DISSERTATION

CANADA THISTLE (*Cirsium arvense* [L.] Scop.) RESPONSE TO MOWING, HERBICIDE, COMPETITIVE GRASSES, AND SOIL AMENDMENTS ON WETLAND, UPLAND, AND MESIC SITES

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JULIE KNUDSON ENTITLED CANADA THISTLE (*Cirsium arvense* [L.] Scop.) RESPONSE TO MOWING, HERBICIDE, COMPETITIVE GRASSES, AND SOIL AMENDMENTS ON WETLAND, UPLAND, AND MESIC SITES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

CANADA THISTLE (*Cirsium arvense* [L.] Scop.) RESPONSE TO MOWING, HERBICIDE, COMPETITIVE GRASSES, AND SOIL AMENDMENTS ON WETLAND, UPLAND, AND MESIC SITES

Canada thistle (*Cirsium arvense* [L.] Scop.) is one of the most problematic weeds of temperate regions and is found throughout North America, Europe, Africa, and across central Asia. Canada thistle's ability to spread quickly and recover from many control methods makes managing Canada thistle a significant challenge for land managers. Herbicide application can be effective, but mixed results, toxicity concerns, and the need for re-application demand new, more efficient strategies that reduce herbicide use.

A greenhouse study tested effectiveness of clipping and grass seeding for Canada thistle control. Grasses used included two natives (western wheatgrass [*Pascopyrum smithii* {Rydb.} A. Löve], streambank wheatgrass [*Elymus lanceolatus* {Scribn. & J.G. Sm.}Gould ssp. *lanceolatus*]) and one sterile hybrid (common wheat [*Triticum aestivum* L.] x tall wheatgrass [*Thinopyrum ponticum* {Podp.} Z.W. Liu & R.C. Wang]) called Regreen™. Grasses were seeded alone or in combination (Regreen+western wheatgrass) in pots with Canada thistle.

Field Study I tested combinations of mowing, herbicide, and grass seeding across two habitats (wetland, upland) and three different local climatic regimes for control of Canada thistle. Grass treatments involved seeding western wheatgrass (upland sites) or prairie cordgrass (*Spartina pectinata* Bosc ex Link) (wetland sites) alone or in combination with Regreen (upland and wetland sites). Six sites (three wetland, three upland) were paired geographically across Colorado with each wetland site in close proximity to an upland site.

Field Study II tested combinations of mowing, herbicide, soil amendment addition (organic matter, manganese), and grass seeding (western wheatgrass, intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] on a Colorado mesic site.

In greenhouse trials, clipping inhibited Canada thistle growth, while grass seeding did not. In Field Study I, herbicide application produced effective control. In Field Study II, tilling enhanced herbicide effectiveness. Organic matter or manganese alone did not reduce Canada thistle growth. Manganese addition reduced herbicide effectiveness. In both field studies, neither mowing nor grass seeding enhanced herbicide effectiveness, and tilling did not increase Canada thistle biomass.

Future research should address restoration of infested wetland sites, the importance of irrigation during drought for restoration, and the mechanism through which manganese sulfate inhibits herbicide effectiveness.

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INTRODUCTION

Canada thistle (*Cirsium arvense* [L.] Scop.) is considered a noxious weed in 31 states (USDA PLANTS 2009) and continues to present a significant problem for land managers across the United States. Vegetative underground spread through creeping roots can result in the plant quickly infesting large areas, and few control methods are consistently effective over the long term. One of the most effective methods of control is herbicide application (Beck 2008), but results vary by site and habitat (Tyser et al. 1998; Beck and Sebastian 2000), multiple return visits to an infested site are often required for re-application (Beck 2008), and public concerns over toxicity issues (Sachs et al. 1987; Pimentel et al. 1991; Freemark and Boutin 1995) are now increasing the demand for new, more efficient control methods that reduce the use of herbicides. The following research was conducted to address this issue, with the goal of developing improved strategies for control of this problematic invader.

One greenhouse study and two field studies were conducted to address the following overall objectives: 1) determine if seeding of desirable competitive grass species in combination with mowing decreases Canada thistle shoot biomass below that of mowing alone, 2) determine if seeding of desirable competitive grass species in combination with herbicide application decreases Canada thistle shoot biomass below that of herbicide alone, 3) determine which of the desirable competitive grass species tested were most useful for further restoration efforts, 4) determine if tilling in preparation for grass seeding exacerbates Canada thistle infestation, 5) determine if mowing in combination with herbicide application decreases Canada thistle shoot biomass below that of herbicide application alone, 6) determine if the addition of soil amendments (organic matter or manganese) reduces Canada thistle shoot biomass below that of the untreated control, and 7) determine if the addition of soil amendments (organic matter or manganese) in combination with herbicide application reduces Canada thistle shoot biomass below that of herbicide application alone.

This dissertation is organized into sections, with the first introductory section containing the Literature Review of Canada thistle. This section is followed by three chapters. Chapter 1 discusses the greenhouse study. Chapters 2 and 3 discuss Field Study I and Field Study II, respectively. The greenhouse study addressed Objectives 1 and 3. Field Study I addressed Objectives 2, 3, 4, and 5 by applying combinations of treatments to Canada thistle-infested upland and wetland sites across the plains of Colorado. Field Study II addressed Objectives 2, 3, 4, 5, 6, and 7 by applying combinations of treatments to a Canada thistle-infested mesic site on the plains of Colorado. These chapters are followed by the Conclusions, which summarize the study findings and discuss possible avenues for further research. Three separate Appendices (one for each of the studies) are included at the end of this dissertation. A small side study ('Root Bud Formation and Root Shoot Emergence') that was performed in conjunction with the greenhouse study is presented and discussed in Appendix 1.

LITERATURE REVIEW

It is estimated that Canada thistle (*Cirsium arvense* [L.] Scop.) invades an additional 40470 hectares every year in the United States (Runyon 2001), at significant cost to land managers and taxpayers. Infesting both private and public lands, this invasive exotic is considered problematic for a variety of reasons including reduced crop yield (Donald and Khan 1996), degradation of native ecosystems and reduction in wildlife habitat (Hutchison 1992; Duncan et al. 2004), reduction in forage (Grekul and Bork 2004), and decreased recreational land use and aesthetics (Morishita 1999; DiTomaso 2000). While much has been written about Canada thistle in an agricultural setting, the following discourse will focus primarily on Canada thistle in a non-cropland setting.

Nomenclature

The earliest name on record for Canada thistle is *Ceanothos Theophrasti* (the Greek term Ceanothos meaning 'a kind of thistle') by Fabius Columna in 1616 (Detmers 1927). In 1623 the name *Carduus in avena proviens* was used, credited to Caspar Bauhin, and was the first recorded use of the Latin term for thistle (*Carduus*) for this species. Other names were used over time: *Carduus serpens laevigatus* (referring to its creeping habit and smooth stem/leaves) in 1651, *Carduus vinearum repens* ('creeping roadside') in 1671, and *Carduus vulgatissimus viniarum* ('common roadside thistle') in 1686. The name *Carduus arvensis* ('thistle of cultivated fields') was first used by Theodorus Tabernaemontanus in 1687, although this name is credited to the Swedish botanist Carl Linnaeus, and English botanist

Edward Robson's use of the name in 1777 (*Carduus arvensis* [L.] Robson) (Detmers 1927; USDA PLANTS 2009).

The plant was finally named *Cirsium arvense* (the Greek term Cirsion meaning 'thistle') in 1700 by the French botanist Joseph Pitton de Tournefort. In 1753, its name was changed to *Serratula arvensis* by Carl Linnaeus, who is credited with being the first to name and describe this plant (*Serratula arvensis* L.). In 1772, the name was changed back to *Cirsium arvense* by the Italian botanist Johann Anton Scopoli. Linnaeus and Scopoli are the two botanists given credit for the current accepted name for Canada thistle, *Cirsium arvense* (L.) Scop.

Other names have since been given to this plant species, including an 1804 use of the name *Cnicus arvensis* by German botanist George Hoffman. More recently, the following names have been assigned to this plant in the United States, although none are used as widely as the name credited to Linnaeus and Scopoli: *Breea arvensis* (L.) Less., *Breea incana* (S.G. Gmel.) W.A. Weber, ined., *Cirsium arvense* (L.) Scop. var. argenteum (Vest) Fiori, *Cirsium arvense* (L.) Scop. var. *horridum* Wimm. & Grab., *Cirsium arvense* (L.) Scop. var. *integrifolium* Wimm. & Grab., *Cirsium arvense* (L.) Scop. var. *mite* Wimm. & Grab., *Cirsium arvense* (L.) Scop. var. *vestitum* Wimm. & Grab., *Cirsium incanum* (S.G. Gmel.) Fisch. ex M. Bieb., and *Cirsium setosum* (Willd.) Besser ex M. Bieb. (USDA PLANTS 2009; Weber and Wittmann 2001). Common names used for *Cirsium arvense* include Canada thistle, creeping thistle, Californian thistle, Canadian thistle, field thistle, corn thistle, and perennial thistle (Detmers 1927; Bendall 1975; Moore 1975; Howes 1979; USDA PLANTS 2009).

Ecotypes and Varieties

There are four different varieties of Canada thistle that are recognized (Moore 1975). Leaf characteristics are the primary means of differentiation. The first variety (var. *horridum* Wimm. and Grab.) has leaves that are thick, subcoriaceous, and the surface is wavy, with marginal spines that are stout and long. The second variety (var. *vestitum* Wimm. & Grab.) has leaves that are gray-tomentose abaxially. The leaves of the third variety (var. *integrifolium* Wimm. & Grab.) are generally glabrous abaxially and are thin and flat, with few fine, short marginal spines and leaves shallowly and regularly pinnatifid or undulating. The fourth variety (var. *arvense*) similarly has leaves that are generally glabrous abaxially and are thin and flat, with few fine, short marginal spines. However, the leaves of this variety are often asymmetrical, and shallowly to deeply pinnatifid. In North America, the most common variety is var. *horridum*, but interbreeding occurs and Detmers (1927) found a specimen of var. *integrifolium* to produce seedlings of all four of the varieties. White-flowered plants have been found in all provinces of Canada (variety *horridum* [f. *albiflorum* {Rand. & Redf.} R. Hoffm.]) (Moore 1975). Robinson and Fernald (1908) also describe a white flowered form in the United States. White flowered plants have been observed in Colorado (J. Knudson, personal observation).

In addition to the separate varieties, many distinct ecotypes of Canada thistle have been found (Hodgson and Moore 1972). Ecotypes can vary significantly by seed dormancy, seed production, seed weight, leaf structure, flower color, timing of bud and flower formation, timing of spring shoot emergence and bolting, lipid deposition, and stomata frequency and area (Hodgson 1964; Hodgson and Moore 1972; Hunter and Smith 1972; Hodgson 1973). Both varieties and ecotypes have been found to respond differently to control methods. All varieties of Canada thistle are diploid ($2n = 34$) (Moore and

Frankton 1974) and the genome size of Canada thistle is 1,519 Mbp per haploid genome (Bennett and Leitch 2004).

History and Origins

Canada thistle likely originated from southeastern Europe and the eastern Mediterranean (Moore 1975). It may also be native to western Asia and northern Africa (Detmers 1927; Amor and Harris 1974). It is believed that Canada thistle first arrived in North America via Europe in contaminated hay and grain seed (Dewey 1901; Hansen 1918; Moore 1975; Erickson 1983). Weed control legislation against Canada thistle was enacted in Vermont in 1795, and in New York in 1831 (Moore 1975). An 1844 Ohio law required landowners to mow their infested lands and adjacent roadsides, and limited the sale of Canada thistle-contaminated seed (Donald 1990). Iowa followed with legislation in 1868 fining landowners for allowing Canada thistle plants to mature after receiving a written warning (Hayden 1934). By 1901, Canada thistle was considered a noxious weed by law in 24 states (Dewey 1901). It was found on the noxious weed seed lists of all states except Arkansas and New Mexico by 1956 (Hodgson 1964).

Distribution

Canada thistle has spread across the globe and is now found throughout Europe, Japan, China, South Africa, western and central Asia, northern India, northern North America, southeastern Australia, Tasmania, and New Zealand (Dewey 1901; Rogers 1928; Hayden 1934; Amor and Harris 1974). The general range of Canada thistle in North America is from 35° N latitude in the United States to 59° N latitude in Canada (Morishita, 1999). In the southern hemisphere, it has been observed at latitudes greater than 37° S latitude (Amor and Harris 1974).

In North America, the northwest and north-central United States and southern Canada have been the most extensively invaded by Canada thistle (Moore 1975). However, the Midwest, Great Plains, and Intermountain West have also experienced heavy infestation (Dewey 1991; Jewett et al. 1996; Nuzzo 1997; Rinehart 2006; Enloe et al. 2007; Oring et al. 2009). Canada thistle is estimated to infest over 2.8 million hectares across 17 western states in the United States (Duncan and Jachetta 2005). In Montana alone, Canada thistle is estimated to infest over 600,000 hectares (Montana Department of Natural Resources and Conservation 2004). Duncan and Jachetta (2005) estimate Canada thistle has been spreading across the U.S. at a historical rate of 10 to 12% per year.

Temperature, precipitation, and daylength are believed to control the range of Canada thistle. Its ideal temperature range is 0°C to 32°C, although low winter temperatures of -27°C to -35°C are common throughout much of its range (Moore 1975). Intolerance of high summer temperatures likely limits its southern range (Moore 1975), and its growth is limited or stopped when temperatures exceed 30 °C for extended periods of time (Haderlie et al. 1991). An ideal precipitation regime for Canada thistle is 41 to 76 cm per year (Hodgson 1968), although its success in drier western states indicates a tolerance for lower precipitation. The greatest infestations in Australia occur with an average precipitation of 70 to 100 cm per year (Amor and Harris 1974). Precipitation for the infested area in Canada ranges from 30 to 100 cm per year (Moore 1975). Canada thistle is believed to be a 'long-day' plant, requiring a long photoperiod for flowering. Photoperiods of 8 to 12 hours will inhibit flowering and bolting, and this day-length requirement may limit its distribution (Hunter and Smith 1972; Moore 1975).

Habitat

Canada thistle can be found along roadsides, railway embankments, irrigation ditches, stream banks, lake shores, croplands, pastures, and rangelands within much of its range (Hodgson 1968; Moore 1975). Additionally, it can be found in most plant communities within this range, including grasslands, wet meadows, cleared swamps, prairie marshes, and muskeg edges, and it frequently invades disturbed areas (Moore 1975; Thompson and Shay 1989; Hogenbirk and Wein 1991; Hutchison 1992; Nuzzo 1997; Henry 2008). Because of its low shade tolerance, it is generally not found in densely forested areas, although it will grow near forest edges and in open clearings (Moore 1975; Nuzzo 1997). However, growth in shaded areas is not impossible, but flower production may be reduced with reduced light availability (Zimdahl et al. 1991).

Detmers (1927) states that Canada thistle grows best in clay soils, but the plant is considered to grow well in silt loams and tolerate a variety of other soils (Rogers 1928; Hodgson 1968; Moore 1975). In Canada, infestations can be found growing in sandy loams, clay loams, sandy clay, and sand dunes (Moore 1975). Germination and growth is possible under low moisture conditions, but moist soils are considered to be favorable for growth (Moore 1975; Wilson 1979). It can survive dry soils better than very wet soils, with prolonged flooding or other extended saturation of the soils inhibiting establishment and growth (Rogers 1928; Moore 1975). Bakker (1960) states that unshaded sites with moist, aerated soils are the ideal environment for Canada thistle growth.

Canada thistle may favor deeply compacted soils (Rietberg 1952; Wallace 2001; Sullivan 2004), or those with poor plant residue decay dominated by anaerobic bacteria (McCaman 1994; Walters 1999). McCaman (1994) observed that Canada thistle presence on a site was often an indicator of soils low in available manganese. Walters (1999) also observed this association, and believes manganese to be the primary nutrient driving

Canada thistle infestation (C. Walters, personal communication, 2005). Korsmo (1930) found Canada thistle to avoid loose, dry, sandy soils in favor of heavier, wetter, and more compacted soils. Canada thistle is also considered to favor calcareous soils (Korsmo 1930). Rogers (1928) stated that in the Central and Northern U.S. Canada thistle grows most abundantly on limestone soils with abundant moisture. Canada thistle is considered tolerant of saline conditions. Wilson (1979) found Canada thistle germination to occur at concentrations of 20,000 ppm NaCl.

Growth, Development, and Reproduction

Canada thistle continues to spread across the United States in large part as a result of its successful reproductive strategies. While it is commonly believed that localized Canada thistle spread occurs primarily through underground vegetative reproduction, more recent evidence suggests that spread by seed may be more important than previously believed. Hettwer and Gerowiit (2004) found significant genotypic variation between neighboring Canada thistle patches, suggesting that spread occurred more through establishment of new clones than by clonal spread of a single genotype (such as might occur via the spread of root fragments as a result of cultivation). The authors concluded that the influx of new clones was likely a product of regular new seedling establishment. On a broader scale, Bodo Slotta et al. (2006) stated that multiple introductions and consistent gene flow among Canada thistle populations is likely the reason for this plant's success across North America. The following describes in detail the growth, development, and reproduction of Canada thistle.

Shoots and Leaves

Shoots

Canada thistle shoots are typically 0.3 to 1.5 m tall (Moore 1975). Emerging shoots in the spring first grow as rosettes for approximately 3 weeks, followed by vertical growth or 'bolting'. In Montana, the most rapid vertical growth occurred in late June, with an approximate growth of 3 cm per day (Hodgson 1968). Shoots typically develop from vertical roots, or from root buds on the horizontal creeping root system (Moore 1975). Shoots are usually green, but can be brownish to reddish purple, and can vary from smooth to ribbed, sometimes with spines below the leaves (Hodgson 1968).

Shoot density can be variable, and depend on a host of factors including soil moisture, soil type, and competition from other species. Hodgson (1964) found shoot density to range from 34 per m^2 to 114 per m^2. Donald (1994*a*) surveyed Canada thistle densities across six countries and found shoot density ranging from 1 per m^2 to 63 per m^2. Shoot density has been observed to be lowest at the edges of a patch (Donald 1994*b*). Conversely, Pavlychenko (1943) found prolonged drought to produce ring-like stands of Canada thistle, with little vegetation in the center.

Leaves

Canada thistle leaves can be highly variable. The leaf surface can range from hairy to glabrous. Usually dark green, they are often deeply lobed with a ruffled margin, but can range from deeply pinnately lobed to entire. Spines often occur around the margin and at the lobe tips, but may be lacking (Hodgson 1968). Leaves consistently alternate on the stem, and the leaf base is sessile and either shortly decurrent or clasping (Moore 1975). Leaf characters such as spininess, texture, segmentation, and vestiture are used to differentiate the varieties.

Roots and Vegetative Reproduction

Roots

While the majority of Canada thistle roots develop within the top 38 cm (Hodgson 1968), they have been found to penetrate as deep as 6.8 meters on the Russian steppe (Rogers 1928). As a seedling, the plant first develops a main root, followed by the development of lateral roots spreading horizontally after several months. Once these roots have spread horizontally, they then grow downwards toward the water table (Moore 1975). Rogers (1928) states that horizontal root spread will extend 0.6 to 1.2 meters before growing downwards, while Moore (1975) states that the horizontal roots will extend only 6 to 12 cm before growing downward. Downward root growth is believed to be inhibited by the water table (Rogers 1928; Hodgson 1968), and may be shallow in wet soils (Moore 1975). Roots can grow quickly in young plants, and produce an average of 111 meters of roots after only 18 weeks (Nadeau and Vanden Born 1989). These authors also found 62.1% of Canada thistle root biomass to occur in the top 40 cm of the soil. Rogers (1928) stated that shoot production is commonly associated with horizontal-growing roots, while water and nutrient uptake is primarily conducted in the vertical roots. Individual roots can live up to 2 years and then are typically replaced by new roots developing from the old ones (Rogers 1928).

The growth and mortality of roots can depend on a host of factors, including soil moisture, soil type, and soil temperature. Lauridson et al. (1983) reported Canada thistle root length to increase with reduced soil moisture. Roots grown in clay soils, muck soils (soils containing 20 to 50% organic matter), limestone, and sand/gravel grew to a maximum depth of 4.5, 3.8, 1.8, and 1.0 m, respectively (Donald 1994*a*). Canada thistle can be damaged under cold temperatures, with the lethal temperature required to kill 50% (LT_{50}) of plant roots considered to be -7°C if directly exposed (Schimming and Messersmith

1988). Root buds may be more susceptible to damage from cold than roots (Carlson and Donald 1988)

Carbohydrate storage in the roots varies with growing season. Sucrose and inulin are believed to be the two principal carbohydrates in Canada thistle roots (Ozer and Koch 1977). Root carbohydrates can be split into 92 different components (Wilson et al. 2006), but components most typically discussed include glucose, fructose, sucrose, and fructans (also referred to as inulin). Fructans are fructose polymers with one terminal glucose molecule and can be categorized into high-degree-of-polymerization (DP) fructans and low-DP fructans (including 1-nystose, 1-kestose, and 1-fructofuranosyl-nystose) (Wilson and Michiels 2003). Carbohydrate analysis of Canada thistle roots in October found free sugars (glucose, fructose, and sucrose) to account for 27% of the total carbohydrates, with sucrose accounting for 87% of the free sugars (Wilson et al. 2006). Fructans (of varying degrees of DP) made up 73% of the total carbohydrates.

Hodgson (1968) found carbohydrate reserves (unspecified type) to be lowest when flower buds began to appear in June in a Montana study. Another Montana study by this author found carbohydrate reserves to decrease from early spring through the end of June, then steadily increase into September, at which point it leveled out. Others have observed a similar decline in carbohydrate reserves from May to early July, followed by an increase into September (Welton et al. 1929; Arny 1932). Tworkoski (1992) found root carbohydrate replenishment (such as occurs in the late summer and fall as transported photoassimilate) to be driven largely by growth stage. Peak photoassimilate movement to the roots occurred during the bolting stage compared to the budding, flower, or post-flower stage. Wilson et al. (2006) observed root concentrations of fructans and sucrose to begin to increase in September/October in a Nebraska field study, continuing to increase from November to December as soils froze. Concentrations remained high through March then declined with initiation of plant growth in April. Higher concentrations of these carbohydrates during the winter are believed to help the plant tolerate colder temperatures. Herbicides that can reduce sucrose and fructan levels in roots in late fall and thus inhibit the plant's tolerance to cold are believed to provide more effective control, and timing herbicide application for when carbohydrate changes begin to occur (after the first frost) is also considered to provide more effective control (Wilson et al. 2006). Herbicide applied 10 days after the first fall frost reduced the levels of fructans carbohydrate in Canada thistle roots and provided better control of Canada thistle than herbicide applied before the first frost (Wilson and Michiels 2003).

Vegetative Reproduction

The most problematic form of reproduction in Canada thistle is vegetative reproduction. Canada thistle populations spread up to 6 m per year (Rogers 1928) through the horizontal spread of its root system, and the network of underground creeping roots that drive this spread often evade most control methods. With even small root fragments (10 mm) able to regenerate into new plants (Hamdoun 1972), control of these spreading roots can be a challenge. Hayden (1934) found pieces of either horizontal or vertical roots to grow into new plants.

Aerial shoots can develop directly from the original vertical root, from lateral buds at the internodes on stem segments, or from root buds on the horizontal roots (Moore 1975; Donald 1994*a*). Survival of plants originating from stem material is higher if the stem source piece is only partially buried in the soil and originates from a plant that has recently flowered (Donald 1994*a*). New plants can also establish from root fragments with no visible root buds (Hamdoun 1972; Nadeau 1988). Root buds that have not yet emerged through the root cortex are not visible on the surface but can be seen through the use of lactic acid to clear the roots (McIntyre and Hunter 1975; Nadeau and Vanden Born 1989). However, Donald (1994*a*) noted that while lactic acid is successful at clearing healthy white roots of greenhouse plants, it may not be effective for clearing thickened, darkened, partially decayed field-grown roots.

Root bud formation and volume has been a focus of research because of its positive relationship with shoot emergence, and the desire to find control methods that will significantly inhibit root bud formation. Treatment of root buds in the literature includes various terminology such as 'adventitious root buds', 'root shoots', or 'underground shoots' (root buds emerged from the cortex and at least 5 mm long) (Nadeau and Vanden Born 1989), and authors sometimes use new shoot growth as a proxy for root bud formation (Baradari et al. 1980; Ziska et al. 2004). The presence of the main shoot (leaves and stem) inhibits root bud activity through apical dominance, but root buds may be released from inhibition through increased relative humidity, implying that root bud inhibition may be driven by competition between the stem and the root buds for water (Hunter et al. 1985). Removal of the main stem (and apical bud) such as through mechanical cutting activates root buds, and new shoots can emerge (Hunter et al. 1985: Nuzzo 1997). Root bud density is highest in late summer compared to spring (Donald 1994*a*), and root bud growth is greatest in late fall and winter following the senescence of aerial shoots, but root bud presence or ability to elongate is unaffected by seasonal variations (McAllister and Haderlie 1985*b*). A Canada thistle plant grown for 18 weeks produced 154 root buds on 111 m of roots > 0.5 mm in diameter (Nadeau and Vanden Born 1989). Regions of greatest aboveground shoot density are often correlated with greater densities of root buds (Donald 1994*b*). Hunter et al. (1985) found root bud number to be unaffected by an increase in relative humidity, but that shoot emergence from the roots increased (in the absence of an attached stem). Nitrogen addition has been found to enhance root bud growth, but inhibit root bud formation (McIntyre and Hunter 1975). Baradari et al. (1980) found that chlorflurenol may increase root shoot number, and in combination with dicamba increase absorption and translocation of the herbicide. French and Lightfield (1990) found inoculating dormant root buds was the most effective means for infecting Canada thistle root cuttings with teliospores of the biocontrol *Puccinia punctiformis.*

Root fragment length, temperature, and photoperiod may also play an important role in root bud formation and root shoot emergence (See Appendix 1 for the summary of a small side study, performed in conjunction with the greenhouse study discussed in Chapter 1, that explored root bud formation and root shoot emergence in Canada thistle).

Sexual Reproduction

Flowers

Canada thistle is normally dioecious, with pollen producing staminate flowers on one plant and seed producing pistillate flowers on the other. However, in a study of ten Montana ecotypes, occasionally seed production occurred on staminate plants (Hodgson 1968). Moore (1975) stated that staminate flowers often contain a vestigial ovary, which may allow seed production in male flowers. Kay (1985) found 15% of clones with apparently male flowers produced 10 to 65 seeds per flowerhead (hermaphrodites), and 11% of plants produced 2 to10 seeds per flowerhead (subhermaphrodites).

The Canada thistle flowerhead consists of many small flowers (florets) clustered into a head approximately 1.5 to 2.0 cm tall and 1 to 1.5 cm wide (Detmers 1927). An average of 110 (staminate flowers) to 120 (carpellate flowers) florets are produced per flowerhead (Detmers 1927). Female flowers have a strong vanilla-like smell. Flowers are pollinated by insects, primarily honeybees (Detmers 1927), but wind pollination can also occur (Derscheid and Schultz 1960). Flower color typically ranges from purple to pink, although pale blue flowers and white flowers have also been observed (Hayden 1934; Hodgson 1964).

Seeds

On a broad scale, seeds are considered to be the primary source for new invasion (Hayden 1934). Seed production generally requires male and female plants to be in close enough proximity to each other for pollination, as mentioned above. Hayden (1934) found pistillate colonies growing within 60 to 90 m of staminate colonies to bear seed. Derscheid and Schultz (1960) found a relatively low level of seed production when male and female plants were 180 m apart. Lalonde and Roitberg (1994) found seed production to be significantly reduced when female plants were more than 50 m from male plants.

