 2008). The presence of flowers immediately after tilling indicates that existing root structures were not destroyed. Future methods that include tilling to an increased depth may result in a decrease of spotted knapweed, while also increasing open sites, and reducing availability of desirable species; all which may reduce the recoverability of a site post-disturbance. As a result, tilling is not recommended as a control strategy for spotted knapweed sites.

Common tansy research plots demonstrated a different response when evaluating the effects of seedbed preparation on percent cover. See Figure 6 for a representation of the distribution of percent cover of common tansy plots that received variable seedbed preparation methods.

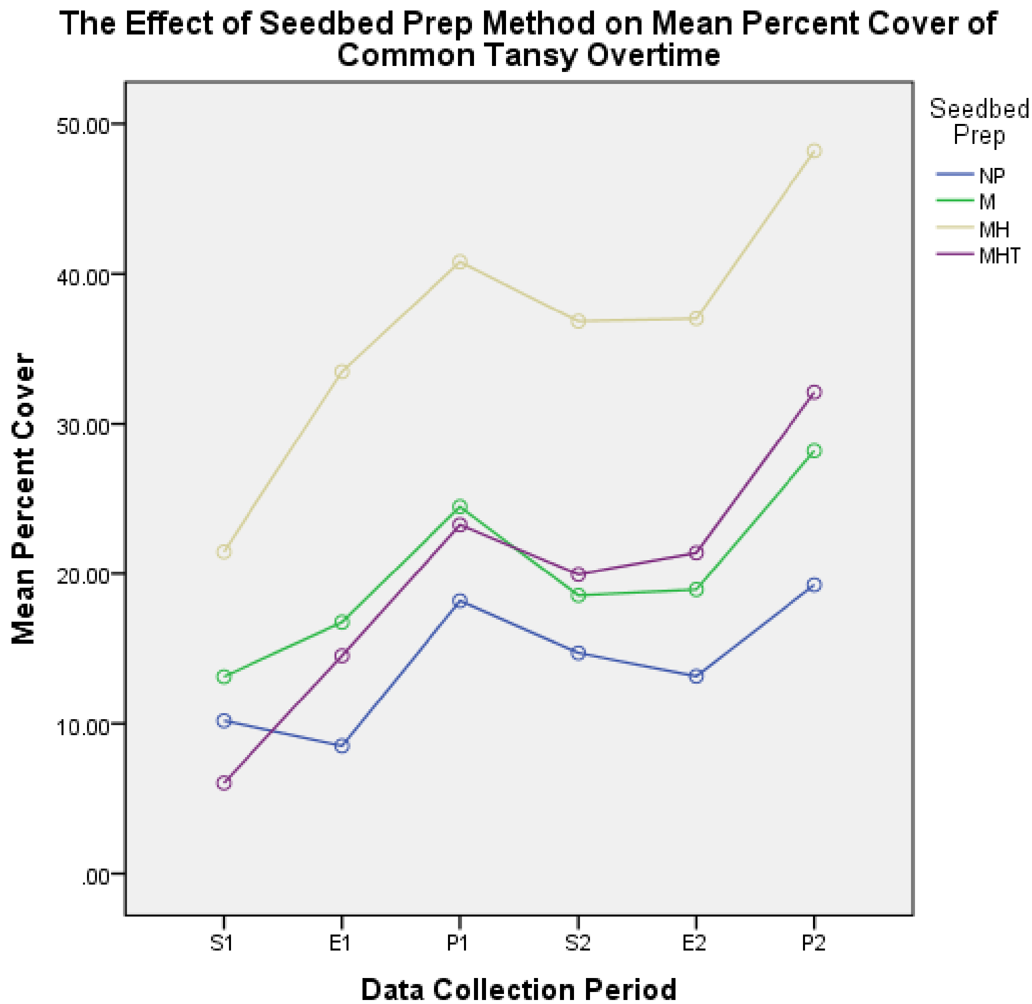

Figure 6: A comparison of spotted knapweed plots which were treated with variable seedbed preparation methods (“NP”= No seedbed preparation ; “M”=Mow only; “MH”= Mow + non-selective herbicide; “MHT”=Mow + non-selective herbicide + till) over the data collection time periods (S1=Senescent stand data 2012; E1=Emergent stand data 2012; P1=Peak flower data 2012; S2=Senescent stand 2013; E2=Emergent stand data 2013; P2=Peak flower data 2013)

One seedbed preparation methods had a significant impact on common tansy percent cover. Plots which included a seedbed preparation method of mow and non-selective herbicide demonstrated significantly higher percent cover of common tansy when compared to any other seedbed preparation method. This is likely driven by the same factors as the spotted knapweed plots; mowing deposits viable seeds onto the soil and non-selective herbicide treatments inhibit the availability and performance of other competing species on the site.