The seed itself is contained in an achene and is straight or slightly curved, straw or light brown in color, and approximately 2.5 to 4 mm long and 1 mm in diameter (Moore 1975). A pappus (cluster of hairs) is attached to the top of the achene to facilitate wind transport. All species of *Cirsium* have a pappus that is distinguished by branching hairs (Moore 1975). Seed production per plant can be variable. Korsmo (1930) reported seed production of 4,600 seeds per plant. Hay (1937) estimated that Canada thistle can produce up to 5,300 seeds per plant, but found average seed production to be 1,530 per plant. When it occurs, Kay (1985) stated that male flowers produce 6 seeds per head on average. Female flowers produced 2 to 3 seeds per head when male plants were 150 to 180 m away, 20 to 30 seeds per head when male plants were 7 to 30 m away, and 50 to 100 seeds per head when two sizeable male populations were present within 7 m (Hayden 1934). Becker et al. (2008) found only 44% of achenes produced by flowering female Canada thistle shoots contained 'normal' seeds, with the rest of the achenes containing either no seeds (38%) or 'shrunken' seeds (17%).

Seed Dispersal

Seed can be dispersed by wind, water, farm machinery, and through contamination of harvested crop seed (Rogers 1928). Historically, contamination of crop seed was viewed as a major mechanism of spread (Donald 1994*a*). Irrigation ditches have also been recognized as important transportation channels for seed (Dewey 1901, Hope 1927). There are conflicting views on the importance of wind as a mode of transportation. Insufficient opening of some seedheads have been observed, preventing the seed and pappus from exiting the head (Donald 1994*a*). Bakker (1960) observed detachment of the pappus plume from the seed, leaving it in the seedhead. At a distance of 10 m from a Canada thistle field, that author found the majority of pappus plumes to not bear any seeds, with most seeds remaining in the fruiting head. Becker et al. (2008) observed greater than 80% of Canada thistle pappi collected did not have a seed attached. However, when the pappus remains attached and seed can exit the seedhead, seed has been observed to travel by wind for 11.4, 7.6, and 3.8 m with winds of 16.4, 10.9, and 5.5 km per hour, respectively (Donald 1994*a*). Bakker (1960) observed seed with pappus attached 1 km from a Canada thistle field. Terminal velocity for Canada thistle seed has been recorded at 26 cm per second (Bakker 1960). Becker et al. (2008) stated that wind dispersal of seed is typically a local event, and that long distance dispersal of any significant amount of seed would be rare. These researchers observed most Canada thistle seeds to fall near their parent plants. Another mechanism of local seed dispersal may involve ants, with Canada thistle seeds discovered to have an elaiosome (fleshy appendage), which may indicate ant dispersal (Pemberton and Irving 1990).

Canada thistle grows near railroad tracks and highways, and can be spread on these vehicles (Hayden 1934). It also often grows near grain elevators and stockyards, and so may contaminate stored seed or spread on animals, and may be mixed in with straw that is sold for bedding or packing. It may also be spread through manure (Donald 1994*a*). Birds have been investigated as direct carriers but little evidence supports this (Hayden 1934). Unfortunately, birds may indirectly contribute to Canada thistle spread. An Oregon State University study found Canada thistle seed, along with nine other noxious weeds, to commonly occur in wild bird seed sold to the public in retail stores (Colquhoun and Mallory-Smith 2004).

While natural water bodies and waterways can be a useful means of natural dispersal, Canada thistle appears to have otherwise poor long-distance natural dispersal mechanisms. Combined with typically low seed viability, the successful spread of this plant throughout the world has likely been largely dependent on repeated introductions of seed dispersed through humans and human-associated activities.

Seed Germination

Some seeds may germinate the same year they are produced, but most will not until the following spring (Rogers 1928). Plants that germinate from seed in the spring can produce stalks but are unlikely to flower the first year. Germination can be affected by a host of factors including temperature, age of seed, planting depth, ecotype, water content, and soil aeration (Bakker 1960; Hodgson 1964). Seeds germinate best with warmer temperatures (25 to 30°C) (Moore 1975). Amor and Harris (1974) found seeds germinated best at 30°C. Fresh seed produced 95% germination (Hayden 1934). The Duvel Buried Seed experiment found no germination of Canada thistle seed 30 years after burial, but found seeds to still germinate 21 years after burial (Toole and Brown 1946). The best sowing depth found by Bakker (1960) was 0.5 cm. Hodgson (1964) found mean germination rates to vary from 0 to 92% depending on seed harvest date and ecotype, with some ecotypes having consistently lower germination rates. Establishment of seedlings in undisturbed areas can be difficult because they are not good competitors and have high light requirements (Hodgson 1968; Moore 1975).

Phenology

Ideal conditions for Canada thistle emergence in the spring is when mean weekly temperatures reach 8°C, although emergence can occur at mean weekly temperatures of 5°C, and emergence can vary by ecotype (Moore 1975). Once the young plant has emerged at the soil surface in the spring, either from seed or vegetatively from pre-existing roots, rosette formation occurs, followed by rapid vertical growth. The plant can produce a stalk as early as 3 weeks after emergence, with emergence occurring in early May in northern states like Montana, or March through May in states like Washington (Rogers 1928).

Flowering begins mid-June to early July in Canada, and may still occur into September (Moore 1975). Canada thistle flowering is driven by day-length, and all ecotypes tested would not flower during an 8 or 12 hour photoperiod (Hunter and Smith 1972). Flowering occurred in some ecotypes with a 14 hour photoperiod, with all tested ecotypes flowering under a 16 hour photoperiod. Temperature may also play a role in flowering. After seed set, the current erect plants begin to die back. New shoot growth that emerges in the fall (either from new seed germination or from pre-existing roots) will stay in rosette form and not bolt. Hunter and Smith (1972) found bolting to be inhibited and plants remaining as rosettes with an 8 and 12 hour photoperiod in all Canada thistle ecotypes tested, but for bolting to occur with a 16 hour photoperiod. Miller and Lym (1998) found a photoperiod of 15 hours or greater was required for most Canada thistle plants to bolt in North Dakota. In that study, 2% of plants bolted with a 13 hour photoperiod, and 25% required a 16 hour day-length. Haderlie et al. (1991) states that 14 hour daylengths or longer are required for Canada thistle to flower. Knowledge of critical day-length windows can be useful for maximizing the efficiency of control methods such as mowing. For example, mowing to control seed production is unnecessary once day-length is short enough that it prevents plants from bolting and producing seed (Dr. K.G. Beck, personal communication, 2005). Fall rosettes will stay green until freezing temperatures cause leaf damage (Wilson et al. 2006).

Plant Interactions and Associations

Canada thistle residue can be autotoxic, inhibiting germination of its own seed (Bendall 1975). Plant residue or residue products of Canada thistle have also been observed to have toxic effects on a variety of other plant species (Putnam 1984; Kovacs et al. 1988; Solymosi and Nagy 1999; Ghosh et al. 2000; Kazinczi et al. 2001), including alfalfa (*Medicago sativa* L. var. 'Dawson'), perennial ryegrass (*Lolium perenne* L.), common barley (*Hordeum vulgare* L.), common wheat (*Triticum aestivum* L. var. 'Centurk'), redroot pigweed (*Amaranthus retroflexus* L.), green foxtail (*Setaria viridis* [L.] P. Beauv.), and subterranean clover (*Trifolium subterraneum* L.) (Bendall 1975; Stachon and Zimdahl 1980; Wilson 1981). Canada thistle can also impact plants through 'aerial allelopathy' (Glinwood et al. 2004), where exposure of common barley to volatiles from Canada thistle plants resulted in reduced insect activity on the barley.

Several researchers have observed infection of Canada thistle roots by arbuscular mycorrhiza. Kovacic et al. (1984) found Canada thistle infected with vesicular arbuscular mycorrhiza growing in a beetle-kill area of a Ponderosa pine (*Pinus ponderosa* C. Lawson) forest. On Vancouver Island, British Columbia, researchers determined Canada thistle plants collected on a local farm site to also be infected with vesicular arbuscular mycorrhiza (Berch et al. 1988).

A host of insects and nematodes have also been found naturally occurring on Canada thistle, as well as several forms of fungi (Moore 1975; Donald 1994*a*). Some of them may have potential as biocontrols (e.g., *Ceutorhynchus litura* [F.]), but others are widespread and associated with important crop species, so their utility as a control tool is limited.

Management

Management methodologies have evolved since the early 1900's when the application of salt (NaCl) to Canada thistle infested soils was considered to be a useful control strategy (Hodgson 1968). However, the development of new control methods has been accompanied by new discoveries about the many ways that this problematic invader can evade eradication. Canada thistle response to a given control measure can vary with climate, weather, habitat, soil type, Canada thistle growth stage, clonal structure, season of application, application technique for a given control method, and ecotype (Hodgson 1964; Hunter and Smith 1972; Donald and Prato 1992; Tworkoski 1992; Donald 1994*b*; Nuzzo 1997; Beck and Sebastian 2000; Krueger-Mangold et al. 2002; Wilson and Michiels 2003; Dupont 2007). Conflicting results for a variety of the control methods discussed below are likely a product of the influence of one or more of these factors.

Chemical Control

Traditional Herbicides

There is a vast array of traditional herbicides that have been used for Canada thistle treatment. The most commonly used appear to be the following: Tordon (picloram) (DowAgrosciences, Indianapolis, IN), Transline (clopyralid) (DowAgroSciences, Indianapolis, IN), Telar (chlorsulfuron) (Du Pont, Wilmington, DE), 2,4-D (Agriliance, St

Paul, MN), Curtail (clopyralid + 2,4-D) (DowAgroSciences, Indianapolis, IN), Redeem (triclopyr + clopyralid) (DowAgroSciences, Indianapolis, IN), Banvel (dicamba) (BASF, Mississauga, Ontario, Canada), and Roundup (glyphosate) (Monsanto, St Louis, MI). Relatively new products that may be useful for Canada thistle control include Hardball or Unison (2,4-D acid formulation) (Helena Chemical, Collierville, TN) (Dr. K.G. Beck, personal communication, 2005). Canada thistle response to these chemicals is variable and depends on a host of factors including timing of application, number of applications, application technique, year of application, and growth stage treated. Plant ecotypes also respond differently to different chemicals (Frank and Tworkoski 1994).

A new herbicide called Milestone (aminopyralid) (Dow AgroSciences LLC, Indianapolis, IN) has also become available that is registered under the Reduced Risk Pesticide initiative of the U.S. Environmental Protection Agency. This means that it is considered to have demonstrated a lower risk to humans and the environment than other currently available alternatives (USEPA 2009). This herbicide can be used up to waters edge on infested sites, which may be particularly useful for control of infestations near water bodies. It has demonstrated significant success against Canada thistle, with greater than 90% control of Canada thistle at the 490 g per ha rate in recent trials (Holen et al. 2007). Its negative effects on the growth of restoration plant species has yet to be fully explored (Henry 2008; Samuel and Lym 2008), but grasses including bluebunch wheatgrass, western wheatgrass, crested wheatgrass, and Idaho fescue have been found to tolerate aminopyralid application of up to 120 g ae per ha (Duncan et al. 2005). Enloe et al. (2007) tested aminopyralid against other commonly used herbicides and found Canada thistle control to be comparable. Despite having greater herbicidal activity than herbicides such as clopyralid, absorption and translocation of aminopyralid applied to Canada thistle plants is lower (Bukun et al. 2009).

Herbicides Approved For Aquatic Environments

Herbicides approved for use on Canada thistle in aquatic environments and containing products such as glyphosate (e.g., Rodeo®, Monsanto Agricultural Company, St. Louis, MO), triclopyr (e.g., Garlon® 3A, DowAgrosciences, Indianapolis, IN), or 2,4-D (e.g., Weedar 64®, Nufarm, St. Joseph, MO) have been successful for control in some situations, but concerns over non-selectivity, lack of long-term successful control, and/or remaining toxicity concerns have limited their use on Canada thistle. Krueger-Mangold et al. (2002) found that a fall wick application of Rodeo© at 1.5 kg ai per ha provided significant Canada thistle control for the 2-year study. However, a longer 5-year study found that 2 years of fall application of glyphosate at 1.7 kg per ha did not maintain Canada thistle densities below that of the untreated control by the fifth year (Donald and Prato 1992). Others have found the non-selectivity of glyphosate to be too damaging to intermixed desirable species, as well as resulting in an increase of weedy annual forbs in place of the original desirables (Grekul et al. 2005). While fall application and wicking may minimize the impact of non-selective herbicides on non-target species, application methods such as wicking are very labor-intensive (i.e. expensive), and may still result in injury to non-targets (Grekul et al. 2005). Selective herbicides such as the triclopyr-based Garlon® 3A are considered less damaging to non-target species (Gardner and Grue 1996), but Garlon® 3A (and other similar triclopyr products approved for use near aquatic environments) must usually be paired with another herbicide to gain significant control of Canada thistle (potentially limiting its aquatic utility). The 2,4-D formulations are also considered to be selective, but their potential toxicity to aquatic environments has been debated (Borges et al. 2004; USEPA 2005), and its use can be heavily restricted in sensitive areas.

'Natural' Herbicides

The use of 'natural' herbicides for long-term control is considered to have limited utility thus far. Two of the most common non-systemic, foliar-applied products used on Canada thistle are Alldown Green Chemistry Herbicide (Summerset Products, Chaska, MN) and Burnout II Weed and Grass Killer (St. Gabriel Laboratories, Orange, VA). Alldown is vinegar (acetic acid)-based, while Burnout II is primarily clove oil-based (although it also contains acetic acid). Card and Saielli (2007) found that both Alldown and Burnout II reduced Canada thistle density and biomass after two seasons of use at 2-week application intervals. Treatment at 8-week intervals, however, resulted in an increase of thistle biomass and density for both herbicides. The USDA-ARS in Beltsville, MD tested high levels of straight acetic acid for control, and found that foliar application of 10 to 20% acetic acid successfully controlled above-ground Canada thistle growth, but showed limited success at below-ground control (Owen 2002; Daniels 2003). Soil drenching with 20 to 30% vinegar was also tested, with a 90% reduction in stem number, but this treatment only slowed regeneration (Radhakrishnan et al. 2003).

Unfortunately, the limited utility for below-ground plant control, necessity of multiple applications per year, and potentially high cost of product often discourages the use of 'natural herbicides' on Canada thistle. Cost per hectare for Alldown and Burnout II (935 L per ha rate) is estimated at $4,448 and $1,977, respectively (Card and Saielli 2007). Broadcast applications of 20% and 30% acetic acid solution are cheaper, at $163 to $244 per hectare (Owen 2002). These herbicides are also generally non-selective, so have potential to negatively impact non-target species. Long-term research is needed to determine if continual top-kill can significantly reduce Canada thistle populations over time.

Alternatives to Chemical Control

A variety of control methods besides traditional herbicide control have been tested on Canada thistle, with mixed results. Tested methods include manual control (hand-pulling/digging), shading, cultivation, mowing, fire, fertilization, grazing, biological control, and plant competition/revegetation.

Manual Control and Shading

Unfortunately, hand-pulling and digging are considered viable control options only for very small infestations (or for first year growth of seedlings), as Canada thistle can easily regenerate from even minute (10 mm) root fragments left behind in the soil (Hamdoun 1972). Hand pulling generally only addresses above-ground growth, leaving below-ground plant material relatively intact for resprouting. Digging is generally considered to be equally as futile because it is virtually impossible to remove all underground roots, leaving remnants to resprout. Because Canada thistle is relatively shade intolerant, shading techniques have been used for control with some success. For example, covering plants with boards, heavy tar or building paper, or sheet metal can be effective (Hansen 1918; Donald 1990; Nuzzo 1997).

Cultivation

Cultivation has demonstrated mixed results for control. Zimdahl and Foster (1993) found that disking 3, 7, 10, 14, or 30 days post-herbicide application did not improve control over herbicide application alone. Bostrom and Fogelfors (1999) found early season plowing followed by late fall harrowing to significantly enhance Canada thistle growth. Conversely, Hodgson (1958) found that six cultivations with duckfoot sweeps at 21-day intervals reduced Canada thistle shoots 99% after one season. Canada thistle has also been found to be more abundant in no-till agricultural systems than in tilled systems (Donald 1990; Miller 1990). However, cultivation is often not appropriate for use in natural areas because of issues with site accessibility, equipment availability and expense for multiple visits throughout the season, as well as the potential for disruption and increased susceptibility of intermixed desirable plant communities.

Mowing

Research on mowing as a treatment for Canada thistle control has shown mixed results, but surprisingly, mowing is still a popular control method. It has been the control strategy of choice for landowners enrolled in the Conservation Reserve Program (Holen et al. 2007), and is still a recommended control strategy for use on its own on many weed control information websites. Ready availability of equipment for large operations or ease of access for small operations (weed-eaters) may be one of its advantages. Mowing is considered useful for control because it may limit the plants' ability to store reserve carbohydrates, thus weakening the plants (Donald 1990). Mowing can also prevent seed dispersal when performed prior to seed set. Mowing should be performed within 6 days of the opening of Canada thistle flowers, as viable seed can develop within 7 to 9 days of flower opening (Derscheid and Schultz 1960).

Grekul and Bork (2007) found a one-time mowing treatment in mid-July (in Central Alberta) resulted in a temporary increase in shoot density that same year, but two years after treatment, shoot density (and biomass) were not different than the unmowed control. Conversely, mowing an infested alfalfa field twice per year reduced Canada thistle populations by 86% after the first year, with 100% elimination after 4 years (Hodgson 1968). Beck and Sebastian (2000) found that mowing three times per year for 2 years in Colorado controlled 85% of Canada thistle at a subirrigated site. On a dryland site, this same treatment did not provide significant control. The authors hypothesized that Canada thistle root growth may have been restricted at the subirrigated site as a result of the high water table, and thus plants were easier to control at this site compared to the dryland site. Amor and Harris (1977) found shoot density to be no different from the unmowed control after the first year of once-annual mowing, but it decreased significantly after the second year of mowing. Two years after the last mowing treatment shoot density was again equal to that of the unmowed control.

Mowing may also improve the effectiveness of biological controls. Demers et al. (2006) found that a late season mowing significantly increased infection of Canada thistle shoots by an obligate rust fungus (*Puccinia punctiformis* [F. Strauss] Rohl.) compared to unmowed plots. Mowed plots also demonstrated a decline in healthy shoots. The authors believe that mowing opens up the plants to infection, and that the mowing equipment may physically distribute the infection. Mowing must be carefully timed with the rust life cycle, however, to coincide with the appropriate infectious spore stage (Demers et al. 2006).

Clearly, the success of a mowing treatment may depend on a variety of factors such as presence of additional competitive species (such as alfalfa), timing of application, number and frequency of mowing events, and soil moisture availability/water table depth. It appears, however, that only in rare cases can mowing alone eliminate infestations, and more often simply prevents the population from spreading (Willard and Lewis 1939).

Fire

The success of burning for Canada thistle control has been limited, and is largely dependent on season of burn and soil moisture. Burning during the dormant season may stimulate the growth of native species, and increase competition. Young (1986) found dormant season burning inhibited Canada thistle flower and seed production. Removal of accumulated Canada thistle plant residue through burning may promote earlier seed germination of native species (Bossard et al. 2000). A spring burn in an infested marsh in Canada encouraged native species growth but did not affect Canada thistle biomass (Thompson and Shay 1989). Early spring fires may only kill aboveground biomass and may increase subsequent sprouting (Harrod and Reichard 2001). Burning dried plants with mature seeds may not destroy viable seed unless seed heads are completely burned (Rogers 1928). Three consecutive years of late spring burning in May or June is suggested as an effective control (Hutchison 1992).

Fertilization

Fertilization has demonstrated limited success as a control measure. Grekul and Bork (2007) found that application of blended complete fertilizer (29-13-3-4, NPKS) at 375 kg per ha reduced Canada thistle infestation density, but increased total biomass. Reece and Wilson (1983) also found thistle density and biomass to increase with application of ammonium nitrate fertilizer (44.7 kg per ha) over a 2 year period. Conversely, Thrasher et al. (1963) found N addition decreased Canada thistle infestation, likely by increasing the competitive ability of seeded grasses. Interestingly, annual spring fertilization (NPKS) has been shown to enhance Canada thistle control when combined with herbicide application (Grekul and Bork 2007), but factors such as expense, intensive equipment/labor requirements, and additional care required when used near aquatic environs (especially N) often make fertilization a relatively unattractive option.

Grazing

Grazing has only proven useful as a control measure in select situations. De Bruijn and Bork (2006) demonstrated significant Canada thistle reduction after 2 to 3 years of high intensity, low frequency rotational grazing with cattle. Cattle have been shown to inhibit flowering by grazing plants before the bud stage (De Bruijn and Bork 2006). A mixture of either 33% goats and 66% sheep or 66% goats and 33% sheep has also been shown to inhibit flower production (Popay and Field 1996). Unfortunately, while the early season tender rosettes may be attractive to both livestock and wildlife, the spiny leaves and relatively tough stalks of the later season plants are generally avoided when other forage is available (Detmers 1927; Leininger 1988; De Bruijn and Bork 2006; Sieberg et al. 2007). Goats are the exception - they will eagerly devour flowering thistle plants, and are less attracted to the vegetative rosette stage (Popay and Field 1996). Palatability of later season plants for other livestock may be increased by spraying plants with a dilute molasses solution (Olson 1999). The logistics of using livestock (e.g., goats, sheep, cattle) as a control measure can be challenging, including lack of animal availability when and where needed, training of livestock to choose weeds as forage, water/fencing/herding costs, management of intensity/duration to avoid overgrazing, and predator management (e.g., mountain lions).

Biological control

Biological control (natural and human-facilitated) has demonstrated limited success thus far. Native North American insects such as the painted lady butterfly (*Vanessa cardui* L.) larvae naturally defoliate Canada thistle, but damage is insufficient to prevent growth and spread of the plant (Moore 1975; Myres 1985). A significant number of non-native insects have been tested for Canada thistle control, including a seed head weevil (*Larinus planus*), a foliage feeder (*Cassida rubiginosa*), a stem mining weevil (*Ceutorhynchus litura*), and a stem/shoot gall fly (*Urophora cardui*). Unfortunately, all have shown limited success on their own (Peschken and Derby 1992; Hein and Wilson 2004; Reed et al. 2006);

or conversely, have proven dangerous to non-target plant species, in the case of *Larinus planus* attacking the native thistle *Cirsium undulatum* (Nutt.) Spreng. var. *tracyi* (Rydb.) S.L. Welsh (Louda and O'Brien 2002).

The bacterium *Pseudomonas syringae pv. tagetis* (PST) shows promise as a disease agent in Canada thistle, but environmental conditions and per bacterium toxin production must still be optimized before this is a viable control avenue (Hoeft et al. 2001; Gronwald et al. 2002; Tichich et al. 2006). Development of a myco-herbicide against Canada thistle is still in its infancy (Guske et al. 2004). According to the Insectary of the Colorado Department of Agriculture, biological controls for Canada thistle are currently available for dispersal, but have not yet been shown to be a consistently effective tool for control in Colorado (M. Stricklan, personal communication, 2009). Some organisms proven to be effective for controlling Canada thistle have also been found to be serious pests of crop plants, limiting their use in or near crop lands.

Plant Competition/Revegetation

A variety of studies have been performed on the potential of using desirable competitive plant species to combat Canada thistle, often in conjunction with other control methods. Many of these studies have focused on the use of exotic plant species for this purpose. Ang et al. (1994) found tall fescue (*Schedonorus phoenix* [Scop.] Holub) and crownvetch (*Securigera varia* [L.] Lassen), seeded at 1.25 times the recommended seeding rate, to successfully reduce Canada thistle when seeded in conjunction with a defoliating insect *Cassida rubiginosa.* Alfalfa and biennial sweetclover (*Melilotus* sp.) have proven to be competitive with Canada thistle, but the grasses redtop (*Agrostis gigantea* Roth), orchardgrass (*Dactylis glomerata* L.), and Timothy (*Phleum pratense* L.) have not proven effective (Detmers 1927; Schreiber 1967; Ominski et al. 1999).

Wallace (2001) stated that Canada thistle control can be gained by establishing a vigorous, dense stand of alfalfa or red clover (*Trifolium pratense* L.). Hodgson (1968) observed alfalfa to consistently control Canada thistle with twice annual mowing. Schreiber (1967) reduced Canada thistle infestations with a combination of alfalfa seeding and 4 years of twice-annual mowing on a grazed pasture. Beck (2008) stated that alfalfa is not a successful competitor with Canada thistle until after it is established, and Canada thistle must be adequately controlled before alfalfa is seeded. Conversely, Hodgson (1968) found that while Canada thistle increased during the first year of establishment of an alfalfa and forage grass mixture, Canada thistle significantly decreased by 89% the next year, and by 100% in the fourth year. When using alfalfa and mowing to control Canada thistle, it is common to see an increase in Canada thistle during the 6th year of alfalfa growth as the alfalfa stand weakens and thins with age (Hodgson 1968). Alfalfa yields may be reduced in 5 year old stands and older, and it is recommended to shift to a different crop after 4 or 5 years. Ominski et al. (1999) found that simply including alfalfa in crop rotations resulted in lower infestation rates of Canada thistle. Other researchers that have found cover crops to successfully inhibit Canada thistle growth have questioned whether it is the competitive aspect of the crop or the physical management practice associated with crop installation (such as tilling) that is the more critical component for success (Donald 1990).

Wilson and Kachman (1999) used western wheatgrass, intermediate wheatgrass, Russian wildrye (*Psathyrostachys juncea* [Fisch.] Nevski), tall fescue, and a hybrid wheatgrass to produce 66, 74, 76, 78, and 85% control of Canada thistle, respectively. The hybrid wheatgrass used was a cross between bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve) and quackgrass (*Elymus repens* [L.] Gould). Quackgrass has allelopathic properties that may suppress the growth of plants like Canada thistle (Weston et al. 1987). Alfalfa also produces allelochemicals called saponins (Miller 1983; Miller et al. 1988), which might contribute to the plants' effectiveness for controlling Canada thistle. Thrasher et al. 1963 also found tall fescue seeding plus mowing to reduce Canada thistle density by 60 to 70%, but fertilization and irrigation also likely contributed to this success. Derscheid et al (1961) used a combination of "bromegrass" seeding and 3 years of mowing to suppress Canada thistle growth by 90%. At a site in Illinois, a Canada thistle infestation was revegetated with transplants and broadcast seeding, then burned 5 years later. Continued annual burning almost completely eliminated Canada thistle after 8 years (Kirt 1996). Unfortunately, factors such as herbivory can reduce the effectiveness of competitive seeding. Edwards et al. (2000) found that grazers on seeded plots preferred the newly seeded species to that of the co-occurring Canada thistle, thus reducing the new competition and favoring Canada thistle growth.

Societal and Ecological Importance

Agricultural Importance

Canada thistle infestation can result in significant financial losses as a result of reduced crop yields (Donald and Khan 1996; Pimentel et al. 2000; Stevenson et al. 2001) and decreased rangeland productivity (Hartley and James 1979; Haggar et al. 1986; Bork et al. 2005). Crop yields decline from increased competition for valuable resources, and harvesting can be made more difficult (Malicki and Berbeciowa 1986; Moore 1975). Donald and Kahn (1996) found spring wheat (*Triticum aestivum* L.) yield decreased significantly as Canada thistle shoot density increased. Mamolos and Kalburtji (2001) observed that Canada thistle presented significant competition with winter wheat. Canada thistle can also contaminate saleable crops such as seed and hay, reducing its value. This invader can also serve as a host for crop pests (Detmers 1927; Moore 1975). Infested rangelands result in reduced forage productivity (Grekul and Bork 2004), and the abrasive plant can discourage grazing in infested areas, reducing forage availability. Handling of stock can be difficult in areas of dense infestation (Popay and Field 1996).

While Canada thistle has primarily negative impacts on agriculture, it may also have some benefits. The strong odor of its flowers attracts a wide range of insects, including moths, bees, wasps and beetles (Proctor et al. 1996; El-Sayed et al. 2008). Researchers are currently exploring the use of volatile compounds produced by these flowers to create insect traps which can be used for monitoring and controlling economically important agricultural pests on-site, as well as for use around cargo facilities and ports of entry for detecting invasive or unwanted insects (Theis 2006; El-Sayed et al. 2008).

Ecological Importance

The invasion of Canada thistle can result in ecological losses such as reduction in wildlife habitat as well as the reduced integrity of native ecosystems (Hutchison 1992; Duncan et al. 2004). Low species diversity often accompanies dense stands of Canada thistle (Stachon and Zimdahl 1980). Prolific seed production by the plant can also impact infested sites for years to come, with almost 25% of the seedbank at one treated site found to be comprised of Canada thistle seed (Travnicek et al. 2005). Aside from the societal value that is placed on the importance of maintaining native ecosystems, the infestation of natural areas by Canada thistle can also translate into a societal (and potentially financial) loss through reduced land use by hunters, naturalists, and other recreationists (Morishita 1999; DiTomaso 2000).

However, when Canada thistle is controlled, ecosystems can begin to recover, with an increase in species richness resulting from effective treatment (Krueger-Mangold et al. 2002). Although its benefits are few for native ecosystems, Canada thistle has been found to provide cover and nesting spots for wildlife (Hammond and Mann 1956; Suring and Vohs 1979). Birds also eat its seed, and grazing animals will eat young thistle shoots (Detmers 1927).

Edible and Medicinal Uses

Canada thistle has been used by Native Americans for a variety of medicinal purposes. The Iroquois used the roots to treat mouth sickness, and the Abnaki used them to treat worms in children (Moerman 1998). The Mohegan employed the leaves as a mouthwash for infants, and used the plant to treat lung infections (Moerman 1998). Both the Mohegan and the Montagnais created a decoction from the plant to treat tuberculosis, and the Ojibwa used Canada thistle as a "bowel tonic" (Moerman 1998). The Chippewa also used it as a tonic and diuretic, as well as an astringent (Densmore 1974).

Canada thistle has been used as a food source in Russia and by Native Americans in the United States (Rogers 1928). It can be used similar to asparagus if the roots and shoots are collected in early spring. Beekeepers consider Canada thistle to be important for honey production, producing high quality honey that is considered comparable to clover honey (Howes 1979). Canada thistle flowers secrete abundant nectar that is easily available to the honey bee and other insects.

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CHAPTER 1: GREENHOUSE STUDY

MANAGEMENT OF CANADA THISTLE (*Cirsium arvense*) UTILIZING CLIPPING AND SEEDING OF GRASSES

ABSTRACT

Chemical restrictions, ecological concerns, liability issues, and public sentiment present increasing challenges to land managers faced with controlling the highly invasive plant Canada thistle (*Cirsium arvense* [L.] Scop.). Although traditional herbicide application can be an effective control strategy, increasing limitations force managers of sensitive environments such as national parks, wildlife refuges, protected waterbodies or waterways, and other conservation lands to search for effective control alternatives. A greenhouse study was conducted to test the effectiveness of clipping (to simulate field mowing) and grass seeding as a possible control alternative for Canada thistle. Two native grasses (western wheatgrass [*Pascopyrum smithii* {Rydb.} A. Löve] and streambank wheatgrass [*Elymus lanceolatus* {Scribn. & J.G. Sm.}Gould ssp. *lanceolatus*]) and one sterile hybrid cross between common wheat (*Triticum aestivum* L.) and tall wheatgrass (*Thinopyrum ponticum* [Podp.] Z.W. Liu & R.C. Wang) called Regreen™ were used. Canada thistle treatments included three levels (clipped, unclipped, Canada thistle absent). Grass seeding treatments included five levels (no grass, streambank wheatgrass, western wheatgrass, Regreen, or Regreen + western wheatgrass). This experiment evaluated 14 treatments (six replicates) applied to potted Canada thistle and grass plants grown for 51 weeks in the greenhouse. Canada thistle and grass shoot biomass were harvested and analyzed to determine the effects of clipping and grass seeding on Canada thistle growth, as well as the effect of Canada thistle on grass growth. Clipping inhibited Canada thistle growth (by 60%), while grass seeding had no effect. Presence of Canada thistle inhibited grass growth for all seeding treatments except the Regreen + western wheatgrass treatment, which was unaffected. My results suggest that considering synergistic effects (Regreen + western wheatgrass treatment) of using multiple species for restoration of Canada thistle-infested sites may be critical, and that cutting of Canada thistle may be useful for reducing Canada thistle growth in restoration efforts.

INTRODUCTION

Canada thistle (*Cirsium arvense* L.), a highly invasive non-native perennial plant, continues to challenge land managers across the United States. Despite our long history with this invader (noxious weed legislation was first enacted against Canada thistle in Vermont in 1795 [Detmers 1927]), truly successful control mechanisms have yet to be established for sensitive areas. Herbicides such as Tordon® 22K (picloram) (Dow AgroSciences LLC, Indianapolis, IN) have proven quite successful in less regulated upland areas, but many areas infested with Canada thistle require an alternative control because of aquatic concerns, herbicide restrictions, threats to endangered species, liability issues, or human/animal-sensitivity issues. These more sensitive areas often occur in wildlife refuges, natural areas, state and national parks, near protected water-bodies or waterways, and on other private or public conservation lands, creating special challenges for successful weed management. While the recently released lower toxicity herbicide Milestone® (aminopyralid) (Dow AgroSciences LLC, Indianapolis, IN) may present a viable control alternative for managers facing herbicide restrictions, additional research on this new product is necessary, and still leaves managers without a solution where herbicide use is prohibited.

Control methods for Canada thistle commonly used or tested in more sensitive areas include handpulling, digging, biological control/grazing, cultivation, fertilizer addition, aquatic labeled herbicides (e.g., Rodeo® [glyphosate], Monsanto Agricultural Company, St. Louis, MO), 'natural' herbicides (e.g., Burnout II Weed and Grass Killer, [citric acid + clove oil], St Gabriel Laboratories, Orange, VA), mowing, or revegetation. Unfortunately, when used alone, most of these methods have demonstrated limited utility for significantly reducing Canada thistle populations over time. Two control measures that may show some promise when used in combination, however, are mowing and revegetation.

Mowing is currently recommended for control of Canada thistle on many weed management information websites (ACDPWD 2003; WIDNR 2004; OSU Cooperative Extension 2008; Plant Conservation Alliance 2009; USACE 2009) and is popular with landowners enrolled in the Conservation Reserve Program (Holen et al. 2007). While the utility of strategically timed mowing to prevent seed production is well documented (Derscheid and Schultz 1960; Thrasher et al. 1963; Moore 1975), research on mowing as a stand alone strategy for Canada thistle management has shown mixed results. Grekul and Bork (2007) reported that a one-time mowing treatment temporarily increased Canada thistle shoot density the year of treatment, and 2 years later shoot density and biomass were not different from the unmowed control. Beck and Sebastian (2000) found that mowing three times annually for 2 years reduced Canada thistle density by 85% at a subirrigated site, but did not provide significant control at a dryland site. Hodgson (1968) reported an 86% reduction in Canada thistle density after the first year as a result of mowing an infested alfalfa (*Medicago sativa* L.) field twice annually. This treatment provided complete control after 4 years, demonstrating the potential role that desirable competitive plant species (e.g., alfalfa) may play in enhancing the success of mowing. Overall, however, these variable results demonstrate the need for further evaluation of mowing as a control strategy.

Revegetation has shown promise as a secondary tool for gaining long-term control of Canada thistle after application of a given control measure, and is becoming increasingly popular as a component of integrated weed management programs in general. Regardless of the weed species targeted, a lack of competition from desirable plants post-control often leads to reestablishment of unwanted weeds (Sheley and Carpinelli 2005; Travnicek et al. 2005). Several forbs and grasses have shown promise as effective competitors with Canada thistle, including tall fescue (*Schedonorus phoenix* [Scop.] Holub) and crownvetch (*Securigera varia* [L.] Lassen) (Ang et al. 1994). Alfalfa and biennial sweetclover (*Melilotus* sp.) have also demonstrated competitiveness with Canada thistle, while grasses such as Timothy (*Phleum pratense* L.), orchardgrass (*Dactylis glomerata* L.), and redtop (*Agrostis gigantea* Roth) have not (Detmers 1927; Schreiber 1967; Ominski et al. 1999). One study found that the use of competitive grasses for Canada thistle control was as effective as herbicide application over a 3-year period (Wilson and Kachman 1999). Unfortunately, most plants tested thus far as competitors against Canada thistle are non-native species. While use of non-natives is understandable because they are frequently more aggressive, seeding of non-native plants is often strongly discouraged or prohibited in more sensitive areas. Clearly, land managers of more sensitive areas face a variety of challenges when attempting to control Canada thistle infestations. Lack of effective long-term control measures combined with little research specific to their needs has left those tasked with restoring native habitat to infested lands with limited options.

The following experiment was developed to test the effectiveness of clipping (to simulate field mowing) and seeding of plant species acceptable for use in more sensitive areas for control of Canada thistle. Specifically, the objectives of this study were to: 1) determine the effects of clipping and grass competition on Canada thistle growth, 2) compare the effectiveness of the different seeding treatments on Canada thistle growth, and 3) determine the effect of Canada thistle on the growth of each of the seeded species. Two native grasses (western wheatgrass [*Pascopyrum smithii* {Rydb.} A. Löve] and streambank wheatgrass [*Elymus lanceolatus* {Scribn. & J.G. Sm.} Gould ssp. *lanceolatus*]) and one sterile commercial hybrid cross between common wheat (*Triticum aestivum* L.) and tall wheatgrass (*Thinopyrum ponticum* [Podp.] Z.W. Liu & R.C. Wang) called Regreen™ were chosen for this research.

It was hypothesized that both clipping and grass competition would reduce Canada thistle shoot biomass, and that the effect of the two factors together would be greater than either alone. It was also predicted that the grass seeding treatments containing Regreen would produce a greater impact on Canada thistle growth than the native grasses seeded alone with Canada thistle because of the more aggressive nature of Regreen. Additionally, it was expected that grass growth would be inhibited by the presence of Canada thistle.

MATERIALS AND METHODS

A greenhouse study was conducted where Canada thistle plants were treated with unique combinations of clipping (used to simulate mowing) and grass seeding. Two response variables were measured: Canada thistle shoot biomass and grass shoot biomass. Canada thistle biomass was analyzed using a two by five factorial design consisting of two levels of Canada thistle clipping (clipped, unclipped) and five levels of grass seeding (no grass, streambank wheatgrass, western wheatgrass, Regreen, or western wheatgrass + Regreen). Grass biomass was analyzed using a three by four factorial design consisting of three levels of Canada thistle (clipped, unclipped, absent) and four levels of grass seeding (streambank wheatgrass, western wheatgrass, Regreen, or western wheatgrass + Regreen). These two analyses combined investigated a total of 14 unique treatment combinations applied to Canada thistle or grass plants (6 replicates per treatment) grown in potting soil for 51 weeks in a greenhouse.

Selection of Grass Species

Western wheatgrass and streambank wheatgrass were chosen for their aggressive underground growth habit, early spring germination prior to Canada thistle growth, wide geographic and habitat range throughout the western United States, tolerance of drought and temperature extremes, and broad availability (Ogle 2000; Ogle 2006; USDA PLANTS 2009). These two species have also demonstrated prior success in control efforts of other weeds including Russian knapweed (*Acroptilon repens* [L.] DC.) (Bottoms and Whitson 1998; Benz et al. 1999), leafy spurge (*Euphorbia esula* L.) (Lym 1998), cheatgrass (*Bromus tectorum* L.), and musk thistle (*Carduus nutans* L.) (Whitson and Koch 1998; Rose et al. 2001). Western wheatgrass has even demonstrated some success against Canada thistle (Wilson and Kachman 1999). The hybrid grass Regreen© was chosen for its ability to establish aggressive root growth more quickly than native grasses, and because of its acceptability for use in more sensitive areas due to its sterile nature.

Source of Plant Material

Western wheatgrass (variety 'Arriba') seed was obtained from Pawnee Buttes Seed Inc. (Greeley, CO, USA). Streambank wheatgrass (variety 'Sodar') seed was obtained from Granite Seed (Lehi, UT, USA). Regreen© seed was obtained from Rainier Seed Company (Davenport, WA, USA). Canada thistle horizontal roots were collected from a site near Fort Collins, Colorado, USA (lat 40°33'46"N, long 105°00'24"W, elevation 1,491m).

Horizontal roots were collected 26 August 2004, and placed in sealed plastic bags with soil collected from the same location. The bags were transported back to the laboratory in a cooler. The bagged soil was moistened, and bags were stored at 6°C in the dark for 8 weeks to prevent sprouting before their use in the experiment. All plant species nomenclature within this paper follows the USDA PLANTS Database (USDA PLANTS 2009).

Plant Preparation and Treatment

On 23 October 2004, refrigerated Canada thistle horizontal root sections were cut into 2.5 cm pieces with a minimum of one bud per piece. The diameter of cut horizontal root pieces ranged from 0.2 to 0.65 cm. Pieces were soaked in 2.54 cm of tap water in a covered tray under refrigeration (6°C) in the dark for 28 h. Horizontal root pieces were planted at a depth of approximately 1.3 cm in 25- x 52-cm plastic flat trays filled with 2.5 cm of completely wetted Scotts MetroMix 350 potting soil (Sun Gro Horticulture, Bellevue, WA). Pieces were planted in rows in two trays for a total of 48 pieces per tray. Each tray was thoroughly watered after planting and kept moist.

Each germinated horizontal root piece used in the experiment was transferred to a 10.2-cm diameter round plastic pot in January 2005 and grown for 7 weeks. Surviving plants were then transferred to 3.8-L plastic pots filled with Scotts MetroMix 350 potting soil (1 plant per pot), and grown there for the remainder of the experiment. After 10 weeks of postemergence growth, 12 grass seeds were added to each of the 60 Canada thistle pots. Each pot received 12 grass seeds (six of each species for the two-species treatment). Western wheatgrass and streambank wheatgrass seeds were planted approximately 1.3 cm deep, while Regreen seeds were planted approximately 0.6 cm deep. More seeds were planted than needed to assure establishment of a sufficient number of plants. Twenty four grass control pots of the same size with the same growth medium as the Canada thistle pots were also seeded with either single grass species or the two-grass species combination. The result was six replicate pots prepared for each treatment combination.

Six weeks after initial seeding, adequate grass seed germination was obtained and pots were thinned to four grass plants per pot. Regreen + western wheatgrass treatments were thinned to two grass plants per species. Because of limited project resources, only one grass species combination treatment could be evaluated. Western wheatgrass was chosen for this combination with Regreen because it is considered to have a more aggressive rhizomatous growth habit than streambank wheatgrass (Ogle 2000). At 17 weeks of Canada thistle plant growth, the single clipping treatment was performed. Canada thistle plants receiving the clipping treatment were clipped with hand shears 9 cm above the soil surface to simulate mowing. This height was chosen for the clipping treatment as it is the approximate mowing height used in field studies for mowing of Canada thistle (Beck and Sebastian 1993, 2000). Clipped shoot biomass for each Canada thistle plant was placed in individual paper bags, dried at 55°C to constant mass and weighed to determine Canada thistle shoot biomass. Grasses were not clipped as they had not yet reached mowing height.

Experimental plants grew in the greenhouse for an additional 34 weeks after the clipping treatment and were watered and weeded for nonexperimental species throughout the study. Plants received natural light supplemented with 400-W high pressure sodium vapor bulbs to obtain a 16-h photoperiod. The supplemental lighting was located 1.5 m above the greenhouse benches. The greenhouse temperature was maintained at approximately 22 ± 5 °C. Pots were randomly assigned to positions on the greenhouse bench and re-randomized and moved every 6 weeks throughout the experiment to minimize effects of potential differences in light or temperature in the greenhouse. For the final harvest, experimental plants were clipped at the soil surface and separated into grass shoot biomass or Canada thistle shoot biomass for each pot and placed in individual paper bags.

It was not possible to accurately separate root biomass when more than one species was grown per pot, thus root biomass was not considered further. Plant material was dried at 55°C to constant mass and weighed to determine the shoot biomass for each plant species.

Statistical Analysis

Two dependent variables were analyzed: *Canada thistle shoot biomass* and *grass shoot biomass*. Treatments applied to Canada thistle plants were organized into a two by five factorial design consisting of two levels of Canada thistle clipping (clipped, unclipped) and five levels of grass seeding (no grass, streambank wheatgrass, western wheatgrass, Regreen, or western wheatgrass + Regreen). Treatments involving grass seeding resulted in a three by four factorial design consisting of three levels of Canada thistle (clipped, unclipped, absent) and four levels of grass seeding (streambank wheatgrass, western wheatgrass, Regreen, or western wheatgrass + Regreen). The data for the dependent variable *Canada thistle shoot biomass* were analyzed using a univariate two-way analysis of variance (ANOVA). The data for the dependent variable *grass shoot biomass* were also analyzed using a univariate two-way ANOVA. The dependent variables *Canada thistle shoot biomass* and *grass shoot biomass* were transformed using natural log transformation to meet assumptions of the analyses. Posthoc pair-wise comparisons of interest were conducted using the Tukey Honestly Significant Difference (HSD) method. All data were analyzed using R 2.8.1 statistical software (R Development Core Team 2009). An alpha level of 0.05 was used for all analyses.

RESULTS

Clipping reduced Canada thistle shoot growth (Fig. 1.1, $F_{1,50} = 126.54$, $P < 0.001$). Mean shoot biomass of Canada thistle in the clipped treatments was lower than the unclipped Canada thistle treatments, regardless of the presence of grass or species seeded. Clipped plants produced less than half the growth of their unclipped counterparts, despite having 34 weeks for regrowth. Grass seeding did not affect shoot biomass of Canada thistle ($F_{4,50} = 0.78$, $P = 0.544$), and there was no interaction between clipping and grass seeding on Canada thistle shoot biomass grown in the greenhouse ($F_{4,50} = 0.85$, $P = 0.500$).

Grass shoot biomass was affected by the presence and clipping status of Canada thistle ($F_{2,56} = 43.47$, $P < 0.001$), and varied with grass species seeded ($F_{3,56} = 37.35$, $P < 0.001$), as indicated by the interaction between Canada thistle treatment and grass species seeded ($F_{6,56} = 2.38$, $P = 0.040$). The presence of unclipped Canada thistle reduced grass shoot biomass below that of the control (Canada thistle absent) when grasses were grown individually (Fig. 1.2), regardless of the species. When grass species were paired (Regreen + western wheatgrass), however, the presence of unclipped Canada thistle had no effect on grass shoot biomass.

With the exception of western wheatgrass grown as a single grass species with Canada thistle, all other single grass species (streambank wheatgrass [$P = 0.124$], Regreen [$P = 0.231$]) and the grass combination ($P = 0.999$) had no detectable difference in shoot biomass when grown in the presence of clipped Canada thistle or the complete absence of Canada thistle (Fig. 1.2). When grown only with Canada thistle, western wheatgrass shoot biomass was lower in the presence of clipped Canada thistle than when grown in the absence of Canada thistle ($P = 0.024$).

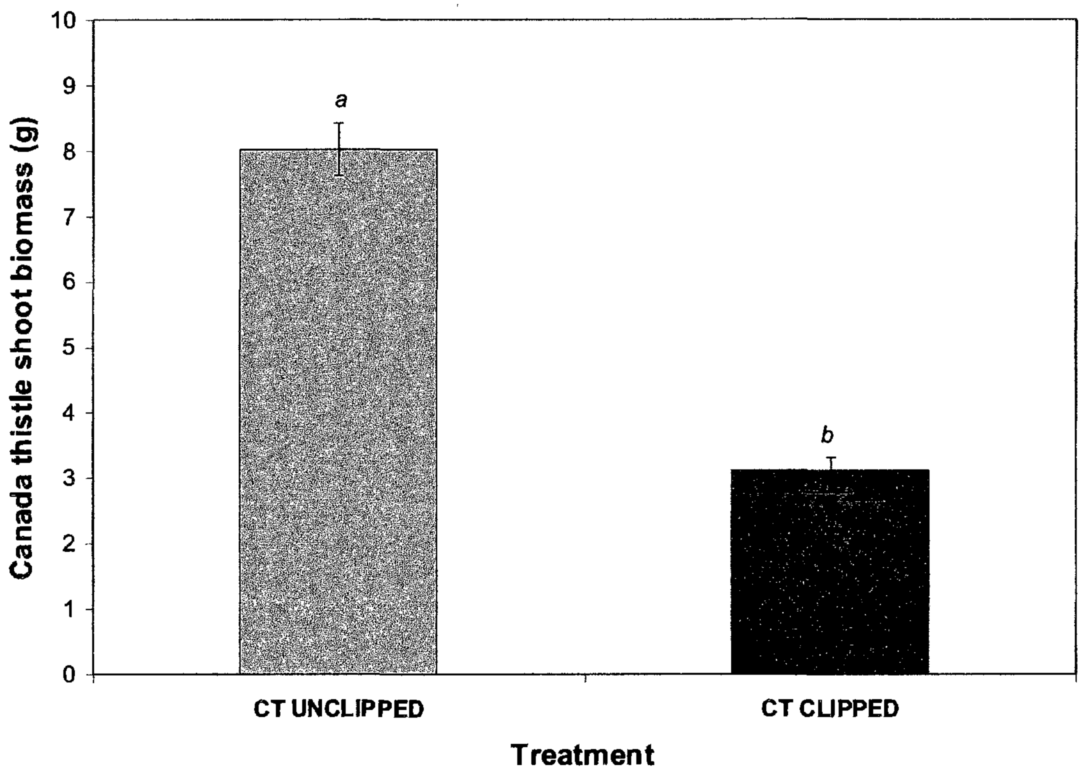

Figure 1.1. Mean (+ SE, n =30) Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass for clipped versus unclipped treatments. Canada thistle (CT) plants were grown alone or with competing grasses. Competing grass species included western wheatgrass (*Pascopyrum smithii* [Rydb. A. Löve) (WW), streambank wheatgrass (*Elymus lanceolatus* [Scribn. & J.G. Sm.] Gould ssp. *lanceolatus*) (SB), Regreen (*Triticum aestivum* x *Thinopyrum ponticum*) (RG), and a combination of Regreen and western wheatgrass (RG+WW). Grass presence did not significantly affect Canada thistle shoot biomass growth. Means are presented here in the untransformed scale, although analysis was conducted on transformed data. Means with letters in common are not significantly different using Tukey's HSD (α = 0.05).

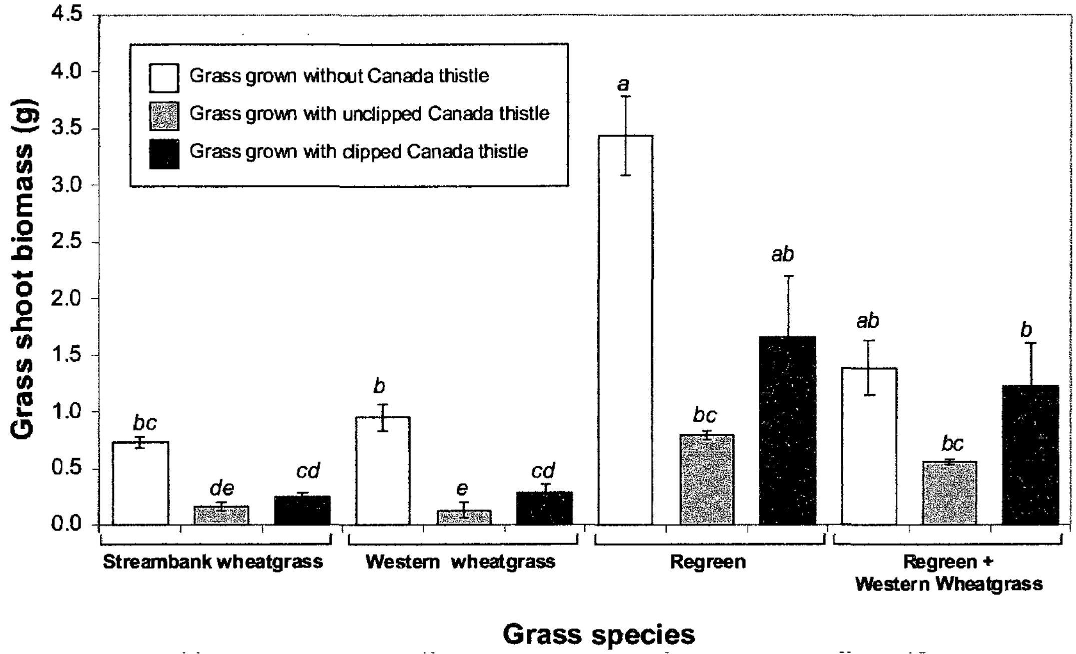

Figure 1.2. Mean (+ SE, n = 5,6) grass shoot biomass by treatment for grass species grown in the presence or absence of Canada thistle (*Cirsium arvense* [L.] Scop.) (CT). When present, the Canada thistle was either clipped at a height of 9 cm to simulate mowing (one-time event at beginning of experiment), or left unclipped. Grass species include western wheatgrass (*Pascopyrum smithii* [Rydb. A. Löve) (WW), streambank wheatgrass (*Elymus lanceolatus* [Scribn. & J.G. Sm.] Gould ssp. *lanceolatus*) (SB), Regreen (*Triticum aestivum* x *Thinopyrum ponticum*) (RG), or a combination of Regreen and western wheatgrass (RG+WW). Means are presented here in the untransformed scale, although analysis was conducted on transformed data. Means with letters in common across all grass species and treatments are not significantly different using Tukey's HSD ($\alpha = 0.05$).

The two most successful grass treatments overall in terms of biomass production appear to be Regreen and Regreen + western wheatgrass. Regreen + western wheatgrass was the only grass treatment where the presence of unclipped Canada thistle did not produce a detectable effect ($P = 0.404$). In fact, Regreen + western wheatgrass was the only grass treatment where no detectable difference in shoot biomass was found regardless of Canada thistle presence or absence or clipping status (Fig. 1.2). All other grass treatments (including western wheatgrass and Regreen grown separately) produced less shoot biomass in the presence of Canada thistle, at least in its unclipped state.

DISCUSSION

Canada Thistle Response to Clipping

The significant reduction in aboveground Canada thistle biomass as a result of clipping, despite adequate time for regrowth, confirms the hypothesis and demonstrates the potential utility of mechanical cutting as a control measure for Canada thistle. Similar results have been demonstrated in field trials conducted by other researchers. In a 2000 field study, Beck and Sebastian reported that mechanical cutting in the form of mowing reduced Canada thistle growth by 85%, in the absence of any other treatment. Amor and Harris (1977) reported a reduction in Canada thistle growth by 95% after 2 years of field mowing. Another study found that mowing could virtually eliminate Canada thistle after 4 years (Welton et al. 1929).

Conversely, Grekul and Bork (2007) found that a one-time field mowing treatment did not affect shoot biomass in the year of treatment, or 2 years after treatment. Another study conducted in Germany similar to my study also had conflicting results. Kluth et al. (2003) conducted a pot experiment in the field over a 2 year period where Canada thistle plants were clipped once annually to simulate mowing. The first year, clipping resulted in an increase in Canada thistle shoot biomass above that of the unclipped control. It was only in the second year (beyond the range of my study) that the clipped Canada thistle plants performed similarly to my study, where clipped plants were found to produce less shoot biomass than the unclipped controls.