Plots which received any other combination of seedbed preparation methods did not result in any significant differences in percent cover of common tansy. The combination of all three methods of seedbed preparation resulted in a similar percent cover when compared to plots that were not altered, or were mowed only. This observed difference between the percent cover in plots that were mowed in combination with non-selective herbicides, when compared to plots which included a seedbed preparation method of mow, nonselective herbicide, and till indicates that the depth of till may have been adequate to destroy belowground root structures, and observed percent cover the result of germination of viable seeds in the soil. This may indicate a reduction in the total viable seeds in the soil body, though additional studies would be necessary to draw firm conclusions. Common tansy, unlike spotted knapweed, produces flower from viable seeds within one year of germination, so the presence of floral structures one-year post treatment is not indicative of the age of the plant; it could be either new from seed or reemerging from existing root mass. As a result, the presence of flowers one year post-treatment does not aid in drawing conclusions regarding the efficacy of the depth of till.

Future work testing variable methods for vegetation control should focus on removing all elements of the targeted invasive species prior to manipulations that result in disturbances to the soil. If removal of targeted species is not completed prior to manipulation, and viable seeds remain in the soil, an increase in the percent cover of the targeted species should be expected.

The combination of disturbances also resulted in an increase in the total number of species counted both in spotted knapweed and common tansy research plots. For spotted knapweed, the only significant increase was detected when comparing plots with no seedbed preparations to plots that received preparations including mow, non-selective herbicide, and tilling. Common tansy demonstrated a significant increase in the number of species identified in a sample as the methods included in the seedbed preparation increased.

Many of the species observed in plots that received all three methods for a seedbed preparation include early-successional species which have rapid life cycles, high reproduction rates and depend on disturbed landscapes. Some representative species include foxtail grasses (*Alopecurus sp.*), smooth brome (*Bromus inermis* Leyss.), Kentucky blue grass (*Poa pratensis* L.) and witch grass (*Panicum capillare* L.*)*. Many of the forb species identified are also disturbance-dependent including cinquefoil species (*Potentilla sp*), yellow wood sorrel (*Oxalis stricta* L.*)*, common mullein (*Verbascum thapsus* L.*)*, and curly dock (*Rumex crispus* L.*)*. These species are listed as weedy, undesirable, or invasive because of their quick colonizing ability. Due to this reversion in the types of species present, seedbed preparation methodology that includes high disturbance pressures may be counteractive to land management goals. This is of particular concern when a historic or native component is desirable. Seedbed preparation methods may not assist with achieving land management goals, even though these treatments result in a higher total species count. This highlights the need for specific land management objectives beyond species counts alone, ideally including a weighted value for desirable species.

Cover Crop

The use of a cover crop was implemented on some of the plots immediately post treatment. Initial work hypothesized that plots which receive a cover crop of Canada wild rye would have fewer open sites available for colonization post-seedbed manipulation. The addition of Canada wild rye was intended as a manipulation to increase species availability and decrease sites available for colonization by targeted species. This was not experimentally supported as the presence or absence of a cover crop did not have a significant effect on spotted knapweed or common tansy percent cover. This is likely due to the presence of a large volume of viable seeds in the soil body. This experiment did not measure a detectable difference in percent cover between plots that did and did not receive the cover crop.

Canada wild rye was recorded in species count samples in many of the plots that had received the cover crop application. This indicates that the seed stock was viable, and successful germination did occur. Due to this, it can be concluded that the use of Canada wild rye as a cover crop at the seeding rate we used does not efficiently utilize available sites to out-compete spotted knapweed. Experimental trials testing the efficacy of the use of a cover crop may find an influence of the susceptibility of a site to reinvasion after removal of a targeted species has been completed. Additional methods of data collection specifically measuring the emergence and density of Canada wild rye would be helpful for quantifying the establishment of this species in future trials.

Native Seeding Method

The method of native seeding did not have significant effect on the percent cover of targeted species in either the spotted knapweed research plots, or in the common tansy research plots. It was hypothesized that native seeds that were drill seeded into the soils would assist with a reduction in the percent cover of targeted species by utilizing some of the available sites that would be colonized by new individuals. Many of the native species that were seeded were observed growing within the research plots, indicating that the seed mix was viable and appropriate for the conditions of the site. The native grass seeding method did not have a measureable effect on percent cover of either targeted species.

All plots received one of the two methods for seed disposal. There is no true control for this variable and therefore, the inability to detect an influence on percent cover of targeted species could be due to two possibilities; both methods of native seeding had the same effect on percent cover; or neither method of native seeding had an effect on percent cover. When comparing the effects of native grass seeding methods, no detectable difference occurred between sites with either method of application. Additional methods of data collection that specifically quantify the occurrence of these species would be useful to measure establishment. It is likely that the native grass seed mix, like the Canada wild rye, is unable to directly out-compete either of the targeted species, and that thorough removal of a targeted species would need to be completed prior to establishment.