One of the factors that differed between the German study and my study was the height of clipping. Clipping height was very high (30 cm) in the German study, leaving a significant amount of photosynthesizing foliage behind, which may have facilitated first year regrowth. The German authors theorize that their mild clipping treatment may have mimicked moderate herbivory, where plant growth would be stimulated, not inhibited, by removal of top growth. Conversely, the clipping treatment in my experiment was performed at a standard mowing height of 9 cm and left little foliage behind, likely inhibiting regrowth by reducing photosynthetic carbohydrate production. Removal of plant top-growth through activities such as mowing is believed to weaken Canada thistle plants over time by inhibiting aboveground photosynthetic carbohydrate production and transport, while also forcing depletion of root carbohydrate reserves to support regrowth after cutting (Boerboom and Wyse 1988). This may explain the inability of the clipped plants to fully recover after the second clipping in the German study.

Another difference between the German study and my study was the timing of clipping. Root carbohydrate reserves of Canada thistle are considered to be at their lowest point at the initiation of flowering (Welton et al. 1929; Arny 1932). In my study, 98.8% of the Canada thistle plants were flowering at the time they were clipped. Clipping my plants at their lowest point of reserves may have enhanced the effectiveness of my clipping treatment. In the German study, clipping was performed in June of each year, before plant flowering. In that study, flowering occurred later in the growing season, with only 6% of control heads beginning to flower by August the first year, and 43% of control heads beginning to flower by August the second year. With flowering occurring earlier the second year, it is likely that the plant root carbohydrate reserves were lower at the time of clipping than the first year, perhaps also contributing to the resulting decrease in biomass of clipped plants the second year. It may be that height of clipping and timing of clipping relative to flowering play a critical role in the relative success of clipping treatments for control of Canada thistle.

Canada Thistle Response to Grass Seeding

Although the presence of grass plants did not inhibit Canada thistle shoot growth in this study (contrary to the hypothesis), the below-ground effect of the grasses on Canada thistle may have been significant. A key requirement for grass species inclusion in this study was an aggressive underground growth habit. Had it been possible to separate the root biomass of Canada thistle and the grasses in each pot, a measurable effect of these grasses on the Canada thistle root biomass may have been observed. Ferrero-Serrano et al. (2008) found the native grass alkali sacaton (*Sporobolus airoides* [Torr.] Torr.) to inhibit Canada thistle root growth when grown together in a greenhouse study, but the grass demonstrated no effect on shoot biomass of Canada thistle. A 1-yr greenhouse experiment such as mine also may not have been long enough for root competition to translate to changes in aboveground Canada thistle plant growth. As the belowground growth is where the strengths of these grasses lie, their true utility may not become apparent for several growing seasons. Ang et al. (1994) found that Canada thistle shoot biomass fluctuated significantly in the first 2 years of their field study when grown in the presence of plant competitors, and they concluded that plant competitors require more than two seasons of growth before they can be effective in suppressing Canada thistle.

Conversely, Friedli and Bacher (2001) found that seeding of competitive exotic grasses such as perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* [Lam.] Husnot), and orchardgrass reduced Canada thistle shoot biomass grown in pots each year of their 2-year study. Again, because native grasses can be slower to establish than exotic grasses (Waldron et al. 2005), the relatively short length of my experiment may not have allowed the native species in my study adequate time to influence the aboveground growth of Canada thistle. As for Regreen, the one exotic grass used in this experiment, it is unclear why it did not produce a significant effect on Canada thistle growth on its own, as hypothesized.

The grasses for this experiment were also selected for their drought tolerance, a benefit that was not tested under greenhouse conditions, but may be important under hot, dry field conditions. For example, Laurialt et al. (2005) found that western wheatgrass established and maintained cover across a range of soil moisture availabilities, while growth of Canada thistle populations may be suppressed when soil moisture availability is reduced (Donald and Prato 1992). Hot, dry years may allow grasses such as western wheatgrass to gain a foothold over Canada thistle under field conditions.

Canada Thistle Response to Grass Seeding x Clipping

Contrary to the hypothesis, there was no interactive effect between clipping and grass seeding in this greenhouse study, although different results may be expected in field trials. Field studies performed by Wilson and Kachman (1999) found that seeding perennial grasses and mowing twice annually for 3 years reduced Canada thistle density by more than 90%. Thrasher et al. (1963) also found in field trials with grass seeding and mowing for control of Canada thistle that in the early stages of the experiment, the competitive ability of the grasses was important in controlling Canada thistle, but that as the age of the grass stand increased and mowing continued, the effects of mowing became more important than competition between the grass and Canada thistle.

Grass Response to Canada Thistle Presence and Grass Species Seeded

The negative effect of unclipped Canada thistle on shoot biomass of each of the grasses grown as single species was hypothesized and expected because previous researchers have demonstrated the negative impacts Canada thistle can have on the growth of other plant species (Bendall 1975; Stachon and Zimdahl 1980; Wilson 1981). What was surprising in this study was the failure of Canada thistle to impact the growth of the paired grass species (Regreen + western wheatgrass). Regardless of clipping status, the presence of Canada thistle produced no detectable effect on the growth of the Regreen + western wheatgrass treatment, while it negatively affected each of these species grown separately. The mechanism of resilience for this pairing is unclear.

One possible explanation for this response may be the cumulative effect of allelochemicals potentially produced by both Regreen and western wheatgrass. Common wheat (one of the hybrid components of Regreen) is believed to have allelopathic potential against weeds in cropping systems (Ma 2005). Additionally, there is some evidence of the production of phytotoxic allelochemicals by western wheatgrass (Bokhari 1978; Kohli et al. 2001). The combination of these allelochemicals may have neutralized the competitive influence of Canada thistle. Although it did not significantly inhibit Canada thistle growth in this greenhouse study, it is expected that under more variable field conditions and if allowed to grow longer, the Regreen + western wheatgrass combination could be a successful competitor against Canada thistle.

This study also elucidated the potential utility of mechanical cutting of Canada thistle for the enhancement of grass growth when only a single desirable species is seeded.

While shoot biomass growth of all of the single grass species was inhibited by the presence of unclipped Canada thistle, the clipping of Canada thistle resulted in greater grass growth for each single species equivalent to grass growth in the complete absence of Canada thistle (with the exception of western wheatgrass). Grass growth may benefit from Canada thistle cutting not only because of the weakened state of the Canada thistle plants, but also from the decrease in competitive plant canopy.

The lack of benefit to western wheatgrass from Canada thistle clipping in this experiment may be a product of short study duration. Regreen is considered to establish and produce growth more quickly than many species considered for revegetation (Glen 1992). Streambank wheatgrass also establishes and matures more quickly than western wheatgrass (Ogle 2000). It may take longer for the benefits of Canada thistle clipping to translate into increased growth in western wheatgrass.

CONCLUSIONS

Controlling Canada thistle in more sensitive areas has become increasingly difficult. Chemical restrictions and ecological concerns are increasing, as are liability concerns. Compliance with public sentiment presents a special challenge for long-term planning and treatment. Many sensitive environments are also faced with increasing pressures from human use and other disturbance that exacerbate Canada thistle infestations. The development of acceptable effective long-term control measures for restoring these Canada thistle infested lands is critical. My results demonstrate the potential for both mechanical cutting and grass seeding as effective tools for restoration of Canada thistle infested sites. The one-time clipping performed in my study resulted in a decrease in Canada thistle biomass, and has implications for the use of mowing as a field control measure. As revegetation tools, the grasses used in this experiment proved to be tolerant of Canada thistle presence, and the combination of Regreen and western wheatgrass demonstrated the ability to grow equally well regardless of Canada thistle presence or cutting status. This is an important finding, as it is generally considered that almost any control measure for Canada thistle requires multiple applications, with complete eradication impossible or at least requiring multiple seasons. Thus any useful revegetation species must be capable of growing in concert with Canada thistle until it can be controlled. The particular success of the combination grass seeding also emphasizes the potential importance of the synergistic effects of using more than one species for restoration of Canada thistle infested sites.

While the seeded grasses did not act as a control measure per se in this study (did not significantly inhibit Canada thistle shoot biomass), it is believed that a study performed over a longer duration, in a field setting, would more clearly demonstrate the additional advantages of these grasses for restoration of Canada thistle infested sites. The aggressive underground growth of these grasses may not translate to observable aboveground effects on Canada thistle for several seasons (regardless of additional control measures used), and the characteristics of these grasses for which they were initially chosen (such as drought tolerance and early season germination) may translate to further advantages in a field setting over Canada thistle.

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CHAPTER 2: FIELD STUDY I

MOWING, HERBICIDE, AND COMPETITIVE GRASSES FOR RESTORATION OF CANADA THISTLE (*Cirsium arvense*) INFESTED WETLAND AND UPLAND SITES

ABSTRACT

Restoration of Canada thistle (*Cirsium arvense* [L.] Scop.)-infested sites presents multiple challenges. Mowing, herbicide application, and seeding of competitive species are three of the most common tools employed to control these infestations, but historically success between sites has been variable, likely in part a result of differences in climate, habitat, and differences in application technique for a given control method. Conflicting results across these conditions make it difficult for land managers to develop consistently successful management plans that minimize the time commitment for a given site. A 3-year field study was conducted in Colorado at six sites across two habitats and three different local climatic regimes to evaluate the efficacy of mowing and seeding competitive desirable plant species in combination with herbicide application to determine the most effective control strategies for Canada thistle. The eight unique treatment combinations were comprised of mowing (mowed, unmown), fall herbicide application (sprayed, unsprayed), and seeding competitive plant species (perennial grass, perennial grass+annual grass, unseeded). Plant species tested were western wheatgrass (*Pascopyrum smithii*

[Rydb.] A. Löve) (perennial grass on upland sites), prairie cordgrass (*Spartina pectinata* Bosc ex Link) (perennial grass on wetland sites), and Regreen™ (*Triticum aestivum* L. x *Thinopyrum ponticum* [Podp.] Z.W. Liu and R.C. Wang) (annual grass). The six sites (three wetland sites, three upland sites) were paired geographically with each wetland site in close proximity to an upland site in three different local climatic regimes near Akron, Denver, and Burlington, Colorado. Results show fall herbicide application of 88 g per ha chlorsulfuron to the rosette growth stage after first frost effectively provided 93 to 100% control of Canada thistle at five of the six sites. Mowing did not improve control above herbicide application alone. Seeded grasses were slow to establish, and had not improved Canada thistle control above herbicide application alone at any site two growing seasons after seeding. Drought may have played an important role in treatment effectiveness and grass establishment.

INTRODUCTION

Canada thistle (*Cirsium arvense* [L.] Scop.), a noxious perennial weed native to Eurasia and estimated to infest 2,856,184 hectares across 17 western states, has been spreading across the U.S. at a historical rate of 10 to 12% per year (Duncan and Jachetta 2005). While this spread can result in significant financial losses through reduced crop yields (Donald and Kahn 1996; Pimentel et al. 2000; Stevenson et al. 2001) and decreased rangeland productivity (Hartley and James 1979; Haggar et al. 1986; Bork et al. 2005), ecological losses through destruction of wildlife habitat and compromised integrity of native landscapes (Hutchison 1992; Duncan et al. 2004) can be equally as devastating, and may translate into financial loss in the form of reduced land use by naturalists, hunters, and other recreationists (Morishita 1999; DiTomaso 2000). Restoration of these infested sites is often considered critical for maintenance of ecological and even financial health (as well as legal compliance), but securing long-term control of Canada thistle on these sites can be challenging.

A host of control methods have been employed to reduce Canada thistle infestations including fire (Young 1986; Thompson and Shay 1989; Travnicek et al. 2005), fertilization (Thrasher et al. 1963; Nadeau and Vandenborn 1990), biological control (Gronwald et al. 2002; Louda and O'Brien 2002), cultivation (Derscheid et al. 1961; Hodgson 1970), and manual control (Sheley et al. 1995). However, few of these have consistently demonstrated long-term success. Two of the most commonly utilized control methods currently are herbicide application and mowing. Herbicide application may be successful for short-term control of Canada thistle with repeated applications, but long-term results are more variable, and toxicity concerns may limit its use. Results vary by chemical used, but also by site or habitat: Beck and Sebastian (2000) found herbicide (chlorsulfuron) application at two different sites, applied at identical rates and methodology, to result in more effective Canada thistle control on the wetter site. The herbicide glyphosate is also reported to be more effective for controlling Canada thistle growing in moist soil (Tworkoski et al. 1998). Mowing can be used to reduce the spread of Canada thistle (Rogers 1928), but is generally not considered an effective tool on its own for long-term reduction of Canada thistle (Willard and Lewis 1939; Amor and Harris 1977; Grekul and Bork 2007). However, mowing may be useful in conjunction with herbicide application. Mowing may weaken Canada thistle plants by reducing carbohydrate reserves (Welton et al. 1929), thus increasing herbicide susceptibility. Beck and Sebastian (2000) found mowing to enhance herbicide (dicamba, clopyralid + 2,4-D) effectiveness, but results were variable between sites.

Another complementary control tool increasingly utilized in conjunction with herbicide application is reseeding infested areas with desirable competitive plants.

Reseeding can reduce bare ground post-treatment, inhibiting the (re) establishment of weedy species, and facilitate restoration of valuable landscapes and wildlife habitat. While some treated sites may be small enough to revegetate naturally and quickly from surrounding desirable vegetation, others are too large or lack desirable on-site vegetation, and require active reseeding. Seeding exotic plant species has proven useful for competition with Canada thistle (Derscheid et al. 1961; Thrasher et al. 1963; Ang et al. 1994*a*; Ominski et al. 1999; Wilson and Kachman 1999), but success can vary by location or observation year. Ang et al. (1994*b*) found that seeding tall fescue (*Schedonorus phoenix* [Scop.] Holub) had a greater negative effect on Canada thistle shoot biomass in a wetter year versus a drier year. Tighter restrictions and improved understanding of wildlife utilization of native vegetation have increased the demand for native plant species (or sterile exotics) in revegetation of Canada thistle-infested sites, but fewer natives have been tested for this purpose. Wilson and Kachman (1999) found that western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve) reduced Canada thistle, although precipitation fluctuations between different years of their study may have affected results. Tyser et al. (1998) found the exotic sterile grass Regreen™ (*Triticum aestivum* L. x *Thinopyrum ponticum* [Podp.] Z.W. Liu and R.C. Wang), a potentially useful plant for restoring infested sites, to establish successfully on some restoration sites, but not others, in Glacier National Park, Montana.

The significant variability in success across time and space for control measures such as herbicide application and mowing, as well as for reseeding efforts, emphasizes the challenges faced by land managers tasked with restoring Canada thistle-infested sites. Differences in climate, weather, habitat, Canada thistle growth stage, season of application, and/or variability in application technique for a given control method also likely confound results between sites, making it particularly difficult for land managers to utilize past research to determine the true effectiveness of different control measures and develop consistently successful management plans. Limited resources and increasing toxicity concerns also demand that managers develop efficient plans that minimize the time commitment and herbicide exposure for a given site, making it all the more critical for managers to have useful information about control measures tested with a minimum of confounding factors.

A field study was developed to test the effectiveness of mowing and seeding competitive desirable plants combined with herbicide application across two habitats and three different local climatic regimes, while holding constant the Canada thistle growth stage treated (rosette), timing of application (after the first frost), and application technique. Other factors such as site soil type (Keys and Friesen 1968), plant gender (Tworkoski et al. 1998), and ecotype (Hodgson 1964; Frank and Tworkoski 1994; Zand et al. 2002) may also play a role in variable success between sites, but it was impossible to hold all these factors constant for the purpose of this study. One additional factor investigated in this study was the use of tilling for seedbed preparation. A concern for using reseeding as a complementary tool for restoration of Canada thistle infested sites is that soils at these sites can be compacted as a result of previous disturbance and some preparation of the seedbed is often required before seeding, especially when broadcast seeding. While soil tilling is a common method of seedbed preparation and can enhance establishment of desirable species (Wilson and Kachman 1999), there is some question as to whether tilling may also inhibit restoration efforts. The act of tilling may result in fragmentation of Canada thistle roots remaining in the soil, which could exacerbate the remaining infestation since Canada thistle plants can (re)establish from root fragments as small as 3 to 6 mm in length (Hayden 1934). While Seely (1952), Derscheid et al. (1961), and Hodgson (1970) found that repeated soil cultivation decreased Canada thistle infestations, a one-time tilling treatment for restoration purposes may have the opposite effect. To test the effect of tilling on Canada thistle infestations, a tilling alone treatment was incorporated into this study.

The objectives of this study were to 1) determine if tilling for seedbed preparation on Canada thistle restoration sites significantly increases Canada thistle biomass, 2) determine if seeding competitive native grasses such as western wheatgrass or prairie cordgrass in combination with herbicide application improves control of Canada thistle biomass above that of herbicide alone, 3) determine if seeding Regreen in combination with either native grass in the presence of herbicide application improves control of Canada thistle over herbicide alone, or each native grass alone, 4) determine if mowing before herbicide application improves Canada thistle control above herbicide alone, and 5) determine if treatment results are consistent across wetland and dryland sites, under different local climatic regimes.

It was hypothesized that tilling would increase Canada thistle biomass, and that seeding western wheatgrass or prairie cordgrass (in the presence of herbicide application) would improve control of Canada thistle above that of herbicide application alone. Regreen addition was predicted to enhance Canada thistle control above that of either native grass alone, and above that of herbicide alone. It was also hypothesized that the herbicide plus mowing treatment would be more effective at controlling Canada thistle biomass than herbicide application alone. Treatment results were predicted to be different for wetland versus dryland sites, and across different local climatic regimes.

MATERIALS AND METHODS

A 3-year field study was conducted at six sites in Colorado across three different local climatic regimes and two habitats where plots of Canada thistle were treated with eight unique treatment combinations including mowing (mowed, unmown), fall herbicide application (sprayed, unsprayed), and seeding competitive plant species (perennial grass, perennial grass + annual grass, unseeded). Plant species used in the study include prairie cordgrass (*Spartina pectinata* Bosc ex Link) (perennial grass for wetland sites), western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve) (perennial grass for upland sites), and Regreen (*Triticum aestivum* L. x *Thinopyrum ponticum* [Podp.] Z.W. Liu and R.C. Wang) (annual grass). The six study sites included three upland and three wetland sites, with members of each upland/wetland pair located in close proximity to one another geographically. Each of the three pairs was located in a different local climatic regime near Akron, Denver, and Burlington.

Study Sites

This study was conducted from 2005 to 2007 at the following six sites: Akron Dry, Akron Wet, Denver Dry, Denver Wet, Bonny Dry, and Bonny Wet. Site selection was driven by the availability of wet and dry habitats in close proximity to each other, with large pre-existing continuous Canada thistle infestations present, growing in typical 'wet' or 'dry' conditions. Wet conditions were defined as infestations growing in settings with relatively high water tables and/or surface water presence such as wetlands or riparian areas. Dry conditions were defined as infestations growing in an upland setting with water tables well below the root zone. The establishment of paired sites was desirable to facilitate comparison of treatment effects between wetland and upland infestations while attempting to control for local weather variability that could otherwise influence treatment results from site to site. Additionally, it was of interest to situate each pair of sites relatively distant from the other pairs to investigate treatment responses on wetland versus upland infestations in different local climatic regimes of Colorado.

Selection of representative 'wet' and 'dry' sites for this study was based on a preliminary investigation of a broad variety of wetland and upland Canada thistle infestations across the state of Colorado, ranging from high altitude mountain meadows to the Front Range and plains. It was determined that a 'representative infestation' must be commonly occurring across Colorado (so that my results would have broad application), must be a significantly large, continuous, relatively uniform infestation of Canada thistle (to allow adequate space for plot establishment), and must be growing on relatively flat terrain (to minimize any slope effects). Mountainous populations of Canada thistle were often relatively unique or located on a hillslope, and were rarely of adequate size. As a result, representative sites were selected from across the Front Range and plains of Colorado. In my preliminary investigation, the most prevalent type of 'wet' Canada thistle infestation of adequate size occurred around the perimeter of cattail (*Typha* sp.)-dominated wetlands slowly receding as a result of drought. The wet sites in this study were all established in these conditions. 'Dry' infestations across Colorado were more variable, with the most common types (of adequate size) being abandoned agricultural fields, dry reservoir beds, and rangelands previously grazed by domestic livestock. Dry sites of all one type could not be found to closely pair with my wet sites geographically, thus one of each of the common types of dryland infestation was selected. The three dryland sites were dominated by similar plant species growing amongst the Canada thistle.

The Akron Wet site (40° 03' 23.39" N, 103°14'47.30"W, 1412 m elevation) was located on private property 11.6 km southwest of Akron, Colorado, and 13.4 km from Akron Colorado Plains Regional Airport. The soil texture of the site was clay, with soils of the general area characterized as a Sampson loam (fine-loamy, mixed, superactive, mesic Pachic Argiustolls) (USDA Soil Survey 2009). This property was previously but not presently grazed by cattle. The plots for this site were situated along the perimeter of a long narrow wetland dominated by cattails (*Typha* sp.). The other most dominant plant in addition to Canada thistle on this site was showy milkweed (*Asclepias speciosa* Torr.).

The Akron Dry site was located on property owned by Akron Colorado Plains Regional Airport (40°10'32.44"N, 103°13'31.49"W, 1437 m elevation), 0.81 km from Akron, Colorado, and 13.4 km north of the Akron Wet site. The soils at this site were a clay loam, with soils of the general area characterized as a Colby-Norka loam (fine-silty, mixed, superactive, calcareous, mesic Aridic Ustorthents) (USDA Soil Survey 2009). The property was previously grazed by cattle, then disturbed and reseeded after airport construction. The site was situated at the bottom of a gentle hillslope. Dominant species present on the site in addition to Canada thistle included western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve), cheatgrass (*Bromus tectorum* L.), and Japanese brome (*Bromus arvensis* L.).

The Denver Wet site (39°47'50.05"N, 104°34'41.06"W, 1637 m elevation) was located on property owned by Front Range Airport, 6.1 km northeast of Watkins, Colorado, and 9.8 km southeast of Denver International Airport. The soils at this site were a silty clay, with soils of the general area characterized as loamy alluvial land (Fine-loamy, mixed [calcareous], mesic Ustic Torrifluvents) (USDA Soil Survey 2009). The plots for this site were situated along the perimeter of a long narrow wetland dominated by cattails (*Typha* sp.). Showy milkweed was the other dominant plant species at this site.

The Denver Dry site (39°46'48.88"N, 104°35'11.29"W, 1662 m elevation) was located on private property 1.9 km southwest of the Denver Wet site, 4.0 km northeast of Watkins, Colorado, and 10.6 km southeast of Denver International Airport. The soil at this site was a loam, with soils of the general area characterized as an Ascalon sandy loam (Fine-loamy, mixed, mesic Aridic Argiustolls) (USDA Soil Survey 2009). The property consisted of a flat abandoned agricultural field, on which the plots were situated in rows.

Dominant species present on the site in addition to Canada thistle included horseweed (*Conyza canadensis* [L.] Cronquist) and cheatgrass.

The Bonny Wet site (39°36'4.33"N, 102°13'11.94"W, 1121 m elevation) was located near the west end of Bonny Reservoir, near Burlington, Colorado. The property was managed by the Colorado Division of Wildlife. The soil at this site was clay, with soils of the general area characterized as a Platte fine sandy loam (sandy, mixed, mesic Mollic Fluvaquents) (USDA Soil Survey 2009). The plots for this site were situated on an open flat shoreline along the edge of a wetland dominated by cattails (*Typha* sp.). Besides Canada thistle, showy milkweed was the other dominant plant at this site.

The Bonny Dry site (39°36'39.68"N, 102°13'2.99"W, 1120 m elevation) was located on the dry uplands of the northwest end of Bonny Reservoir, approximately 70 m above the level of the reservoir at the time, and 1.1 km north of the Bonny Wet site. The site property was managed by Colorado State Parks. The soil at this site was a clay loam, with soils of the general area characterized as a Las Animas fine sandy loam (coarse-loamy, mixed, superactive, calcareous, mesic Typic Fluvaquents) (USDA Soil Survey 2009). Plots at this site were established in rows on flat terrain. Dominant species present on the site in addition to Canada thistle included horseweed, cheatgrass, and Japanese brome. See Table 2.1 for temperature and precipitation data for the three locations (Akron, Denver, Bonny).

Plant Materials

The native grass western wheatgrass was chosen for its early spring emergence as well as its aggressive underground growth habit (Ogle 2000). The cool-season sod-forming perennial western wheatgrass is considered to have good drought tolerance and be adaptable to a variety of soils, making it a favorable choice for seeding on the dry sites

Table 2.1. Climatologic data for the three study locations: Akron, Denver, and Bonny.

	Location		
	Akron[a]	Denver[b]	Bonny[c]
Long-term (30-yr) Mean Total Annual Precipitation (mm)	422	394	457
Mean Annual Temperature (°C)			
2005	10.6	10.9	10.8
2006	10.7	11.0	11.5
2007	10.3	10.4	10.4
Mean Total Annual Precipitation (mm)			
2005	378	352	472
2006	265	219	411
2007	346	356	404

[a]For the Akron location, 30-yr precipitation data is from the AKRON4E station; mean annual temperature and precipitation data is from the Akron Colorado Plains Regional Airport (AKO) station (National Climatic Data Center 2009).
[b]For the Denver location, 30-yr precipitation data is from the DENVERSTAPLETON station; mean annual temperature and precipitation data is from the Denver International Airport (DEN) station (National Climatic Data Center 2009).
[c]For the Bonny location, 30-yr precipitation data is from the BONNYDAM2NE station; mean annual temperature and precipitation data is from the BONNYDAM2NE station (National Climatic Data Center 2009).

in this study (Ogle 2000). The native grass prairie cordgrass was chosen for its aggressive underground growth habit and ability to tolerate seasonally dry sites as well as temporarily high water tables once established (Jensen 2006), favorable characteristics for seeding on the wet sites in this study. This warm-season sod-forming perennial grass also produces rapid growth and establishes dense mats, often resulting in pure stands to the exclusion of other species (Hijar 2002), desirable for inhibiting Canada thistle regrowth post-treatment. The non-native desirable species, Regreen, a sterile commercial hybrid cross between common wheat and tall wheatgrass, was chosen for its purported drought tolerance (Glen 1992) and rapid establishment early in the season, typically before other native species (Morris and Schupp 2009). This characteristic made it particularly desirable, as native species can take longer to establish on restoration sites, and it was imperative that desirable vegetation be established quickly post-treatment to inhibit reinfestation. The sterile nature of this non-native grass ensures that its residency is temporary, although Regreen has been observed to persist for a second growing season (Beyers 2008), and may even persist for as long as 3 years (Glen 1992). Western wheatgrass (variety 'Arriba'), prairie cordgrass, and Regreen seed was obtained from Arkansas Valley Seed, Longmont CO, USA. All plant species nomenclature within this paper follows the USDA PLANTS Database (USDA PLANTS 2009).

Experimental Design

This two by two by three fractional factorial study was designed to assess the response of Canada thistle to eight unique treatment combinations of mowing (mowed, unmown), fall herbicide application (sprayed, unsprayed), and seeding of competitive plant species (perennial grass, perennial grass + annual grass, unseeded) on wetland versus upland Canada thistle infestations. The six sites (three wetland sites, three upland sites) were paired geographically, with members of each wetland/upland pair in close proximity to one another at three locations in Colorado, U.S.A.

At each site, thirty two 3- x 3-m plots were established across a large, relatively uniform population of Canada thistle. One-meter buffer strips were left between plots. Four replicate plots were randomly assigned to each of the eight unique treatments. Treatment components consisted of mowing (MOW), herbicide (HERB) application, and seeding competitive plant species. Seeding treatments consisted of habitat specific perennial grasses seeded alone, or paired with the annual grass Regreen (RG). The upland-specific perennial grass was western wheatgrass (WW) and the wetland-specific perennial grass was prairie cordgrass (PC). Regreen was considered to be tolerant of both upland and wetland conditions for the short period of time it would reside on site (due to its sterile nature), and was considered to be critical in both habitat types to obtain rapid plant cover to inhibit Canada thistle reinfestation while the natives established more slowly. Tilling of the soil was conducted at each site in each plot (except where noted) to prepare the soil for seeding. The eight treatment combinations were untilled control, tilled control, WW(or PC)+RG, HERB, HERB+WW(or PC), HERB+WW(or PC)+RG, HERB+WW(or PC)+ MOW, and HERB+WW(or PC)+RG+MOW.

Procedures

Two mowing treatments per season (approximately Jun 15 and Jul 15) are considered to be the best mowing strategy for Canada thistle (Dr. K.G. Beck, personal communication, 2005). The first mowing treatment in this study occurred Aug 2005 out of necessity, directly after the pre-treatment harvest of plant biomass. In 2006, mowing treatments were applied approximately Jun 15 and Jul 15 at each site. In 2007, a mowing treatment was applied in Aug, directly after the post-treatment biomass harvest was conducted. Mowing was performed at a standard 7 to 10 cm height using a handheld motorized weed cutter. This mowing height was chosen because it is one of the lowest settings available on large equipment likely to be used on large scale Canada thistle restoration sites in the future, and because it is a mowing height that has been used in other Canada thistle mowing studies (Beck and Sebastian 1993, 2000).

Herbicide was applied as chlorsulfuron (Telar [Dupont, Wilmington, DE]) at a rate of 88 g per ha plus 0.25% v/v nonionic surfactant using a backpack sprayer with a 3-m boom. This herbicide was chosen because it is effective on Canada thistle over a wide range of conditions. Herbicide application occurred at each site as broadcast spraying on Canada thistle rosettes in the fall of 2005 (Oct 22 through 23) and 2006 (Oct 7 through 8) after the first frost. Wilson and Michiels (2003) and Wilson et al. (2006) both achieved improved control of Canada thistle with a fall herbicide application after the first frost. A third application of herbicide was conducted at each site post-clipping in the fall of 2007, as this is intended to continue as a long-term study.

Tilling and seeding of plots at each site occurred Feb 8 through 12, 2006. All plots at each site were tilled to a depth of 10 cm (except for the untilled control) using a 173-cm wide rototiller attached to a Bobcat 763. In preparation for tilling, a shank-toothed bucket was used on the Bobcat 763 to remove dead accumulated aboveground vegetation, using care not to remove more than 2.5 to 5 cm of surface soil. Soil was then ripped, and tilled. Post-tilling, a roller was attached to the Bobcat 763 and used on each plot to increase the firmness of the seedbed at the dry sites. The plots at the wet sites were not rolled due to concern that these damper heavier soils would become too compacted with rolling. Grass seed was broadcast by hand on each of the seeded plots. Specifically, seeding treatments included unseeded, western wheatgrass (44.8 kg PLS per ha, or 1140 seeds PLS per m^2), prairie cordgrass (22.4 kg PLS per ha, or 970 seeds PLS per m^2), western wheatgrass (22.4 kg PLS per ha, or 570 seeds PLS per m^2) with Regreen (22.4 kg PLS per ha, or 60 seeds PLS per m^2), and prairie cordgrass (11.2 kg PLS per ha, or 490 seeds PLS per m^2) with Regreen (22.4 kg PLS per ha, or 60 seeds PLS per m^2). Once applied, seed was covered with soil using a handheld rake. All tilled plots were hand-raked in the same manner, regardless of presence of seed or species seeded. Plots were not watered or mulched. In 2005, 2006, and 2007, the 1-meter buffers between each plot were mowed once mid-summer to reduce the influence of Canada thistle plants growing around the perimeter of each plot.

Sampling

Pre-treatment data collection was performed for each plot at each site (192 plots) in Aug 2005, at the peak of Canada thistle biomass growth. A 0.5-m-interval grid map for each plot was developed with thirty six 0.25-m^2 subplots. Four 0.25-m^2 subplots were randomly selected for harvest from each plot. In each subplot, all aboveground plant biomass was clipped at ground level and placed in individual paper bags by species. Species in common between subplots for a given plot were bagged together. A square 0.25-m^2 PVC frame was used to delineate each subplot, and a Stihl HS45 handheld motorized hedge trimmer with a 0.5-m bar was used for clipping. Hand shears were used when necessary. Subplot locations for each plot were recorded in 2005. All clipped plant material was dried at 55°C to constant mass and weighed to determine shoot biomass.

For final post-treatment data collection in Aug 2007, the type and method of data collection was identical, but previously clipped subplots for each plot were avoided, along with all other 0.5-m^2 subplots located around the perimeter of each plot, to minimize edge effects. Composite soil samples were collected at each site and analyzed for soil texture using the hydrometer method (Klute 1986) by the Colorado State University Soil Testing Laboratory, Fort Collins, CO, USA.

The portion of the study presented here includes data analyses for Canada thistle shoot biomass for each site and western wheatgrass shoot biomass for the Denver Dry and Bonny Dry sites. No western wheatgrass growth was observed at the Akron Dry site, and no prairie cordgrass growth was observed at any of the three wet sites. Regreen growth was observed at several sites, but heavy herbivory prohibited clipping this plant in Aug 2007.

Statistical Analysis

A preliminary three-way analysis of variance (ANOVA) indicated that the dependent variable *Canada thistle shoot biomass* should be analyzed separately by site, because of the highly significant 3-way interaction of location (site), habitat, and treatment. A square root transformation was used for these data at the Akron Wet, Denver Dry, Denver Wet, Bonny Dry, and Bonny Wet sites. A one-way ANOVA was used for data analyses at each of these sites, and Tukey's Honestly Significant Difference (HSD) method was used to perform posthoc pair-wise comparisons of treatment means. The data set for the Akron Dry site contained many zeroes, therefore treatment means were compared using the Kruskall-Wallis Rank Sum test followed by the Wilcoxon Rank Sum test with exact p-values for posthoc pair-wise comparisons. The data for the dependent variable *grass shoot biomass* were analyzed separately by site for consistency with the previous analyses. *Grass shoot biomass* data were analyzed using a one-way ANOVA and square root transformation for each of the two sites it was collected on, Denver Dry and Bonny Dry. No further pairwise comparisons for these sites were required. The statistical software R 2.8.1 was used for analyses of all data (R Development Core Team 2009). An alpha level of 0.05 was used for all analyses.

RESULTS

Significant treatment effects were detected at each site for Canada thistle shoot biomass. At the Bonny Wet site, Canada thistle biomass was not different for the unsprayed treatments: untilled, tilling alone, and prairie cordgrass + Regreen (Fig. 2.1). The five herbicide treatments (herbicide alone, western wheatgrass/prairie cordgrass + herbicide, western wheatgrass/prairie cordgrass + Regreen + herbicide, western wheatgrass/prairie cordgrass + mowing + herbicide, and western wheatgrass/prairie cordgrass + Regreen + mowing + herbicide) were not different from each other. Canada thistle mean shoot biomass from the unsprayed treatment 'prairie cordgrass + Regreen' was greater than each of the herbicide treatments. Interestingly, the untilled control treatment was not different from three of the herbicide treatments (prairie cordgrass + herbicide, prairie cordgrass + Regreen + herbicide, and prairie cordgrass + Regreen + mowing + herbicide). Additionally, the tilling alone treatment was not different from the herbicide treatments 'prairie cordgrass + herbicide' and 'prairie cordgrass + Regreen + mowing + herbicide'.

Results for Canada thistle biomass analyses at the Akron Wet, Denver Wet, Denver Dry, and Bonny Dry sites were similar (Figs 2.1 and 2.2). Specifically, at each of these sites, Canada thistle biomass was similar for the untilled, tilling alone, and western wheatgrass + Regreen treatments, but each of these treatments had greater Canada thistle biomass than the five herbicide treatments. These five herbicide treatments (herbicide alone, western wheatgrass/prairie cordgrass + herbicide, western wheatgrass/prairie cordgrass + Regreen + herbicide, western wheatgrass/prairie cordgrass + mowing + herbicide, and western wheatgrass/prairie cordgrass + Regreen + mowing + herbicide) were not different from each other.

At the Akron Dry site, five of the eight treatments (those treated with herbicide) resulted in no Canada thistle biomass production post-treatment, and thus were not different from each other (Fig. 2.2). The untilled, tilling alone, and western wheatgrass + Regreen treatments yielded Canada thistle biomass, but were not different from each other. The untilled and western wheatgrass + Regreen treatments were greater than the zero-biomass (herbicide) treatments, but the tilling alone treatment was not different from the zero-biomass treatments.

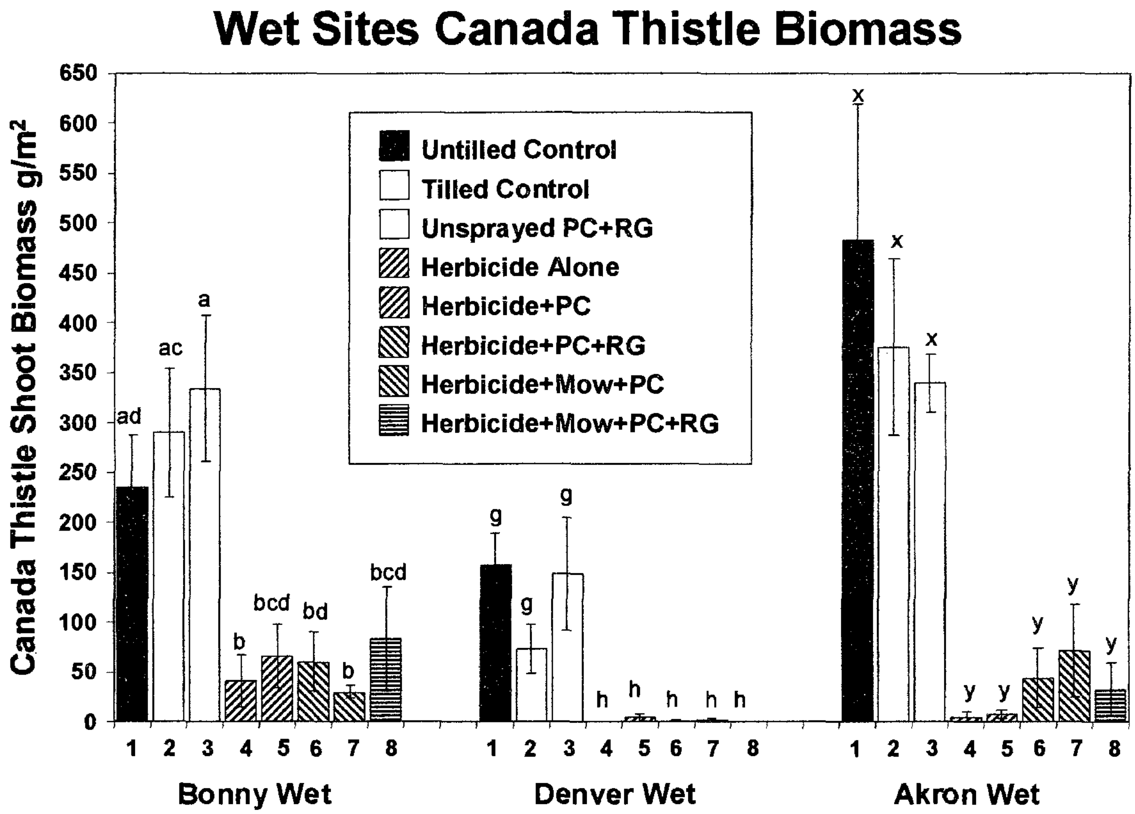

Figure 2.1. Mean (+ SE, n =4) Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass by treatment grown for two field seasons at the Akron Wet, Bonny Wet, and Denver Wet sites. Treatments applied to Canada thistle were comprised of unique combinations of seeding of grasses, herbicide application, and mowing. Grasses seeded included prairie cordgrass (*Spartina pectinata* Bosc ex Link) (PC) or a combination of prairie cordgrass and Regreen (*Triticum aestivum* L. x *Thinopyrum ponticum* [Podp.] Z.W. Liu & R.C. Wang) (PC+RG). Herbicide (HERB) applied was chlorsulfuron, at a rate of 88 g per ha. Mowing (MOW) was performed at a standard height of 7 to 10 cm. All treatments were tilled unless otherwise stated. The treatments are displayed as follows: (1) untilled control, (2) tilled control, (3) PC+RG, (4) HERB, (5) HERB+PC, (6) HERB+PC+RG, (7) HERB+MOW+PC, and (8) HERB+MOW+PC+RG. Means are presented here in the untransformed scale, although analysis was conducted on transformed data. Means with letters in common within each site are not significantly different using Tukey's HSD ($\alpha = 0.05$).

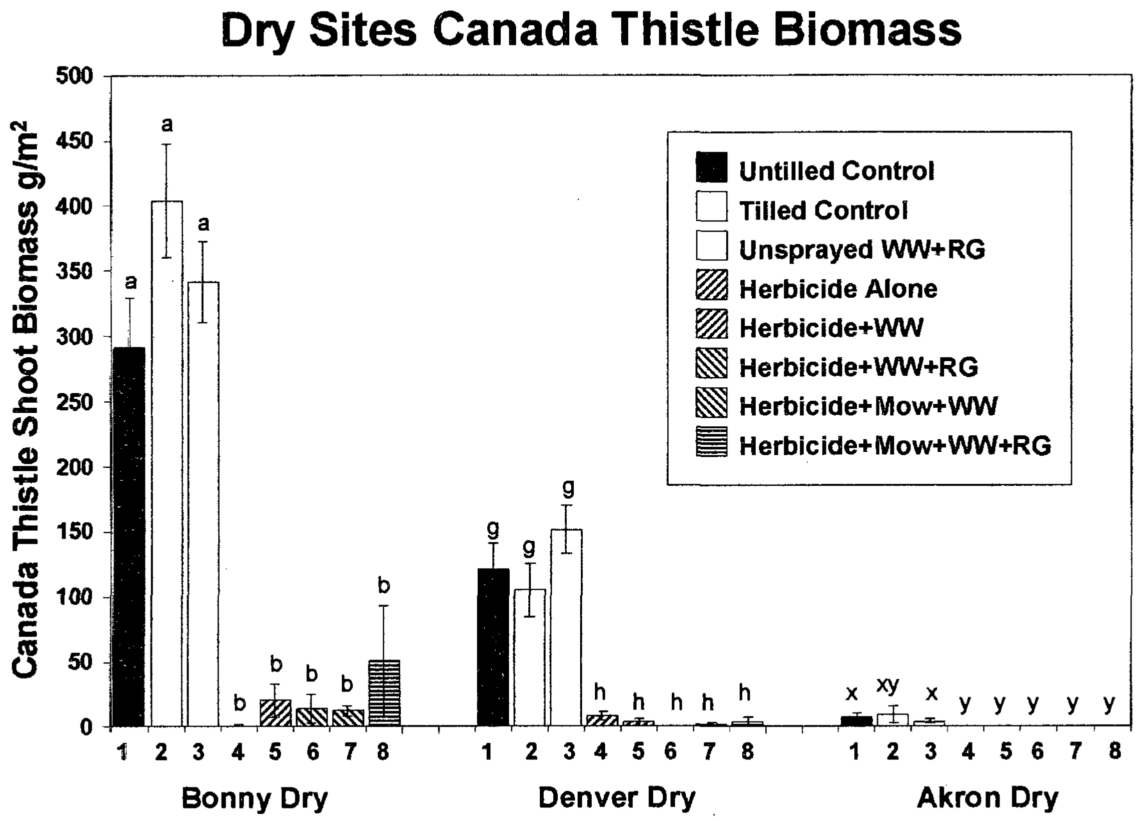

Figure 2.2. Mean (+ SE, n =4) Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass by treatment grown for two field seasons at the Akron Dry, Bonny Dry, and Denver Dry sites. Treatments applied to Canada thistle were comprised of unique combinations of seeding of grasses, herbicide application, and mowing. Grasses seeded included western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve) (WW) or a combination of western wheatgrass and Regreen (*Triticum aestivum* L. x *Thinopyrum ponticum* [Podp.] Z.W. Liu & R.C. Wang) (WW+RG). Herbicide (HERB) applied was chlorsulfuron, at a rate of 88 g per ha. Mowing (MOW) was performed at a standard height of 7 to 10 cm. All treatments were tilled unless otherwise stated. The treatments are displayed as follows: (1) untilled control, (2) tilled control, (3) WW+RG, (4) HERB, (5) HERB+WW, (6) HERB+WW+RG, (7) HERB+MOW+WW, and (8) HERB+MOW+WW+RG. Means are presented here in the untransformed scale, although analysis was conducted on transformed data. Means with letters in common within each site are not significantly different using Tukey's HSD (Bonny Dry, Denver Dry) or Wilcoxon Rank Sum (Akron Dry) ($\alpha = 0.05$).

Grass shoot biomass (western wheatgrass) was not different among treatments at either the Denver Dry ($F_{4,15} = 2.72$, $P = 0.069$) or Bonny Dry site ($F_{4,15} = 0.87$, $P = 0.506$). Mean shoot biomass was not different regardless of mowing, herbicide application, or additional species seeded. Overall mean shoot biomass was 4.17 g per $m^2 \pm 1.07$ SE (n = 20) at the Denver Dry site (mean range of 1.5 to 9.8 g per m^2), and 1.99 g per $m^2 \pm 1.10$ SE (n = 20) at the Bonny Dry site (mean range of 0.5 to 5.7 g per m^2).

The dominant plant species to replace Canada thistle at most sites in herbicide treated plots was kochia (*Bassia scoparia* [L.] A.J. Scott). Kochia shoot biomass made up 88% (Akron Dry), 92% (Akron Wet), 86% (Denver Dry), 76% (Denver Wet), 85% (Bonny Dry), and 2% (Bonny Wet) of the total aboveground biomass in herbicide treated plots at each site in Aug 2007. At the time of project initiation (2005), kochia was only found in herbicide plots (pre-treatment) at the Denver Dry site, comprising 0.1% of the total aboveground biomass.

DISCUSSION

Canada thistle biomass

Tilling

Tilling alone did not increase Canada thistle biomass at any of the six sites, and was not different from the untilled control (Figs 2.1 and 2.2). This result is an important indication that tilling for seedbed preparation may be an acceptable step in the restoration process. Tilling is considered useful for establishment of seeded species because it reduces surface soil compaction and removes or incorporates pre-existing vegetation, creating open space for new plant establishment, as well as increasing soil water infiltration to facilitate growth (Brady and Weil 1996). This may be particularly important on Canada thistle-infested sites, where the dense pre-existing above and below ground biomass can inhibit establishment of desirable species. Wilson and Kachman (1999) found greater establishment of seeded species (including western wheatgrass) on Canada thistle infested sites with tilling (compared to no tilling). A tilling depth of 10 cm was used in my study, and may be critical to avoid significant increase in Canada thistle biomass. Primary vegetative spread of Canada thistle occurs through its horizontal root system, most typically found 15 to 30 cm below the soil surface (Rogers 1928; Pavlychenko 1943). Fragmentation of these horizontal roots could increase Canada thistle biomass (Moore 1975), so a tilling depth that avoids the majority of horizontal underground roots is likely critical for minimizing Canada thistle regeneration.

Interestingly, at the Akron Dry site, while the tilling alone treatment did not differ from the untilled Canada thistle control, it was also no different from those treatments with herbicide application (which produced significantly less Canada thistle biomass than the untilled Canada thistle control). One of the tilling alone treatment plots produced zero Canada thistle biomass, similar to all plots treated with herbicide. While this lack of difference between tilling alone and the herbicide treatments may simply be a product of low replication (n=4), it is also possible that tilling alone can negatively impact Canada thistle biomass in some situations. Tilling may decrease stored soil moisture (compared to untilled soils), which can increase water stress for plants, particularly under drought conditions (Blevins et al. 1983; Bonfil et al. 1999). In 2006, the Palmer Drought Severity Index (National Weather Service Climate Prediction Center 2008) classified all site location areas in this study as experiencing severe to extreme drought for a majority of the growing season, and moderate to severe drought for the rest of the growing season. Over the course of the study, all site locations received below average precipitation and experienced hot drying temperatures (an average of 52 [2006] and 56 [2007] days with temperatures above 32°C, and multiple days above 40.5°C [2007]) throughout the growing seasons. The Akron

Dry site, however, was the 'driest' of the three dryland sites in 2007, receiving less precipitation (346 mm) than either the Bonny (404 mm) or Denver Dry (356 mm) sites. Additionally, much of the untreated aboveground Canada thistle biomass growing *outside* the treatment plots at the Akron Dry site disappeared in 2007, unlike any of the other sites (J. Knudson, personal observation). Because Canada thistle outside the plots was untreated, it is likely that lack of soil moisture played a role in its disappearance from the plots. Pavlychenko (1943) stated that under drought conditions a Canada thistle stand may retreat underground until more soil moisture is available. Others have also found moisture stress to inhibit emergence and new shoot regrowth of Canada thistle (Forsberg 1967; Hamdoun 1972; Donald 1993). On sites this dry, tilling may have a negative effect on Canada thistle biomass.

At the Bonny Wet site, Canada thistle biomass in the tilling alone treatment was also not different from two of the five herbicide treatments (prairie cordgrass + herbicide, prairie cordgrass + Regreen + mowing + herbicide). The reason for this is unclear, but appears to be more a product of the reduced effectiveness of the herbicide treatments than a tilling effect (see *Herbicide* section).

Herbicide

Any treatment involving herbicide resulted in a significant decrease in Canada thistle biomass below that of the untreated control for all sites except the Bonny Wet site (Figs 2.1 and 2.2). The success of this herbicide in my study was not surprising, because chlorsulfuron has previously demonstrated success for reducing Canada thistle biomass (O'Sullivan 1982; Donald and Prato 1992; Sprague et al. 1999). Additionally, my fall herbicide application on Canada thistle rosettes, after the first frost, likely contributed significantly to my success. Wilson et al. (2006) found fall herbicide application (clopyralid) to decrease Canada thistle density 92% (versus 33% with a spring herbicide application), and Wilson and Michiels (2003) achieved improved control of Canada thistle with herbicide application after the first fall frost. It is important to note, however, that the success of this and other herbicides for long-term control of Canada thistle biomass is often dependent on repeated application over multiple years, with reinfestation likely if this regime is not followed. The intent of my study was to determine the best tools to complement and minimize herbicide application to reduce the number of herbicide applications required. As of Aug 2007, mean Canada thistle biomass for the herbicide treated plots was decreased by 100% (Akron Dry), 93% (Akron Wet), 97% (Denver Dry), 99% (Denver Wet), 93% (Bonny Dry), and 76% (Bonny Wet) compared to the untilled controls. While these results are encouraging, the continued presence of Canada thistle in most of the herbicide sprayed plots demonstrates the need for additional herbicide application to avoid reinfestation at these sites even after two applications (2005, 2006).

It is important to note the potential role of local weather conditions on the effectiveness of herbicide at the Akron Dry site, and perhaps interpret this result with care. Akron Dry was the only site where 100% control of Canada thistle was achieved with herbicide. Unfortunately, this was also the only site where much of the Canada thistle outside the plots disappeared during the same study period. Because plants outside the plots were untreated, and likely suffered from a lack of soil moisture (no biocontrol agents were observed), it is also likely that the Canada thistle plants inside the plots were weakened by lack of soil moisture, thus making the plants more susceptible to herbicide application. Donald and Prato (1992) believe drought conditions improved effectiveness of herbicide treatments on Canada thistle in their study. Others have found moisture stress to have no effect on herbicide effectiveness on Canada thistle (Lauridson et al. 1983).

All treatments involving herbicide application produced less Canada thistle biomass than each of the non-herbicide treatments at all sites except Bonny Wet and Akron Dry. This lack of difference between several of the sprayed and unsprayed treatments at Bonny Wet and Akron Dry is surprising. At Bonny Wet, two of the five herbicide treatments (prairie cordgrass + herbicide, prairie cordgrass + Regreen + mowing + herbicide) were not different from the unsprayed tilling alone treatment. While Canada thistle biomass was generally higher in the tilling alone treatment plots versus these two herbicide treatments, lack of herbicide effectiveness in one replicate of each of the two herbicide treatments resulted in a lack of statistical difference between the three treatments. At Akron Dry, it was also the unsprayed tilling alone treatment that was not different from any of the herbicide treatments. At this site, however, these results appear to be more a product of the effect of tilling (discussed earlier) than a poor herbicide effect.

Mowing

Mowing did not significantly reduce Canada thistle biomass below that of herbicide application alone at any of the sites (Figs 2.1 and 2.2). These results are similar to those of Beck and Sebastian (2000), where mowing did not enhance chlorsulfuron control of Canada thistle on a wet or a drier upland site. Conversely, these same authors found mowing to significantly improve herbicide control of Canada thistle when using herbicides such as dicamba and clopyralid + 2,4-D, and other investigators have found mowing to enhance chlorsulfuron control of invasive species such as perennial pepperweed (*Lepidium latifolium* L.) (Renz and DiTomaso 2006). Other researchers have found no significant increase in Canada thistle control above herbicide (MCPB) alone with the addition of mowing (Amor and Harris 1977).

The Bonny Wet exception to the above findings was surprising. Three herbicide treatments at this site (prairie cordgrass + herbicide, prairie cordgrass + Regreen + herbicide, and prairie cordgrass + Regreen + mowing + herbicide) supported the same amount of Canada thistle biomass as the untilled control. Why chlorsulfuron failed to reduce Canada thistle biomass at this site is unclear. The Bonny Wet site demonstrated the lowest level of herbicide control (76%) relative to all other sites. This anomaly is contrary to findings by Beck and Sebastian (2000), where chlorsulfuron application resulted in 100% control on wetter sites. In that study, it was hypothesized that the high water table on the wetter site may have inhibited Canada thistle root growth, increasing the susceptibility of stunted roots to herbicide. Rogers (1928) reported weak and shallow root development on wet soils with a high water table. Unfortunately, the Bonny Wet site became drier throughout the course of the study, and declining water levels here may have confounded the results. While the plots at the other two wetland sites were situated directly around the perimeter of the wetland, the plots at the Bonny Wet site were necessarily situated on a slight rise above the wetland, because extreme water fluctuations were expected at this site. While water levels were similar at all three wet sites at the initiation of the study, water levels dropped more at the Bonny site than the other two sites, likely increasing the desiccation of the Bonny Wet plots. Perhaps the receding water levels stimulated downward extension of Canada thistle roots, thus reducing the plants' susceptibility to herbicide treatment. Lauridson et al. (1983) reported an increase in Canada thistle root length with reduced soil moisture. Beck and Sebastian (2000) hypothesized that greater herbicide effectiveness on sites with higher water tables may be a result of Canada thistle root growth being restricted (as a result of the high water table), thus causing the Canada thistle plants to be less vigorous and more easily controlled than on a site with a deeper water table.

The lack of difference in mowing response between wet and dry sites was surprising because authors such as Beck and Sebastian (2000) reported greater success of mowing alone on wetter versus drier sites. It is worth noting that while the mowing effect was not significant, herbicide application was much easier in mowed plots, and it appeared that mowed plots had larger and denser coverage of Canada thistle rosettes in the fall (J. Knudson, personal observation).

Seeding

Seeding competitive grasses alone, without herbicide application, did not reduce Canada thistle biomass at any of the sites below that of the untilled control (Figs 2.1 and 2.2). This was expected, but inclusion of this treatment was necessary as a grass seeding control. This lack of a competitive grass effect without additional control measures was consistent across the wet and dry sites. Limited grass establishment or the short duration of grass competition likely explain the lack of difference between this treatment and the unseeded tilled control at each of the six sites (Figs 2.1 and 2.2).