Future Work

During analysis and interpretation, some limitations became very clear due to established methodology. One significant limitation that was encountered includes a large amount of pseudo-replication. Each of the thirty-two research combinations were treated with one of each of the four variables, and due to the stacked nature of the experimental design only four true replicates exist for each multivariate combination. Analysis of the impacts of any single variable intrinsically includes the influence of other variables as they were applied to the same site. If significance was detected during this study, it includes variability caused by interactions. Data collection methods utilized in this experimental study included two measures of percent cover during three different times of the year, resulting in a total of 12 individual percent cover datum for each of the 32 combinations. This was not enough total data to detect multi-level interactions. Alteration of the data collection method to include an increased number of samples per individual research combination during peak flowering would be more beneficial for determining the interactive effects of each of the variables in-combination.

The use of non-selective herbicides appeared to increase the percent cover of each of the targeted species, which was counter to experimental goals. Because of this dramatic effect, I would not recommend the use of a single application of non-selective herbicide as a tool to reduce any targeted invasive species. The increase in the availability of open sites for targeted species outweighed the value of non-selective treatment. Selective herbicides are engineered to control specific groupings of plants and to reduce or minimize the impact on non-targeted species. A duplication of this experimental design testing a seedbed preparation method that includes a selective herbicide (as opposed to a non-selective herbicide) may demonstrate a significant reduction in targeted species by specifically reducing the availability of targeted invasive species only. In this study, the use of non-selective glyphosate is likely reducing or eliminating other associated species. The use of a selective herbicide may preserve valuable vegetative components that would then contribute to the establishment of desirable native species post-treatment.

The combination of mowing and tilling may have caused a reduction in the total number of viable seeds in the soil body due to the deposition of seeds and the creation of a large amount of available sites. This increase in availability of open sites likely resulted in increased germination of seeds in the soil. The following treatment of Milestone® may then selectively remove recently germinated individuals. This may have reduced the total volume of viable spotted knapweed seeds in the soil though additional experimental studies quantifying seed viability would be necessary to confirm this. The combination of an increase in the germination of viable seeds and a treatment of Milestone® on emergent individuals may reduce the total number of viable seeds in the seed bank, and have a longer-term influence on the percent cover of targeted species than was measureable in this four-year study. Additional experimental research projects that analyze the effects of variable seedbed preparation methods on targeted species percent cover are needed.

One major issue with using a total species count as an indication of the effects of experimental manipulations is a lack of information regarding the value of the species that occurred within the site. Native and non-native species receive the same single value, even though the value of a native species to a site may be greater than the value of a non-native species. Both targeted species, when present within a sample, increases the total diversity of the site. The presences of the targeted species in a plot is counter to land management goals, but is positively quantified. This indicates that total number of species within a plot, without consideration of species identity, is not a representative of site quality. It is extremely difficult to quantify the value of any single species on a site, and individual land managers should determine desirable species for variable sites based ecological occurrences and land management objectives. One useful way that land managers and ecologists divide species identity is into functional groups or guilds, as outline by Kindscher and Wells (1995). Future work examining the impact of land management techniques should define specific species objectives, and not use species counts exclusively due to a lack of valuable information.

One other issue became obvious as analysis continued. Initial measurements taken before any experimental trials identified general occurrences of targeted species and species diversity within the research blocks as a whole, but did not include specific measurement of total species number or percent cover of targeted species present in each plot prior to manipulation. As a result, there are not baseline measurements that can be used to make comparisons to research plots before and after manipulation, and only between-group differences are testable. Future experimental studies should be sure to have an adequate volume of baseline measurement to allow for scientifically valid comparisons, as well as aim to have true controls for each tested variable.

Conclusions

This study tested a variety of methods available for managing vegetative species including seedbed preparation methods, application of a cover crop, native seeding methods, and the application of a selective herbicide. Each of these methods manipulated a characteristic that influences succession change: availability of species, performance of species, or the availability of open spaces. One variable that significantly decreased species percent cover was the application of selective herbicide Milestone®. Selective herbicide directly reduces the species availability and performance of targeted invasive species, and indirectly increases the availability of open sites. This study found that only land management strategies that selectively reduce the performance and availability of targeted invasive species are effective at reducing the percent cover of targeted invasive species. Land management strategies which manipulate additional factors which influence succession including availability of non-target species, the performance of non-target species, and the availability of open sites are not likely to have a significant effect on targeted species percent cover.

Additional variables beyond selective herbicide application may be influencing the succession pathway of the site, though additional metrics and time would be needed to determine the influence treatments may have on future maturation of the site. Overall, sites dominated by invasive species are considered to be stable in a succession pathway. Thorough removal of the targeted invasive species should be completed prior to experimental manipulation in order to measure the influence land management strategies have on succession pathways.

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