Competitive grass seeding without herbicide application also produced more Canada thistle biomass than any of the herbicide treated plots at the six sites (Figs 2.1 and 2.2). These results were also expected. Wilson and Kachman (1999) found competitive grass seeding into tilled soil to be as effective as herbicide application for controlling Canada thistle, but mowing treatments before tilling may have played a significant role in that study. Others have demonstrated the limited utility of seeding desirable species for restoration of weed-infested sites without the use of adequate control measures in addition to seeding (Evans et al. 1970; Goebel et al. 1988; Jacobs and Knudsen 2006).

For treatments containing herbicide, Canada thistle biomass was not affected by the different competitive grass seeding combinations at any of the sites (Figs 2.1 and 2.2).

Only western wheatgrass produced harvestable biomass in this study, but only in the second growing season (2007). Although established at two sites (Bonny Dry and Denver Dry), western wheatgrass had little effect on Canada thistle shoot biomass, likely a result of limited grass establishment and the limited time for competitive interactions to occur. Ogle (2000) stated that western wheatgrass stands can be slow to develop and can be entirely absent the first year due to poor germination. More than 50% of western wheatgrass stands are established after four growing seasons (Ogle 2000). Ang et al. (1994*a*) reported that plant competitors require more than two seasons of growth before they can effectively suppress Canada thistle.

Grass biomass

As mentioned above, none of the seeded grasses produced significant aboveground biomass during the first growing season (2006) at any of the sites. The second growing season (2007), western wheatgrass produced harvestable biomass at Bonny Dry and Denver Dry. Grass response to the different treatments did not differ between the two sites, and grasses grew equally as well in the presence or absence of mowing, herbicide application, or seeding of Regreen. It is important to note, however, that western wheatgrass was never directly exposed to mowing (i.e. cut), since it did not germinate until 2007 and that years' mowing treatment was performed after this grass was harvested. Canada thistle vegetation mowed in 2006, however, would have decomposed on top of the western wheatgrass seed that year. Canada thistle residue can be autotoxic to its own seed and inhibit germination (Bendall 1975). Its residue (or residue products) has demonstrated toxic effects on plants such as alfalfa (*Medicago sativa* L. var. 'Dawson'), perennial ryegrass (*Lolium perenne* L.), common barley (*Hordeum vulgare* L.), and common wheat (*Triticum aestivum* L. var.

'Centurk') (Bendall 1975; Wilson 1981). Evidence that Canada thistle mowing residue does not appear harmful to western wheatgrass seed is promising.

Western wheatgrass seed was also exposed to herbicide application (chlorsulfuron, 88 g per ha) in fall 2006. In mowed plots, direct application of herbicide to seeded soil would have been unavoidable. This same seed was exposed to very hot dry conditions during the 2006 growing season, with an average of 56 days above 32°C and drought. Reseeding after this first season was considered, because of the lack of germination and potentially reduced seed viability from exposure to such harsh conditions. Its germination in 2007 despite herbicide and heat exposure demonstrates the resilience of this grass species.

It is unclear why no western wheatgrass established at the Akron Dry site in 2007, although precipitation timing may have been critical. Despite adequate temperatures for western wheatgrass germination by early Mar 2007 at all sites (optimum temperature for germination of this grass is 15 to 20°C [Qiu 2005]), the Akron Dry site may have had inadequate soil moisture to support it, with only 35 mm of precipitation received from Oct 06 to Feb 07 (1.3 mm in Feb), compared with Bonny and Denver receiving 184 mm and 89 mm from Oct 06 to Feb 07 (24 and 9 mm in Feb), respectively. Significant precipitation did not occur until later in the spring in Akron and was soon followed by increasingly hot temperatures likely damaging to young seedlings. Conditions throughout the rest of the 2007 growing season at the Akron Dry site also appear to have been more extreme than at the other two dry sites, with even Canada thistle plants desiccating from a lack of soil moisture at this site (J. Knudson, personal observation).

The lack of growth of prairie cordgrass was surprising. This grass was initially selected for its aggressive underground growth and tendency to grow in dense mats to the exclusion of other species (such as Canada thistle), but also because of its natural occurrence and successful growth outside the plot area at the Bonny Wet site. Unfortunately, growing conditions for 2006 and 2007 may have been inadequate for this wetland species. Minimum annual precipitation required for this grass is 356 mm (USDA PLANTS 2009); precipitation totaled 219 mm (Denver), 265 mm (Akron), and 411 mm (Bonny) in 2006, and 356 mm (Denver), 346 mm (Akron), and 404 mm (Bonny) in 2007. Depending on timing, the below average precipitation (combined with hot drying temperatures) for these 2 years may have been inadequate for growth at all but the Bonny Wet site.

Dropping ground water levels at the wet sites (Bonny Wet in particular) over the course of the study may also have inhibited growth. Once established, prairie cordgrass grows well on seasonally dry sites, but significant soil moisture is required for germination and establishment (Jensen 2006), and irrigation during this critical time is considered useful (Hijar 2002). High ground water levels were expected at my sites in spring to facilitate grass establishment, but drought conditions resulted in lower water levels than predicted. A final factor that may have inhibited prairie cordgrass establishment on my sites is inadequate seedbed firmness. This grass favors a firm seedbed for establishment, but high soil moisture levels at my wet sites prohibited the use of a roller post-tilling.

The lack of harvestable Regreen in 2007 was unexpected. Key issues potentially affecting Regreen presence were precipitation, seeding conditions, timing of seeding, and herbivory. Regreen growth was observed but production was sporadic among seeded plots in 2007. Tyser et al. (1998) had similar results, where poor Regreen germination was observed across all sites where it was seeded, despite previous success in nearby areas in previous years. Those authors hypothesized inadequate precipitation as a possible cause. While total precipitation for 2006 and 2007 should have been sufficient for Regreen establishment on my sites (305 mm minimum annual precipitation required [H. Wood, personal communication, 2005]), timing of precipitation may have been inadequate. Beyers (2008) found broadcast seeding to produce poor Regreen establishment, and concluded that drill seeding may be more useful. Glen (1992) recommends a seeding depth of 5 to 7.5 cm in dry conditions to inhibit germination until sufficient precipitation is present (a depth that would generally require drill seeding). Densmore et al. (2000) determined the optimum seeding rate for Regreen to be 150 kg per ha. Regreen was seeded at a rate of 23 kg PLS per ha because it was seeded in combination with another species, but perhaps a higher seeding rate would have been useful. Ang et al. (1994*a*) found Canada thistle biomass decreased significantly with increased seeding rates of plant competitors above recommended rates.

Regreen was seeded in Feb 2006 at my sites. A fall seeding was desired but not possible due to early enduring snow cover in fall 2005. The wheat variety used for the Regreen hybrid cross (¾ common wheat [*Triticum aestivum*] x ¼ tall wheatgrass [*Thinopyrum ponticum*] [Glen 1992]) is believed to be winter wheat (R. Gilbert, personal communication, 2009). Winter wheat is generally adapted to fall planting and germination, overwintering until spring when growth can continue. Because Regreens' lineage is so heavily weighted towards winter wheat, fall plantings may be more successful. Regreen seed producers use a fall planting for seed production in eastern Washington (R. Gilbert, personal communication, 2009).

Regreen that did establish in 2007 incurred heavy herbivory (deer tracks in plots) and was not harvestable. Herbivory is often a challenge in restoration projects (Belnap and Sharpe 1995; Opperman and Merenlender 2000; Sweeney et al. 2002), with no ideal solution. Fencing was not financially feasible here, but may be critical on restoration sites with little other palatable vegetation. Seed consumption by wildlife is another concern, especially with large energy-rich seeds like Regreen (Nelson et al. 1970; Hoffmann et al.

1995). Additionally, Wilson (1981) found toxic effects of Canada thistle residue on the winter wheat variety 'Centurk'. Because winter wheat is a major component of Regreen's lineage, it may be that Canada thistle residue is toxic to Regreen seed.

The successful establishment of western wheatgrass in this study may have been facilitated by a volunteer 'nurse' plant, kochia (*Bassia scoparia* [L.] A.J. Scott). Only one herbicide was applied in my study to avoid confounding herbicide effects, but land managers often add a second herbicide to spray mixtures when treating Canada thistle infested sites to target weedy annuals that would otherwise flourish after Canada thistle has been controlled. Failure to treat these weedy annuals can present challenges for restoration, but their presence may also be helpful, particularly in dry conditions. The weedy annual kochia became the dominant plant to replace Canada thistle in herbicide treated plots in my study, with kochia found in all herbicide treated plots at Akron Dry, Akron Wet, Denver Dry, and Denver Wet sites, and most herbicide treated plots at the Bonny Dry site. This is not unusual, and others have observed an increase in kochia on field sites as Canada thistle decreased (Wilson 1981). The increase was significant in my study; at the time of study initiation (2005) kochia was found only at the Denver Dry site, making up 0.1 % of the total biomass for the herbicide plots (pre-treatment). By 2007, kochia made up 88% (Akron Dry), 92% (Akron Wet), 86% (Denver Dry), 76% (Denver Wet), 85% (Bonny Dry), and 2% (Bonny Wet) of the total biomass for the herbicide treated plots at each site.

At the Denver Dry and Bonny Dry sites where western wheatgrass grew, this grass was frequently observed growing close to the base of kochia plants in herbicide treated plots, despite large areas in most of these plots devoid of any vegetative cover. Kochia has deep roots that can draw otherwise inaccessible water up towards the soil surface (Phillips and Launchbaugh 1958), and its large surface area can provide shade from desiccating heat as well as wind protection and snow and rain accumulation (Iverson and Wali 1982).

Kochia also has a high protein content and can serve as forage when necessary (Sherrod 1971), potentially protecting new plants from herbivory. Interestingly, kochia seems to increase in drier years of low rainfall and above average temperatures (Wiese and Vandiver 1970; Blackshaw et al. 2001), which is also when it may be most useful as a 'nurse' plant. Iverson and Wali (1982) state that kochia makes an excellent nurse crop because it protects seedlings from excessive drying and wind damage. An additional advantage is that this annual is typically a temporary component of the ecosystem, reported to thrive for 2 years post-disturbance on mined land in North Dakota, but disappear by the end of the fourth year (Iverson and Wali 1979). However, because kochia is considered to be more drought tolerant than cultivated crops or native grasses (Phillips and Launchbaugh 1958), its continued presence on a site (e.g., if significant site disturbance continues) could be detrimental to restoration goals, and should then be managed accordingly.

CONCLUSIONS

Tilling at a depth of 10 cm did not compromise restoration efforts on Canada thistle infested sites in this study, and may even be beneficial. Mowing was not useful for enhancing chlorsulfuron control of Canada thistle on wet or dry sites. Chlorsulfuron application at a rate of 88 g per ha applied to fall rosettes after the first frost successfully controlled Canada thistle, reducing Canada thistle biomass by 93 to 100% at most sites.

Western wheatgrass grew well on two of the three dry sites where seeded, and is recommended for further use. Its tolerance to herbicide application and decaying Canada thistle residue demonstrates its resilience and utility for these endeavors. In drought conditions, deeper seeding and supplemental watering may be necessary for Regreen establishment on infested sites, regardless of habitat. Fall seeding may also be important for Regreen success. Prairie cordgrass did not perform well at the wetland sites, likely a result of inadequate soil moisture for germination and a failure to meet minimum precipitation requirements. Irrigation and perhaps a firmer seedbed may be useful for prairie cordgrass establishment on such sites in the future. Wetland infestations of Canada thistle present a difficult challenge for restoration. While there are many species yet to be explored for restoration purposes on infested wetland sites, western wheatgrass may be worth consideration, as it is thought to be tolerant of poor drainage, relatively high water tables, and early spring flooding. It is otherwise recommended that species seeded in these wetter habitats be 'facultative', that is, tolerant of both wet and dry conditions, or that a mixture of species be seeded that can more broadly address changing conditions in these unpredictable environments.

While often considered troublesome and cost-prohibitive, supplemental watering in wet and dry habitats may be worth consideration under drought conditions. To shorten the window of time spent on a site and establish productive populations of desirable species quickly after application of a given Canada thistle control method, supplemental watering may be critical. Additionally, protection against herbivory during the early establishment period may be important. Post-treatment invasion by undesirable weedy annuals is typically considered to be problematic on restoration sites, but certain weedy annuals may act as 'nurse' plants on these sites in harsh conditions and benefit restoration efforts.

The challenges for restoration of Canada thistle infested sites are many, but increasing awareness of the utility of revegetation in conjunction with control application on many of these sites continues to improve success rates and spawn new discoveries.

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CHAPTER 3: FIELD STUDY II

SOIL AMENDMENTS, MOWING, HERBICIDE AND COMPETITIVE GRASSES FOR RESTORING A MESIC CANADA THISTLE (*Cirsium arvense*) INFESTED SITE

ABSTRACT

The noxious weed Canada thistle (*Cirsium arvense* [L.] Scop.) spreads aggressively through its underground root system and presents a significant financial and ecological threat to farmers, ranchers, and land managers. Herbicide application is an effective control tool, but often requires multiple site visits for reapplication and is increasingly met with public outcry over toxicity issues. An alternative control strategy must be found that efficiently restores Canada thistle infested sites while minimizing herbicide use. A mesic site near Platteville, Colorado was selected for a 3-year field study to determine the effect of 21 unique treatment combinations applied for Canada thistle management. Treatment combinations included fall herbicide application (sprayed/unsprayed), tilling (tilled/untilled), soil amendment addition (organic matter, manganese [Mn], unamended), seeding competitive grasses (western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Löve], intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey], absent), and mowing (mowed/unmowed). Tilling enhanced herbicide (88 g per ha chlorsulfuron) effectiveness. In the absence of herbicide, organic matter and manganese

amendments did not inhibit Canada thistle growth. Manganese addition to herbicide sprayed plots reduced herbicide effectiveness by 25%. Intermediate wheatgrass was 95% more productive than western wheatgrass, and both grasses were generally unresponsive to changes in other treatment factors. Herbicide application was only effective where plots had been tilled. Tilling did not increase Canada thistle shoot biomass.

INTRODUCTION

The noxious weed Canada thistle (*Cirsium arvense* [L.] Scop.) can germinate after 17 to 21 years of seed burial (Toole and Brown 1946; Burnside et al. 1996), extend its root system horizontally up to 6 m in one season (Rogers 1928; Hayden 1934), and is estimated to produce up to 5,300 seeds per plant per year (Hay 1937). Although this plant may provide cover for wildlife (Suring and Vohs 1979), attract honey bees and play an important role in honey production (Howes 1979; El-Sayed et al. 2008), and treat tuberculosis and worms in children (Moerman 1998), it is a noxious weed that presents a significant financial and ecological threat to farmers, ranchers, and land managers. Herbicide application is generally recognized as the most effective tool for management of this invasive exotic, but often requires multiple visits to an infested site for reapplication (Becker et al. 2007; Gover et al. 2007; Beck 2008), and increased regulation and public scrutiny over toxicity can limit its use. An improved management strategy is needed that minimizes the use of herbicide application while efficiently restoring Canada thistle infested sites.

One control strategy considered useful for enhancing restoration efforts on infested sites is mowing. While generally not considered a useful tool on its own for reducing Canada thistle (Amor and Harris 1977; Grekul and Bork 2007), mowing may have some utility if used in conjunction with herbicide application. Mowing may reduce carbohydrate reserves in Canada thistle plants, weakening them (Welton et al. 1929) and increasing their vulnerability to herbicide application. Beck and Sebastian (2000) found mowing improved herbicide (dicamba, clopyralid + 2,4-D) effectiveness on Canada thistle for some rates of application.

Another avenue of interest for enhancing Canada thistle control is the addition of soil amendments. Although previous research investigating the utility of nutrient addition for reducing Canada thistle biomass has been largely unsuccessful, a few treatments have proven to be effective. Grekul and Bork (2007) found application of blended complete fertilizer (29-13-3-4, NPKS) at 375 kg per ha to reduce Canada thistle density. Thrasher et al. (1963) reported that N addition on an irrigated pasture decreased Canada thistle abundance, although grass seeding may have affected these results. Annual spring fertilization (NPKS) also improved Canada thistle control when combined with herbicide application (Grekul and Bork 2007).

Canada thistle has been observed to be favored by soils that are deeply compacted (Rietberg 1952; Wallace 2001; Sullivan 2004), and/or have poor plant residue decay dominated by anaerobic bacteria, and low soil manganese content (McCaman 1994; Walters 1999). In fact, manganese is considered by some to be the primary nutrient driving Canada thistle infestation (C. Walters, personal communication, 2005). Interestingly, many of the crops that commonly experience soil manganese deficiencies (e.g., soybeans, winter wheat, sugar beets) also often have problems with Canada thistle (Farley and Draycott 1973; Gettier et al. 1985; Miller et al. 1994; Donald and Khan 1996; Mamolos and Kalburtji 2001; Gronwald et al. 2002; Reid 2006). It has also been observed that when alfalfa is established as a cover crop in areas of previous Canada thistle infestation, it is successful at preventing Canada thistle from re-establishing (Wallace 2001), or conversely, the establishment of alfalfa can significantly reduce current Canada thistle infestations

(Hodgson 1958). Manganese deficiency in alfalfa is rare, and more commonly manganese toxicity is observed in alfalfa, as it is known to accumulate significant amounts of manganese (Skinner and Peterson 1928; Rubenstein et al. 1962; Graven et al. 1965; Ouellette and Dessereaux 1958; Barr et al. 1997; Poniedzialek et al. 2005; Koenig et al. 2009).

I conducted a small pilot field study in 2005 in Colorado which confirmed that Canada thistle was often found growing on compacted soils, frequently in combination with poor plant residue decay. Analyses of soil manganese in Canada thistle plots at seven sites across the plains of Colorado found mean available soil manganese to be 5.8 ppm (unpublished data). In Colorado, soil manganese availability of 0 to 0.5 ppm may indicate low soil manganese (Soltanpour and Follett 2005). Soil manganese availability above 5.0 ppm is considered adequate for crop production. While this pilot study did not find available soil manganese to be deficient in Canada thistle infested soils from the perspective of crop production, it was determined that manipulation of soil manganese in infested soils was still of interest for further research.

It was hypothesized that soil manganese manipulation and improvement of soil structure and health on infested sites may significantly inhibit Canada thistle growth. Specifically, it was proposed that soil manganese be manipulated through addition of a manganese amendment, and that soil structure and health be improved through the use of tilling and organic matter addition. Choice of soil amendments was based on several factors. Manganese addition was chosen because of previous observations of Canada thistle's preference for low manganese soils (McCaman 1994; Walters 1999) and because other weedy plants such as field bindweed (*Convolvulus arvensis* L.) and redroot pigweed (*Amaranthus retroflexus* L.) have also shown inhibited plant growth with manganese addition (Bilsky and Foy, 1988). Organic matter was chosen for a variety of reasons.

Organic matter addition can improve soil structure by increasing soil aggregation, which in turn can increase air and water infiltration into the soil (Brady and Weil 1996), potentially creating conditions less favorable for Canada thistle. Addition of organic amendments has also previously demonstrated some utility for inhibiting weed growth (Fennimore and Jackson 2003). It was also suspected that organic matter addition could negatively affect Canada thistle indirectly, by enhancing growth of desirable plant species and thus increasing their ability to compete with Canada thistle. Organic matter can act as a reservoir of nutrients to slowly improve soil fertility, and can also increase soil water holding capacity (Ozores-Hampton et al. 2005; Brady and Weil 1996). Increased soil water availability could be a critical benefit to potential competitors, especially during drought conditions. Additionally, humic compounds in organic matter may enhance the competitive ability of native plants by improving plant nutrient uptake through increased cell membrane permeability, as well as by increasing the growth and metabolism of beneficial organisms in the soil (Valdrighi et al. 1996). Organic matter amendments can also serve as a source of mycorrhizal inoculum, which may improve native plant competitiveness on Canada thistle infested soils over time (Noyd et al. 1996).

Soil tillage was considered useful for reducing soil compaction, improving soil aeration, and improving plant residue decay by increasing activity of aerobic soil organisms (Brady and Weil 1996). Additionally, it could be useful for preparation of the soil for amendment incorporation or seedbed preparation. Although soil tillage can have a variety of benefits for restoration purposes, a possible disadvantage is that it could result in the cutting of thistle roots into many small pieces, potentially exacerbating the existing Canada thistle infestation by creating a multitude of new plants (new Canada thistle plants can establish from root fragments as small as 0.3 cm). Edwards et al. (2000) found cultivation created multiple root fragments from which significant shoot recruitment occurred.

Regardless of the control treatment(s) selected, seeding desirable competitive plants post-treatment may be a critical component of improved restoration of Canada thistle infested sites. Rapid establishment of competitive plant cover in treated areas helps to prevent re-infestation of Canada thistle and other weedy species. While native plants may be preferable for restoration in some areas, native species can be slow to establish, which can be detrimental to restoration efforts. Exotic plant species may establish more quickly than natives, minimizing re-infestation and additional herbicide application. Exotic plant species also have a long history of providing significant competition against Canada thistle (Derscheid et al. 1961; Thrasher et al. 1963; Ang et al. 1994; Ominski et al. 1999). One exotic grass that has demonstrated utility in competition with Canada thistle is intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey). Wilson and Kachman (1999) found intermediate wheatgrass reduced Canada thistle density by 74% when seeded on an infested site in Nebraska along the North Platte River. Intermediate wheatgrass has a very aggressive root system, and can produce rapid growth early in the season, making it a good competitor against Canada thistle (which also begins growth early in the spring, and spreads primarily through its vigorous underground root system). This grass also provides good forage for livestock and wildlife, and habitat for wildlife (Duebbert and Lokemoen 1977; Hijar 2002; Ogle 2003). In areas where the use of native plant species is required, restoration may be more challenging. Investigation of native plant species for revegetation of Canada thistle infested sites has been limited. One native grass that has previously proven successful against Canada thistle is western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve) (Wilson and Kachman 1999). Its early spring growth, relatively aggressive root system, tolerance of a wide range of soil conditions, and ability to withstand significant drought in addition to poor drainage and early spring flooding are all characteristics that should benefit this grass as a Canada thistle competitor.

Western wheatgrass is also useful as forage for livestock, and provides both forage and habitat for wildlife.

The following study was conducted to test the effect of tilling, soil amendment addition (organic matter, manganese) and seeding competitive plant species (intermediate wheatgrass, western wheatgrass) in the presence or absence of herbicide application on Canada thistle shoot biomass. Mowing before herbicide application was also investigated. The objectives of this study were to 1) determine if organic matter or manganese addition would reduce Canada thistle shoot biomass production below that of the untreated control, 2) determine if organic matter or manganese addition in combination with herbicide application could improve Canada thistle control compared to herbicide application alone, 3) determine if seeding a native grass (western wheatgrass) or an exotic (intermediate wheatgrass) in combination with herbicide application could significantly reduce Canada thistle shoot biomass below that of herbicide application alone, 4) determine if the native or exotic grass was more effective at inhibiting Canada thistle shoot biomass, 5) determine the effect of tilling on Canada thistle shoot biomass, and 6) determine if mowing before herbicide application would significantly decrease Canada thistle shoot biomass compared to herbicide application alone.

In this study, it was hypothesized that organic matter and manganese addition would significantly reduce Canada thistle shoot biomass compared to the untreated control, and that in combination with herbicide application would improve Canada thistle control compared to herbicide application alone. Seeding western wheatgrass or intermediate wheatgrass in association with herbicide application was predicted to significantly reduce Canada thistle shoot biomass compared to herbicide application alone, but the exotic intermediate wheatgrass was predicted to be more effective than western wheatgrass. Tilling was predicted to be useful for restoration, but was predicted to increase Canada thistle shoot biomass in the absence of any other control treatment. Mowing before herbicide application was predicted to significantly decrease Canada thistle shoot biomass compared to herbicide application alone.

MATERIALS AND METHODS

Study Site

This study was conducted from 2005 to 2007 on a mesic site near Platteville on the plains of northeastern Colorado. Study plots were established on flat terrain in a dense, pre-existing, relatively uniform infestation of Canada thistle located at the north end of a formerly irrigated abandoned agricultural field. The site was directly adjacent to a permanent wetland and the South Platte River, and had also previously been used for grazing. The site (40°10'46.32"N, 104°49'46.89"W, 1470 m elevation) was located on private property 3.1 km southwest of Platteville, and 21 km north of Brighton, Colorado. The soil texture for this site was a clay loam, with soils of the general area characterized as Aquolls and Aquents, gravelly substratum (USDA Soil Survey 2009). Mean total annual long-term (30-yr) precipitation for Brighton, Colorado (BRIGHTON 3 SE station) is 362 mm, and mean annual long-term (30-yr) temperature is 10.04°C (National Climatic Data Center 2009), with total annual precipitation of 343 mm (2005), 260 mm (2006), and 310 mm (2007) for the study period at BRIGHTON 3 SE station (National Climatic Data Center 2009). Mean annual temperature for this same period was 9.5°C (2005), 10.7°C (2006), and 10.2°C (2007) (National Climatic Data Center 2009). Other than Canada thistle, the most dominant plant species on this site were field bindweed (*Convolvulus arvensis* L.), cheatgrass (*Bromus tectorum* L.), and poison hemlock (*Conium maculatum* L.). The adjacent wetland was dominated by cattails (*Typha* sp.).

Plant Materials and Soil Amendments

Plant species chosen for this study consisted of western wheatgrass and intermediate wheatgrass. Both of these cool-season sod-forming perennial grasses were chosen for their potential to outcompete Canada thistle with their early season emergence and vigorous underground growth, as well as their broad availability and common usage for revegetation purposes. The native western wheatgrass is also highly tolerant of drought and a wide range of soil conditions, although its establishment can be slow (Ogle 2000). Conversely, the introduced species intermediate wheatgrass generally requires greater moisture and soil fertility than western wheatgrass, but can establish relatively quickly and its massive root system can provide a competitive edge (Ogle 2003). Although native species are increasingly preferred for restoration, their establishment can be slow (Bell 2001; Smart et al. 1998). Certain exotic plant species can similarly provide forage and habitat for wildlife, while establishing more quickly than natives, providing faster ground cover to inhibit (re)infestation (Duebbert and Lokemoen 1977; Hijar 2002; Ogle 2003; Waldron et al. 2005). This in turn could reduce the need for herbicide reapplication. Western wheatgrass (variety 'Rosanna') and intermediate wheatgrass (variety 'Oahe') were obtained from Arkansas Valley Seed, Longmont, CO, USA. The organic matter amendment (compost, variety 'Yard Pride') was obtained from Hageman Earth Cycle Inc., Fort Collins, CO, USA. The manganese soil amendment selected for the study was manganese sulfate 32% ($MnSO_4$) (RSA MicroTech LLC, Marysville, WA) obtained from Simplot Grower Solutions, Timnath, CO, USA. All plant species nomenclature within this paper follows the USDA PLANTS Database (USDA PLANTS 2009).

Experimental Design

A 3-year field study was conducted to determine the effectiveness of different combinations of tilling, soil amendment addition, seeding competitive grasses, herbicide application, and mowing for the control and restoration of a Canada thistle infested site. Tilling was initially of interest in this study for its use in reducing soil compaction, but it was soon determined that tilling would be necessary for all treatments involving soil amendment addition or seeding. Tillage was necessary to incorporate soil amendments and to prepare the seedbed. Thus, it was determined that all treatments should be tilled for consistency. The study was thus initially designed as a complete two by three by three factorial experiment comprised of 18 unique treatment combinations of herbicide application, competitive grass seeding, and soil amendment addition (organic matter or manganese) applied to tilled Canada thistle plots. Mowing was not a component of the study at this point.

Subsequently, it was determined that an untilled control should be added to the experiment, to determine the effects of tilling. Another control, untilled herbicide application, was also added because herbicide application on untilled Canada thistle is a common method of treatment for Canada thistle infestations, and it was considered important to compare the relative success of my tilled treatment combinations against this standard practice. A third treatment was added to the project design at this point as well (untilled herbicide application + mowing) at the request of the private landowner who donated the property for this experiment. The landowner was interested in using a combination of mowing and herbicide application on untilled Canada thistle. This resulted in 18 tilled treatments and three untilled treatments for a total of 21 unique treatment combinations for this study.

Treatments were applied to completely randomized 3 x 3 meter plots with four replicates per treatment for a total of 84 plots. Treatment combinations included the following: tilling (tilled/untilled), soil amendment addition (organic matter, manganese, unamended), seeding competitive plant species (western wheatgrass, intermediate wheatgrass, unseeded), fall herbicide application (sprayed with chlorsulfuron, unsprayed), and mowing (mowed/unmowed). All plots were established in a grid pattern across the Canada thistle stand, and buffer strips (1-m wide) were established between plots. All plots contained a similar amount of Canada thistle pre-treatment. The three untilled treatments consisted of the untilled control, untilled herbicide application, and untilled herbicide application+mowing. The 18 tilled treatments consisted of the tilled control (tilling alone) plus all possible combinations of amendments, seeded grasses, and herbicide treatment (Appendix 3, Table A3.1).

Procedures

The first mowing treatment for mowed plots was implemented immediately after the pre-treatment plant biomass harvest in Aug 2005. In 2006, two mowing treatments were conducted on mowed plots, one on approximately Jun 15 and the second on approximately Jul 15. A successful mowing strategy for Canada thistle on the plains of Colorado employs two mowing treatments per season on these approximate dates (Dr. K.G. Beck, personal communication, 2005). In 2007, a mowing treatment was applied in Aug, immediately after the post-treatment biomass harvest (an early season mowing treatment in 2007 was not possible because it would have affected the biomass harvest). A motorized handheld weed cutter was used to mow the Canada thistle to a height of 7 to 10 cm. This mowing height has been used in other Canada thistle mowing studies (Beck and Sebastian 1993, 2000), and it is one of the lowest height settings available on large mowing equipment that might be preferred for future use on larger Canada thistle restoration projects.

Herbicide treated plots received 88 g per ha chlorsulfuron (Telar [Dupont, Wilmington, DE]) plus 0.25% v/v nonionic surfactant applied through broadcast spraying using a backpack sprayer and a 3-m boom. Chlorsulfuron was chosen for this study because of its effectiveness for control of Canada thistle over a wide range of conditions. The herbicide aminopyralid (Milestone®[Dow AgroSciences LLC, Indianapolis, IN]) became available after my study was initiated, and may present a viable alternative, although its potentially negative effects on the growth of desirable restoration plant species has yet to be fully explored (Henry 2008; Samuel and Lym 2008). Herbicide was applied to fall Canada thistle rosettes after the first frost in 2005 and 2006. Applying herbicide after the first fall frost is considered effective for Canada thistle control (Wilson and Michiels 2003; Wilson et al. 2006).

Tilling, seeding, and application of soil amendments to designated plots occurred on Apr 20 through 21, 2006. Except for the three treatments specifically requiring no tillage, all plots for this study were tilled to reduce soil compaction, facilitate incorporation of soil amendments and for seedbed preparation. A 173-cm rototiller attached to a Bobcat 763 was used to till each plot to a depth of 10 cm. Before tilling, it was necessary to remove dead accumulated aboveground vegetation. This was accomplished using a shank-toothed bucket on the Bobcat 763, and no more than 2.5 to 5 cm of surface soil was removed. This was followed by soil ripping, and finally tilling. A roller attached to the Bobcat 763 was used on each plot after tilling to increase seedbed firmness.

Grass seed was hand broadcast on each of the seeded plots at a rate of 44.8 kg PLS per ha with either western wheatgrass (1140 seeds PLS per m^2) or intermediate wheatgrass (790 seeds PLS per m^2). A handheld rake was used to cover the seeds with soil. Soil amendments were applied as a one-time treatment in the following manner. Manganese was applied to each designated plot at a rate of 28 kg Mn per ha as $MnSO_4$. Organic matter was applied to designated plots at a rate of 44.8 Mg per ha of compost, variety 'Yard Pride'. A handheld rake was used to spread the amendments evenly across each treated plot, and incorporate the amendment into the top 5 cm of soil. For consistency, plots not seeded or treated with soil amendments were raked in a similar manner. No mulching or watering of plots was conducted. Plot buffers were mowed once per summer mid-season (2005 through 2007) to minimize effects of Canada thistle plants growing around the plot perimeter might have.

Sampling

In Aug 2005 at the peak of Canada thistle biomass growth, the pre-treatment plant biomass harvest was conducted. Plant biomass was collected from a 1 m^2 area in each plot. A 0.5-m-interval grid map for each plot was developed with thirty six 0.25-m^2 subplots. Four 0.25-m^2 subplots were randomly selected for harvest from each plot. All aboveground plant biomass was clipped from each subplot at ground level and placed in separate paper bags by species. Species in common between subplots were bagged together for a given plot. Each subplot was delineated for clipping using a square 0.25-m^2 PVC frame. Clipping was accomplished through the use of a Stihl HS45 handheld motorized hedge trimmer with a 0.5-m bar, or hand shears when necessary. Locations of 2005 subplots within each plot were recorded. Clipped plant material was dried at 55°C to constant mass and weighed to determine shoot biomass.

Final post-treatment biomass harvesting was conducted in Aug 2007. Biomass collection and processing methodology was similar to the pre-treatment harvest. Four subplots within each plot were again randomly selected for harvest, but previously clipped

$0.25\text{-}m^2$ subplots and all $0.25\text{-}m^2$ subplots located around the perimeter of each plot were excluded from selection to avoid edge effects.

A composite soil sample for the site was collected in 2005 from four randomly selected plots and analyzed for soil texture using the hydrometer method (Klute 1986) by the Colorado State University Soil Testing Laboratory, Fort Collins, CO, USA. A composite soil sample comprised of three samples per plot was also collected from each plot from a depth of 0 to 15 cm in fall of 2005 (pre-treatment) and in fall 2007 (post-treatment). The composite soil samples for individual plots were analyzed for manganese, % organic matter, pH (saturated paste), sulfate, electrical conductivity (EC), nitrate (NO_3-N), phosphorus (P), potassium (K), zinc (Zn), iron (Fe), copper (Cu), and calcium (Ca) by the Colorado State University Soil Testing Laboratory, Fort Collins, CO, USA.

Statistical Analysis

The following dependent variables were analyzed separately for this experiment: *Canada thistle biomass*, *grass biomass*, and specific soil parameters associated with the treatments. Although this experiment was originally designed as a complete two by three by three factorial experiment with 18 treatment combinations (all tilled), the necessary incorporation of three additional (untilled) treatments into the study design (to monitor the effects of tilling alone, untilled herbicide application, and untilled herbicide application + mowing) resulted in a 21 treatment study that presented more complexities for statistical analyses. Because of the incorporation of untilled treatments and the sheer number of treatments in the final study design, a step-wise approach was determined necessary for analyses of the *Canada thistle biomass* data. A preliminary one-way analysis of variance (ANOVA) was performed on *Canada thistle biomass* for the 21 treatments. This preliminary analysis was used to evaluate the three added (untilled) treatments in conjunction with their tilled counterparts. Specifically, Tukey's Honestly Significant Difference (HSD) post-hoc pairwise comparisons were used to determine the effect of tilling, and the effect of herbicide application and herbicide application + mowing in untilled soil. The following five treatments were evaluated for this purpose: untilled control, tilled control, untilled herbicide, tilled herbicide, and untilled herbicide + mowing. Following this preliminary analysis, a three-way ANOVA was performed on *Canada thistle biomass* for the 18 tilled treatments to determine the effects of grass seeding, soil amendment addition, herbicide application, and their possible interactions. The three-way interaction was not significant, and grass seeding had no effect alone or as an interaction. Thus the analysis was simplified to a 3 x 6 two-way ANOVA (grass seeding [western wheatgrass, intermediate wheatgrass, none] x treatment [none, herbicide, organic matter, manganese, herbicide + organic matter, herbicide + manganese]). The Tukey's HSD method was used to perform posthoc pair-wise comparisons of treatment means. Finally, within the original one-way ANOVA, the untreated (untilled) control was compared to each of the six (tilled) treatment means resulting from the two-way ANOVA. A square root transformation was utilized for analyses of these data (Appendix 3, Tables A3.2 – A3.8).

Grass shoot biomass data were comprised only of tilled plots, resulting in a simple three-way ANOVA with a log transformation. Tukey's HSD method was used to perform posthoc pair-wise comparisons of treatment means.

Soil data analyses focused primarily on confirming the presence of added soil amendments and their potential effect on soil acidity. Soil parameters investigated for these analyses include *manganese*, *% organic matter*, *pH*, and *sulfate*. To be consistent with the *Canada thistle biomass* analyses, each soil parameter was analyzed using a 3 x 6 two-way ANOVA (grass seeding [western wheatgrass, intermediate wheatgrass, none] x treatment [none, herbicide, organic matter, manganese, herbicide + organic matter, herbicide +

manganese]), followed by pair-wise comparisons averaging across grass. The exception was *sulfate*, which was analyzed using a one-way ANOVA (followed by pair-wise comparisons averaging across grass). A two-way ANOVA was not possible because laboratory analyses for soil sulfate content was only performed on a subset of treatments due to limited funding. A Bonferoni adjustment was used with the pair-wise comparisons. A log transformation was used for analyses of *% organic matter*. The statistical software R 2.8.1 was used for analyses of all data (R Development Core Team 2009). An alpha level of 0.05 was used for all analyses, including serving as the baseline alpha level for the Bonferoni adjustment.

RESULTS

Untilled Canada thistle treatments (untilled herbicide, untilled herbicide + mowing) were not different from each other, and were not different from the tilled or untilled controls for Canada thistle shoot biomass (Fig. 3.1). The tilled herbicide alone treatment had less Canada thistle shoot biomass than the tilled control, and less than all three of the untilled treatments: untilled herbicide, untilled herbicide+mowing, and the untilled control.

Tilled Canada thistle shoot biomass was unaffected by grass seeding ($F_{2,49} = 1.99$, $P = 0.147$), and grass seeding did not interact with the other treatments ($F_{10,49} = 0.91$, $P = 0.531$). Averaging over grass seeding, the following tilled treatments were not different from each other: tilled control, organic matter alone, and manganese alone (Fig. 3.2). Canada thistle shoot biomass was lower in tilled treatments with herbicide application. Canada thistle shoot biomass for the tilled herbicide alone treatment was also lower than the three tilled but unsprayed treatments: tilled control, organic matter alone, and manganese alone (Fig. 3.2). The tilled herbicide + organic matter treatment was not different from the tilled herbicide alone treatment. The tilled herbicide + organic matter treatment similarly

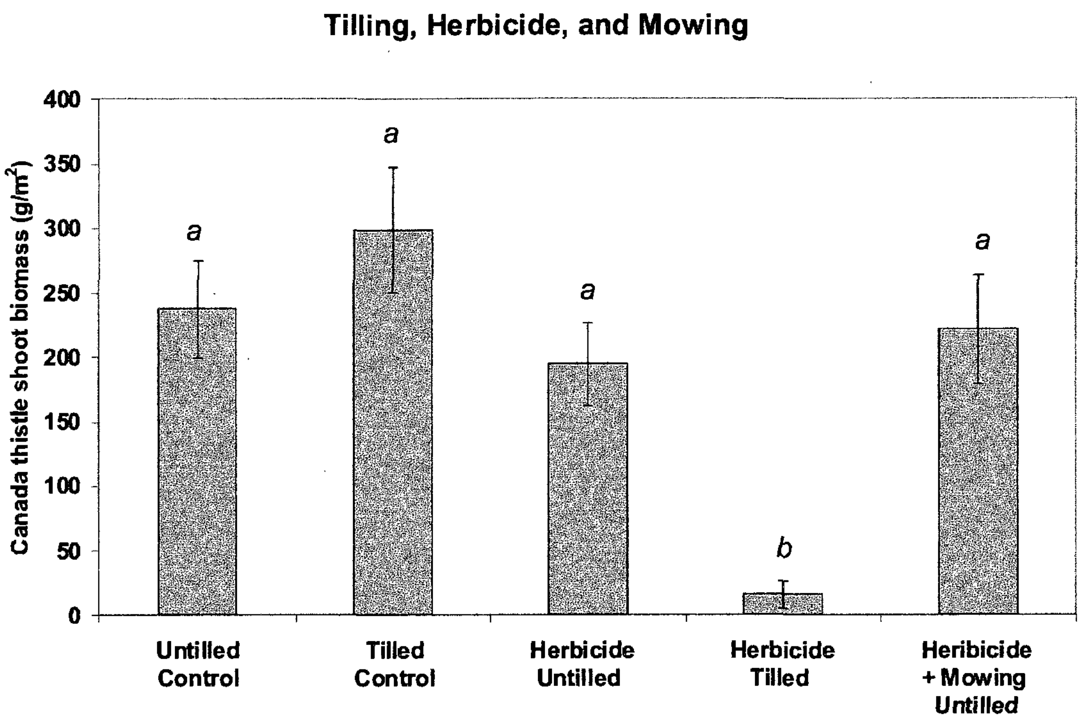

Figure 3.1. Mean (+ SE, n =4) Canada thistle shoot biomass treated with unique combinations of tilling, herbicide, and mowing and grown for two field seasons (2006, 2007) at the Platteville, CO (W2) site. Herbicide application (chlorsulfuron) was conducted at a rate of 88 g per ha. Mowing was performed at a standard height of 7 to 10 cm. Shoot biomass was harvested in Aug 2007. Means are presented here in the untransformed scale, although analysis was conducted on transformed data. Means with letters in common are not significantly different using Tukey's HSD ($\alpha = 0.05$).

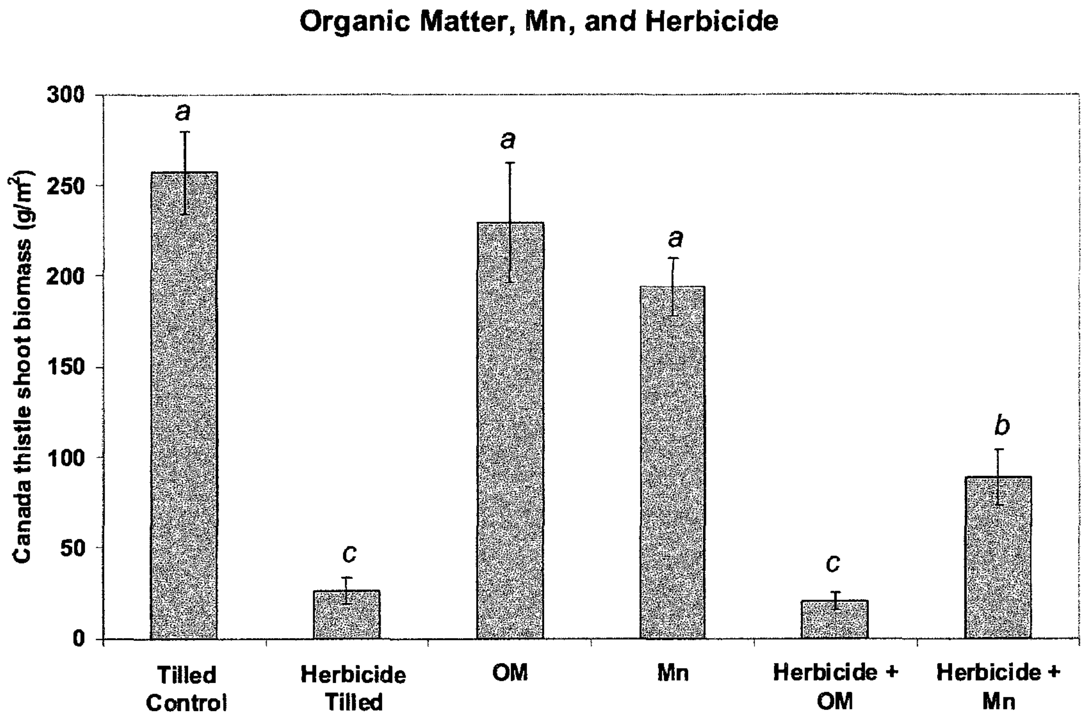

Figure 3.2. Mean (+ SE) Canada thistle shoot biomass treated with unique combinations of organic matter (OM), manganese (Mn), herbicide, and tilling, and grown for two field seasons (2006, 2007) at the Platteville, Colorado (W2) site. All treatments presented here were tilled and means presented are averaged over grass seeding treatments. Organic matter was applied as compost at a rate of 44.8 Mg per ha. Manganese was applied at a rate of 28 kg Mn per ha as $MnSo_4$. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. Shoot biomass was harvested in Aug 2007. Means are presented here in the untransformed scale, although analysis was conducted on transformed data. Means with letters in common are not significantly different using Tukey's HSD ($\alpha = 0.05$).

resulted in less Canada thistle shoot biomass than the tilled control, organic matter alone, and manganese alone. Interestingly, the tilled herbicide + manganese treatment produced less shoot biomass (88.72 g per m^2 ± 15.40 SE [n = 12]) than the tilled control, organic matter alone, and manganese alone treatments, but produced more Canada thistle shoot biomass than the tilled herbicide alone and the tilled herbicide + organic matter treatments.

Canada thistle shoot biomass in the untilled control was not different from shoot biomass in the following tilled treatments: tilled control, organic matter alone, and manganese alone. Canada thistle shoot biomass in the three tilled treatments that received herbicide application (herbicide, herbicide + organic matter, herbicide + manganese) was lower than in shoot biomass in the untilled control.

Grass shoot biomass varied significantly with grass species seeded ($F_{1,33} = 260.2$, $P = <0.001$), herbicide application ($F_{1,33} = 32.11$, $P = <0.001$), and soil amendment addition ($F_{2,33} = 5.52$, $P = 0.009$) (Fig. 3.3). A three way interaction between these three factors was also significant ($F_{2,33} = 5.12$, $P = 0.012$). Western wheatgrass shoot biomass remained the same, regardless of treatment. Intermediate wheatgrass shoot biomass was not different for five of the six tilled treatments: herbicide alone, organic matter alone, manganese alone, herbicide + organic matter, and herbicide + manganese. These five intermediate wheatgrass treatments produced more grass shoot biomass than any of the western wheatgrass treatments. The intermediate wheatgrass alone treatment produced less shoot biomass (12.47 g per m^2 ± 4.48 SE [n = 4]) than the other intermediate wheatgrass treatments, and was not different from the western wheatgrass treatments. Mean grass shoot biomass across all intermediate wheatgrass plots was 128.0 g per m^2 ± 18.8 SE [n = 23], or 1,279.0 kg per ha. Mean grass shoot biomass across all western wheatgrass plots was 5.79 g per m^2 ± 1.1 SE [n = 22], or 57.9 kg per ha.

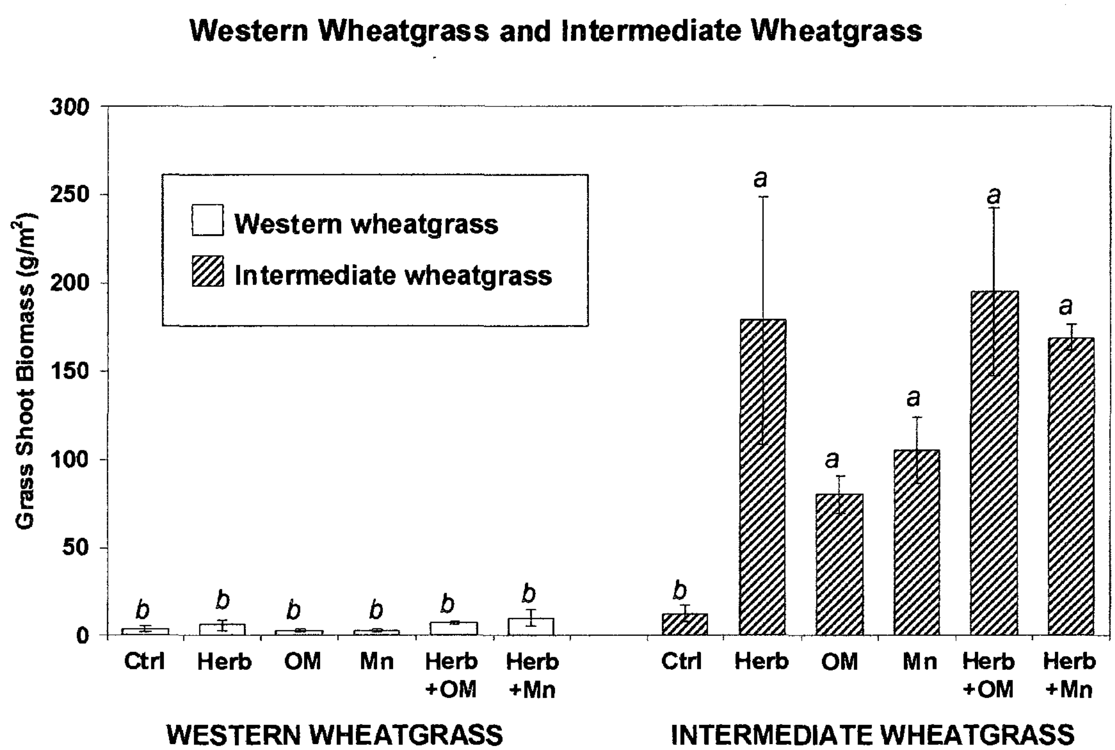

Figure 3.3. Mean (+ SE) grass shoot biomass grown for two field seasons with Canada thistle at the Platteville, Colorado (W2) site. All treatments presented here were tilled. Grass species seeded in treatment plots was either western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve) or intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey). Canada thistle plots were also treated with unique combinations of organic matter (OM), manganese (Mn), and herbicide (HERB). The control (Ctrl) is tilled but otherwise untreated. Organic matter was applied as compost at a rate of 44.8 Mg per ha. Manganese was applied at a rate of 28 kg Mn per ha as $MnSo_4$. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. Means are presented here in the untransformed scale, although analysis was conducted on transformed data. Means with letters in common across both grass species are not significantly different using Tukey's HSD (α = 0.05).

Significant differences were observed among treatments in the tilled plots for the soil parameters manganese, % organic matter, pH, and sulfate. Soil manganese post-treatment was higher in the manganese alone (6.68 ppm Mn ± 0.31 SE [n = 12], P = 0.003) and herbicide + manganese (6.83 ppm Mn ± 0.54 SE [n = 12]; P = 0.001) treatments compared to the tilled control (5.09 ppm Mn ± 0.23 SE [n = 12]). Soil manganese was also higher in the herbicide + manganese treatment compared to the herbicide alone treatment (4.21 ppm Mn ± 0.51 SE [n = 12]; P < 0.001).

Soil % organic matter did not increase in tilled plots post-treatment in the organic matter alone (7.59 % OM ± 1.19 SE [n = 12]; P = 0.106) or herbicide + organic matter (6.39 % OM ± 0.31 SE [n = 12]; P = 0.577) treatments compared to the tilled control (6.02 % OM ± 0.28 SE [n = 12]). Soil % organic matter was not different between the herbicide + organic matter treatment and the herbicide alone treatment (5.7 % OM ± 0.29 SE [n = 12]; P = 0.179). Pre-treatment mean soil organic matter across all plots was 7.30 % OM ± 0.12 SE [n = 84]).

Soil pH in tilled plots post-treatment for the herbicide alone (7.06 ± 0.04 SE [n = 12]; P = 0.620), manganese alone (7.15 ± 0.02 SE [n = 12]; P = 0.172), and herbicide + manganese (7.07 ± 0.03 SE [n = 12]; P = 0.803) treatments was not different from the tilled control (7.08 ± 0.03 SE [n = 12]). Soil pH was not different between the herbicide alone and herbicide + manganese treatments (P = 0.799). Interestingly, soil pH was higher in the organic matter alone (7.43 ± 0.02 SE [n = 12]; P < 0.001) and herbicide + organic matter (7.42 ± 0.02 SE [n = 12]; P < 0.001) treatments compared with the tilled control. Pre-treatment soil pH for these plots was: tilled control (6.63 ± 0.04 SE [n = 12]), organic matter alone (6.65 ± 0.04 SE [n = 12]), and herbicide+organic matter (6.70 ± 0.04 SE [n = 12]).

Soil sulfate (SO_4-S, mg per kg) in tilled plots post-treatment was lower in the manganese alone (27.28 mg per kg SO4 ± 7.64 SE [n = 12]; $P < 0.001$) and herbicide + manganese (17.93 mg per kg SO4 ± 1.13 SE [n = 12]; $P < 0.001$) treatments compared to the tilled control (82.72 mg per kg SO4 ± 12.42 SE [n = 12]). Soil sulfate was also lower in the herbicide + manganese treatment compared to the herbicide alone treatment (87.97 mg per kg SO4 ± 7.10 SE [n = 12]; $P < 0.001$).

The pH of the compost amendment was 7.2 ± 0.06 SE (n = 3) and the organic matter content was 30.4 % OM ± 4.58 SE (n = 3). The compost amendment also contained 688.4 mg per kg available N [NO_3-N] [n=3]), and 12.49 kg N per Mg. Pre-treatment (2005) soil pH for selected plots was: tilled control (6.63 ± 0.04 SE [n = 12]), organic matter alone (6.65 ± 0.04 SE [n = 12]), and herbicide + organic matter (6.70 ± 0.04 SE [n = 12]).

Pre-treatment (2005) levels of soil nutrients for the study site were: nitrate (4.61 ppm NO_3-N ± 0.26 SE [n = 84]), phosphorus (26.33 ppm P ± 0.73 SE [n = 84]), potassium (722.91 ppm K ± 9.54 SE [n = 84]), copper (11.59 ppm Cu ± 0.25 SE [n = 84]), calcium (2583.68 mg per kg Ca ± 14.28 SE [n = 84]), zinc (8.38 ppm Zn ± 0.13 SE [n = 84]), iron (29.48 ppm Fe ± 0.63 SE [n = 84]), and manganese (5.77 ppm Mn ± 0.37 SE [n = 84]). The pre-treatment (2005) electrical conductivity value for the site was (1.09 mmhos per cm EC ± 0.02 SE [n = 84]). Pre-treatment (2005) levels at the study site for soil pH and soil % organic matter were (6.64 ± 0.02 SE [n = 84]) and (7.30 % OM ± 0.12 SE [n = 84]), respectively.

DISCUSSION

Canada thistle biomass

Tilling

Canada thistle shoot biomass did not increase with tilling (Fig. 3.1). Shoot biomass was not different between the untilled and tilled controls, demonstrating that tilling can be useful for restoration purposes while not exacerbating existing Canada thistle infestations. While tilling was predicted to be a useful tool for restoration of Canada thistle infested sites by reducing soil compaction and preparing the soil for amendment addition or seeding, there was some concern that tilling could hinder restoration efforts by exacerbating the existing Canada thistle infestation. When Canada thistle roots are cut into pieces (which could happen with tilling), the many resulting root fragments (as small as 0.3 cm) can produce new plants (Hayden 1934), which could make the infestation worse. My study demonstrates that tilling is not detrimental to the restoration process on a mesic site.

Soil tilling enhanced herbicide effectiveness. Canada thistle shoot biomass decreased when plots were tilled prior to herbicide application compared with untilled herbicide application (Fig. 3.1). This may be a product of the good growing conditions present at this mesic site, which resulted in a very dense stand of Canada thistle, and significant plant litter accumulation. Because one of the strengths of chlorsulfuron herbicide is its soil activity (Walker and Brown 1983), it is considered most effective when the herbicide can reach the soil. While fall rosettes were present for herbicide application in both the tilled and untilled plots, they were accompanied by dense accumulated plant material and litter in the untilled plots, which likely prevented the herbicide from reaching the soil. In the tilled plots, previously accumulated plant litter and dense root material was removed and/or broken up at the initiation of the study, resulting in relatively bare ground and less accumulated plant material accompanying the fall rosettes. Soil compaction was also relatively high in the area of the Canada thistle infestation at this site, and the untilled plots remained so throughout the study (unlike the tilled plots). This compaction may also have inhibited migration of the herbicide into the soil.

Herbicide

Herbicide application significantly affected Canada thistle shoot biomass in this study, but was dependent on tilling status (as mentioned above). All untilled herbicide treatments (herbicide alone, herbicide + mowing) resulted in no difference in Canada thistle shoot biomass relative to the untilled control (Fig. 3.1), and higher biomass than the tilled herbicide alone treatment, likely a result of the soil compaction and/or dense soil cover discussed above. Conversely, all tilled treatments containing herbicide (herbicide alone, herbicide + organic matter, herbicide + manganese) resulted in less Canada thistle shoot biomass than the tilled and/or untilled controls (Figs. 3.1 and 3.2). Canada thistle shoot biomass was reduced to 18.6% of the total biomass in the tilled herbicide treated plots compared to 72.9% of the total biomass in the tilled unsprayed control. This was not surprising, as chlorsulfuron is generally considered an effective herbicide for controlling Canada thistle (O'Sullivan 1982; Donald and Prato 1992; Sprague et al. 1999). Additionally, the timing of herbicide application likely enhanced its effectiveness, as others have observed improved control with fall herbicide application (Wilson et al. 2006) and with application after the first frost (Wilson and Michiels 2003). However, it is likely that herbicide application would need to continue at this site to achieve successful restoration. The first herbicide application at this site was conducted in fall 2005 on all herbicide treatment plots, before plot tilling in the spring of 2006. Based on the ineffective control of herbicide on untilled plots, it appears that the 2005 herbicide application on tilled plots before tillage occurred likely had little effect. Therefore, tilled plots had only received one (fall 2006) herbicide application that was likely useful before the final biomass harvest in Aug 2007. To reduce Canada thistle shoot biomass to 18% after only one herbicide application should be considered a success, particularly because good growing conditions at this site likely made Canada thistle plants more resilient to herbicide application.

Soil amendment addition

Canada thistle shoot biomass was not significantly affected by the addition of organic matter alone or manganese alone. There was no difference in Canada thistle shoot biomass between these two treatments, and no difference from the tilled control (Fig. 3.2). These results are contrary to those hypothesized, that the soil amendments would significantly inhibit Canada thistle shoot biomass either directly (by reducing Canada thistle growth) or indirectly (by favoring the growth of the other species present, thus increasing competition against Canada thistle). Organic matter addition was predicted to be beneficial in these compacted infested soils in a variety of ways, including increasing water infiltration and aeration of the soil, serving as a reservoir for soil nutrients, increasing soil water holding capacity, potentially improving plant nutrient uptake, and increasing beneficial microorganisms in the soil – all of which could have enhanced the growth and competitive ability of the other species growing at the site, as well as potentially shifting soil conditions to those less favorable for Canada thistle. Additionally, organic matter amendments had previously demonstrated utility for inhibiting the growth of weedy species (Fennimore and Jackson 2003). Perhaps an increase in nutrient availability as a result of organic matter addition benefitted Canada thistle as much as or more than the desirable plant species present. The organic amendment used in this study was a compost containing a significant amount of available nitrogen (688.4 mg per kg available N [NO_3-N] [$n=3$]), and was applied at a rate of 44.8 Mg per ha compost, or 559 kg N per ha. While nitrogen fertilization can improve the growth of desirable plant species (Wight 1976; Mortvedt et al. 1996; Gillen and Berg 1998), it can also benefit undesirable plant species - it has been specifically found to enhance Canada thistle growth (Reece and Wilson 1983; Lowe et al. 2002). Because soil nitrate levels at this site were initially low at the beginning of the study (4.61 ppm NO_3-N ± 0.26 SE [n = 84]), amending these plots with compost may have benefitted Canada thistle through nitrogen addition. Interestingly, Canada thistle is also considered to be a good accumulator of nitrate, and is one of the more common plants associated with nitrate poisoning in livestock (Knight and Walter 2001; Robinson and Alex 2002; Stoltenow and Lardy 2008).

Another possible reason that organic matter addition failed to reduce Canada thistle biomass in this study is that, with the exception of soil nitrate, the study site was relatively fertile, with a sufficient amount of organic matter present (7.30 % OM ± 0.12 SE [n = 84]) before amendment addition. Soil organic matter of 4 to 5% is considered sufficient for crop production (Whiting et al. 2008). Pre-treatment (2005) levels of soil nutrients such as phosphorus, potassium, copper, calcium, zinc, iron, and manganese were all adequate or high for crop production (Soltanpour and Follett 2005; SDSU Soil Testing Laboratory 2009). Pre-treatment (2005) electrical conductivity was also found to be adequate for crop production. Nitrate was the only nutrient found to be low at the initiation of the study (Mortvedt et al. 1996). Adding more organic matter and nutrients to this site through compost addition may have been of limited utility.

An alternative reason that organic matter addition may not have produced a significant effect on Canada thistle biomass is that in post-treatment soil analyses, organic matter content in the organic matter alone treatment was found to be the same as that in the unamended tilled control. Perhaps an insufficient amount of organic matter was added to treatment plots. The organic matter content of the added compost material was 30.4 % OM

± 4.58 SE ($n = 3$). While the organic matter content of finished compost (dry weight basis) can range from 30 to 70 %, 50 to 60 % organic matter is considered desirable for most compost uses (Agricultural Analytical Services Laboratory 2009). Conversely, the use of tilling in this experiment likely improved aeration of the soil, and may have caused a significant increase in the decomposition rate of added organic matter through oxidation and microbial activity (Brady and Weil 1996). Because the organic matter amendment in this study was incorporated into the soil soon after tilling was complete, it may be that the added organic matter decomposed relatively quickly, and its benefits were short-lived.

Why manganese addition alone did not affect Canada thistle biomass is unclear. Soil analyses confirmed that manganese was significantly higher in the manganese alone treatment compared to the unamended tilled control, but perhaps the amount of manganese added was too low to affect Canada thistle. Conversely, it may be that Canada thistle simply does not respond to an increase in soil manganese, unlike weeds such as redroot pigweed and field bindweed that have shown reduced plant growth with manganese addition of 15 and 25 mg Mn per kg, respectively (Bilsky and Foy 1988). Canada thistle shoot biomass was also significantly higher in the organic matter alone or manganese alone treatments compared to the three tilled herbicide treatments. This was not surprising, as herbicide application is often a very effective tool for Canada thistle control, although it frequently requires multiple applications.

Herbicide x Soil Amendment addition

The addition of organic matter to herbicide sprayed plots did not enhance herbicide effectiveness (Fig. 3.2). Again, this was surprising, because it was expected that organic matter addition would reduce Canada thistle biomass, either directly or indirectly. Post-treatment soil analyses indicated that organic matter content in the herbicide + organic matter treatment was no different from the unamended tilled control. Perhaps too little organic matter was added to treatment plots, or perhaps its effects were relatively short-lived as a result of high degradation rates post-tilling.

Soil pH was higher in the organic matter treated plots compared to the unamended tilled control. Soil pH for all other tilled treatments was not different from the unamended tilled control. The likely reason for this increase in soil pH was the source of the organic matter amendment, a compost material with a relatively high pH. That the addition of organic matter to herbicide sprayed plots (and subsequent increase in pH) did not affect Canada thistle shoot biomass is of interest. Fredrickson and Shea (1986) found the degradation rate of chlorsulfuron to slow considerably as pH increased (from 5.6 to 7.5) in a silty clay loam soil. Mersie and Foy (1985) also found phytotoxicity of chlorsulfuron to increase for some plants with increasing pH (from 4.2 to 6.9), with optimal phytoxicity at a pH of 6.9 in a silt loam.

Unlike organic matter, the addition of manganese to herbicide sprayed plots resulted in an increase in Canada thistle shoot biomass, above that of the tilled herbicide alone treatment, but below that of the manganese alone treatment and the other unsprayed treatments (tilled control, organic matter alone). Soil analyses also confirm that manganese content of the herbicide + manganese treatment was higher than in the tilled control, and higher than the tilled herbicide alone treatment. Why manganese addition would enhance Canada thistle growth and/or reduce herbicide effectiveness is unclear.

Soil pH in the two manganese treatments was not different from the unamended tilled control. A change in soil pH with the addition of the manganese amendment was initially of concern, since the manganese amendment was applied as manganese sulfate. Sulfate is generally quite acidic, and it was predicted that this amendment might affect the soil pH in these treatments. Interestingly, soil sulfate content was lower in each of the manganese ($MnSO_4$) treatments compared to the unamended tilled control. This may be a result of Canada thistle taking up significant amounts of sulfate from the soil when available. Canada thistle can accumulate large amounts of sulfate, which can be toxic to livestock (Loneragan et al. 1998; Knight and Walter 2001; Kahn 2005). It may be that increased accumulation of sulfate by the Canada thistle plants in the manganese ($MnSO_4$) treatment plots enhanced Canada thistle growth in some way that improved its tolerance to herbicide application, but did not directly enhance Canada thistle growth (since Canada thistle growth was not enhanced in the manganese [$MnSO_4$] alone treatments). The mode of action for chlorsulfuron in plants is to act as a branched-chain amino acid biosynthesis inhibitor (Ross and Lembi 1999). Specifically, the herbicide interferes with the chloroplast enzyme acetolactate synthase (ALS) in plants by binding with it and inhibiting enzyme function. This prevents the formation of key amino acids (leucine, isoleucine, valine), leading to a reduction in protein synthesis in the plant, followed by reduced plant growth and eventual mortality. Interestingly, most sulfur taken up by plants is found in proteins, specifically the amino acids cysteine and methionine, which are protein building blocks (Salisbury and Ross 1992). Tien Le et al. (2003) found that mutations of several methionine residues important for catalytic function of ALS in tobacco (*Nicotiana tabacum* L.) showed strong herbicide resistance to a sulfonylurea (chlorsulfuron is a sulfonylurea), and they believe these methionine residues may be located near a common sulfonylurea binding site of the ALS. Perhaps the increased availability of sulfur in Canada thistle plants in treated plots inhibits herbicide binding or function in some way, thus conferring increased herbicide tolerance to the plants. Sulfur is also used in plants in the vitamins thiamine and biotin, and in coenzyme A, which is a compound critical for respiration and fatty acid synthesis and breakdown (Salisbury and Ross 1992). Proteins can also contain disulfide bonds, for which sulfur is important (Jez 2008). More research is needed as to the possible utility of increased sulfur uptake in Canada thistle plants, and its potential role in herbicide tolerance.

Conversely, it may be that the increased availability of manganese confers some advantage for Canada thistle growth in the presence of herbicide. There is little known about the possible benefits of increased manganese availability on herbicide tolerance in Canada thistle. Some researchers have observed that the addition of liquid manganese to glyphosate herbicide spray tanks can reduce herbicide effectiveness on weeds such as common lambsquarters (*Chenopodium album* L.) and smooth pigweed (*Amaranthus hybridus* L.) (Bailey et al. 2002). These authors postulated that reduced control was a product of insoluble salt complex formation of the herbicide in the tank when combined with manganese, resulting in a solution that could not as readily be absorbed by the treated plants. However, the addition of manganese to the herbicide formulation also raised the pH of the solution, and Buhler and Burnside (1983) found that glyphosate phytotoxicity can be greater with more acidic solutions (although it can depend on the source of acidity). In my study, soil pH did not change with the addition of the manganese amendment, so it appears likely that the effect on Canada thistle biomass was caused by manganese or sulfate, or the result of some soil chemical interaction between the herbicide chlorsulfuron and soil manganese or sulfate. The herbicide + manganese treatment also produced greater Canada thistle biomass than the herbicide + organic matter treatment. Because organic matter appeared to have no effect on herbicide success, this result was not surprising.

Seeding

Grass seeding did not affect Canada thistle growth, regardless of the presence or absence of herbicide or soil amendments. This is contrary to results observed by others, who found grass seeding to inhibit Canada thistle growth (Hodgson 1958; Wilson and

Kachman 1999). However, after only two seasons of growth, perhaps neither grass species had sufficiently established to produce a significant effect on Canada thistle. Other researchers have found that more than two seasons of growth are required for plant competitors to successfully inhibit Canada thistle growth (Ang et al. 1994). The primary benefit of these grasses for restoration of infested sites is their aggressive root system, hypothesized to provide significant underground competition against Canada thistle and prevent re-establishment and spread. Perhaps underground competition from these grasses did begin to inhibit below-ground Canada thistle growth, but had not yet translated to a reduction in above-ground Canada thistle biomass.

Herbicide + Mowing

The untilled herbicide + mowing treatment did not reduce Canada thistle shoot biomass below that of the untilled control (Fig. 3.1). These are similar to the results of Beck and Sebastian (2000), who found mowing to have no effect on chlorsulfuron control of Canada thistle. Unfortunately, the lack of Canada thistle response to both the herbicide + mowing and the herbicide alone treatments in untilled plots may have been a result of inadequate herbicide application to the soil (as discussed above). While mowing opened up the vegetative canopy by reducing standing vegetation on the herbicide + mowing plots, and thus likely improved herbicide contact with fall rosettes, mowing would not have reduced the significant amount of plant litter on the ground that had accumulated in previous years in these untilled plots. In fact, mowing may have simply added to this accumulation with deposition of the mowed plant residue, thus further inhibiting herbicide application to the soil.

Grass biomass

Intermediate wheatgrass produced more biomass than western wheatgrass, with the exception of the untreated intermediate wheatgrass treatment (Fig. 3.3). This was not surprising, since intermediate wheatgrass is generally considered to be more productive than western wheatgrass under adequate conditions (Ogle 2000; Ogle 2003). Intermediate wheatgrass is also considered to thrive under conditions of ample moisture and high fertility (McGinnies and Nicholas 1980; Smoliak et al. 1990; Hijar 2002). This mesic site likely provided adequate moisture for intermediate wheatgrass with its relatively high humidity and relatively shallow water table (approximately 2.5 m deep in Apr 2006), products of the directly adjacent permanent wetland and the South Platte River. The addition of soil amendments such as organic matter and manganese likely increased soil fertility on what was already a relatively fertile site. As mentioned above, the organic matter amendment contained a high level of available N, and studies have shown significant increase in intermediate wheatgrass biomass with N fertilization (Lavin 1967; Lutwick and Smith 1979). A review of the literature found no specific information available on the response of intermediate wheatgrass to soil manganese addition, but exotic grasses such as redtop (*Agrostis gigantea* Roth) have been shown to respond favorably to manganese addition, with a significant increase in shoot biomass above that of the untreated control found with increasing manganese addition up to 4,000 mg per L MnSO4 (Paschke et al. 2005).

In the absence of significant moisture or highly fertile soils, reduced productivity of intermediate wheatgrass can be expected (Smoliak et al. 1990; Ogle 2003). Additionally, reduced vigor and yield of intermediate wheatgrass has been observed in the presence of weedy competitors (Carnahan and Hull 1962; Kay and Evans 1965; Saskatchewan Forage Council 1998). My study found that in the presence of additional nutrients (in the form of soil amendments) and/or the suppression of the primary competitor (herbicide application), intermediate wheatgrass was highly productive. When forced to compete directly with Canada thistle (absence of herbicide application) and/or without additional nutrient amendments (absence of organic matter or manganese addition), intermediate wheatgrass growth was inhibited, and shoot biomass in the intermediate wheatgrass alone treatment was equivalent to that of the less productive grass western wheatgrass.

Western wheatgrass, although less productive, was more consistent and produced the same amount of growth regardless of treatment (Fig. 3.3). Why western wheatgrass did not demonstrate the same trend as intermediate wheatgrass is unclear, although others have observed tilling plus herbicide application to produce a significant increase in intermediate wheatgrass density in Canada thistle infested plots, but not produce a significant increase in western wheatgrass density (Wilson and Kachman 1999). Increased nutrient availability, particularly nitrogen, did not appear to affect western wheatgrass growth at this site. This is contrary to findings by others that have observed increased western wheatgrass biomass production with increased nitrogen availability (Wight 1976; Lowe et al. 2002). Goetz (1969) found that western wheatgrass cover increased with nitrogen fertilization at some sites but not others. The reduction of a weedy competitor (Canada thistle) through herbicide application also did not appear to affect western wheatgrass growth. This is contrary to results of others who report increased western wheatgrass growth with the removal of a weedy competitor (Haferkamp and Heitschmidt 1999).

Hybner et al. (2003) found 'Rosanna' western wheatgrass produced 1,009 kg per ha and 'Oahe' intermediate wheatgrass produced 1,254 kg per ha (when harvested one year after planting) on dryland sites in Wyoming receiving an average of 25 to 35 cm of precipitation per year. My study produced 57.9 kg per ha western wheatgrass and 1,279.0 kg per ha 'Oahe' intermediate wheatgrass. While my intermediate wheatgrass production was comparable to theirs, western wheatgrass production in my study was low. It may be that western wheatgrass establishment was slower at my site. Ogle (2000) states that western wheatgrass can be slow to establish, and can take up to four seasons or longer for stand establishment. My study was also conducted under drought conditions. Although the groundwater at this site was relatively shallow (approximately 2.5 m deep in Apr 2006), perhaps western wheatgrass, which produces most of its root system near the soil surface and has few deep roots (Ogle 2000), could not access this water. Conversely, intermediate wheatgrass produces a massive root system that can extend deep into the ground (Ogle 2003), and could likely access the groundwater at this site. Perhaps inadequate moisture was the key factor inhibiting western wheatgrass growth at this site, thus reducing the importance of the relatively fertile soils and the addition of other soil amendments/nutrients.

CONCLUSIONS

Tilling may be advantageous before herbicide application in dense Canada thistle stands with a significant accumulation of plant litter, particularly if control is dependent on soil activity of the herbicide. Without tillage, herbicide soil activity may be inhibited under such conditions. Tilling is useful for seedbed preparation, and in my study did not result in an increase in Canada thistle biomass. Fall herbicide application with 88 g per ha chlorsulfuron to Canada thistle rosettes after the first frost is an effective control treatment, and in my study reduced subsequent shoot biomass to 18.6% of the total biomass in tilled herbicide treated plots compared to 72.9% of the total biomass in unsprayed plots (tilled, no soil amendments). Mowing did not enhance chlorsulfuron effectiveness for Canada thistle control on this mesic site.

Organic matter addition at the rate of 44.8 Mg per ha of compost was not useful for reducing Canada thistle shoot biomass and did not enhance herbicide effectiveness, although the presence of relatively fertile soils pre-treatment may have affected results. Manganese ($MnSO_4$) addition alone at a rate of 28 kg Mn per ha does not appear to be useful for reducing Canada thistle biomass on its own. However, the reduction in herbicide effectiveness with manganese sulfate application requires further research. Increased availability of manganese (or sulfate) in the presence of herbicide may confer some advantage to Canada thistle, or interacts with soil herbicide to reduce effectiveness.

Intermediate wheatgrass produced significant growth on this mesic site, and generally produced more biomass than western wheatgrass. Intermediate wheatgrass flourished in the presence of herbicide and or soil amendment addition, likely because of the improved fertility and reduced competition. Western wheatgrass produced the same amount of growth with or without herbicide and/or soil amendments. Neither grass inhibited Canada thistle shoot biomass production, but two seasons of growth may not have been long enough to observe significant effects. Because western wheatgrass grew equally as well in the presence of Canada thistle regardless of treatment, it is recommended for future restoration efforts. Intermediate wheatgrass would also likely be useful, although its growth may be inhibited (at least initially) in the absence of herbicide application or in less fertile soils. While native plants are increasingly preferred for restoration purposes, the more abundant aboveground biomass production (under adequate conditions) and more aggressive underground root production for exotic grasses such as intermediate wheatgrass may make them superior competitors against Canada thistle. Additionally, exotic grasses may establish and spread more quickly across treated areas, which could significantly reduce the need for herbicide reapplication.

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CONCLUSIONS

In these studies, clipping (to simulate mowing) reduced Canada thistle shoot biomass in the greenhouse, but in combination with herbicide application in field studies, mowing did not improve Canada thistle control over chlorsulfuron application alone on wetland, upland, or mesic sites. Seeding competitive grass species did not reduce Canada thistle shoot biomass (in combination with mowing or herbicide application) below that of mowing or herbicide application alone. The addition of soil amendments (organic matter or manganese) alone did not reduce Canada thistle growth compared to the untilled control. Manganese addition in combination with herbicide application reduced the effectiveness of the herbicide on Canada thistle.

Tilling proved to be useful for seedbed preparation, did not increase Canada thistle abundance, and enhanced herbicide effectiveness for Canada thistle control at the mesic field site. The Canada thistle stand was very dense at this site (with significant litter accumulation) and vegetation and litter removal by tilling may have enhanced soil contact and absorption of the herbicide (chlorsulfuron), which has significant soil activity. Two of the grass species established significant biomass by the second season in field studies (western wheatgrass and intermediate wheatgrass) and show promise for future restoration efforts. Both western wheatgrass and intermediate wheatgrass tolerated repeated herbicide application, and in Field Study I, western wheatgrass stand establishment was not affected by Canada thistle residues (which can be toxic to some plants) placed on top of the seed as a result of the mowing treatment. Additionally, western wheatgrass alone and intermediate wheatgrass alone in field trials grew equally well in the presence or absence of Canada thistle, suggesting that they are good competitors for future restoration efforts on Canada thistle-infested sites. The treatment combination of Regreen+western wheatgrass in the greenhouse study also resulted in equal biomass production in the presence or absence of Canada thistle, with potential implications for the utility of seeding multiple species to restore infested sites.

Drought was likely a significant factor in experiment results. No prairie cordgrass established at any of the wetland sites. In Field Study II, western wheatgrass and intermediate wheatgrass produced significant harvestable biomass the second season. Field Study II was conducted during the same drought window, but mesic conditions at this site may have favored grass growth. The drought may have affected Regreen as well, which was slow to establish at any of the sites in Field Study I. Unfortunately when it did establish, it was intensely grazed, preventing biomass harvesting of this grass.

Although grass seeding did not significantly affect Canada thistle shoot biomass in these studies, the relatively short duration of the field studies likely did not provide adequate time for the grass stands to fully establish and reach their full potential for inhibiting Canada thistle growth. Western wheatgrass and intermediate wheatgrass appear to be good candidates for future efforts. Prairie cordgrass did not appear to be successful in this study, but its aggressive underground growth and tendency to produce dense stands to the exclusion of other species begs further research for utilization of this species in Canada thistle restoration efforts. Similarly, Regreen establishment was relatively low in this study, but fall seeding and greater moisture availability may enhance its success in future restoration efforts. Mowing does not appear to be useful for restoration efforts and can be time consuming and expensive. Depending on the density of the Canada thistle stand, the accumulation of litter, and the herbicide used, tilling may be useful for future control efforts to enhance soil herbicide activity, as well as for seedbed preparation.

Future research should also address the restoration of wetland infested sites and the importance of irrigation during drought for establishing seeded species on infested sites. It may be that restoration of wetland infested sites should focus on using facultative species (plant species that can tolerate wet or dry conditions) for reseeding, or a mixture of species that ensure establishment of some vegetative cover regardless of moisture availability. Grass species should be chosen over forb species, however, so that forb-specific herbicide use can continue on the infested site while desirable plant species establish until an acceptable level of Canada thistle control is reached.

In drought conditions, establishment of any seeded plants can be difficult. Because the goal of reseeding treated areas is to establish quick plant cover to inhibit re-infestation of Canada thistle, irrigation may be particularly critical for restoration of these sites during drought conditions, or perhaps regardless of drought status. While time consuming and potentially expensive, a small investment in irrigation during the first and possibly second year of restoration may significantly shorten the amount of time it takes to successfully revegetate the site and provide a more effective barrier against weed reinvasion, saving time and money in the long run and potentially reducing the need for herbicide reapplication. Further research should focus on determining whether irrigation can significantly reduce Canada thistle reinfestation by enhancing growth of seeded desirable species, whether it significantly reduces the need for herbicide application in the long run, and the effect of increased moisture availability on the resilience of the remaining Canada thistle plants.

Future research should also explore the mechanism through which manganese (or sulfate, as the manganese amendment was applied as $MnSO_4$) either inhibits herbicide activity or conveys some herbicide tolerance to Canada thistle, in order to either guide future herbicide development, or to educate future efforts for soil fertility manipulation as a possible Canada thistle control measure. Although future research should likely consist of both greenhouse and field trials, genetic level research in the laboratory may also be critical to sufficiently understand this mechanism.

APPENDIX 1

Data and Analyses for Greenhouse Study

Table A1.1. Unique treatment combinations applied to Canada thistle (*Cirsium arvense* [L.] Scop.) plants grown in the greenhouse for 51 weeks.

Treatment Number	Treatment Application
1	Canada Thistle
2	Western wheatgrass
3	Streambank wheatgrass
4	Regreen
5	Regreen + western wheatgrass
6	Canada Thistle + western wheatgrass
7	Canada Thistle + streambank wheatgrass
8	Canada Thistle + Regreen
9	Canada Thistle + Regreen + western wheatgrass
10	Canada Thistle + clipping
11	Canada Thistle + clipping + western wheatgrass
12	Canada Thistle + clipping + streambank wheatgrass
13	Canada Thistle + clipping + Regreen
14	Canada Thistle + clipping + Regreen + western wheatgrass

Table A1.2. Two-way analysis of variance (ANOVA) summary for Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass grown for 51 weeks in the greenhouse. Data were analyzed using a log transformation.

Source	df	F	P
Grass Seeding	4	0.779	0.544
Clipping	1	126.54	<0.001
Grass x Clipping	4	0.850	0.500
Error	50		

Table A1.3. Two-way analysis of variance (ANOVA) summary for grass shoot biomass grown for 51 weeks in the greenhouse in the absence of Canada thistle or in the presence of clipped or unclipped Canada. Data were analyzed using a log transformation.

Source	df	F	P
Canada thistle	2	43.47	<0.001
Grass Species	3	37.35	<0.001
Canada thistle x Grass	6	2.38	0.040
Error	56		

GREENHOUSE SIDE STUDY: ROOT BUD FORMATION AND ROOT SHOOT EMERGENCE

A small side study performed in conjunction with the greenhouse study discussed in Chapter 1 in this dissertation explored root bud formation and root shoot emergence in the greenhouse. Horizontal roots collected 26 Aug 2004 near Fort Collins, Colorado, were placed in sealed plastic bags containing soil collected from the same location. The soil was moistened and then refrigerated in the dark at 6°C for 8 wk. On 23 Oct 2004, the horizontal roots were cut into 2.5 cm pieces ranging in diameter from 0.2 to 0.65 cm. Root pieces showing no visible root buds were separated from those that presented visible root buds. The two groups were soaked separately in 2.54 cm of tap water in covered trays under refrigeration (6°C) in the dark for 28 h. Of the 164 root pieces demonstrating no visible root buds prior to soaking, 18 of these pieces produced visible root buds by the end of the soaking period.

Remaining (144) root pieces with no visible root buds were planted at a depth of approximately 1.3 cm in 25 x 52 cm plastic flat trays filled with 2.5 cm of completely wetted Scotts MetroMix 350 potting soil (Sun Gro Horticulture, Bellevue, WA, USA). Pieces were planted in rows in three trays for a total of 48 pieces per tray. Root pieces with visible root buds (96) were planted in the same manner. Each tray was thoroughly watered after planting and kept moist.

Twenty days after planting in flats, only one (0.7 %) of the bud-less root pieces resulted in a shoot emerging above the soil surface. After the same length of time, 36 (37.5%) of the root pieces with buds resulted in a shoot emerging above the soil surface. These results contrast with those of Hamdoun (1972), who found growth of root pieces at 20°C for 3 wk resulted in 88% of root pieces with pre-existing visible buds producing shoots and 72% of root pieces without pre-existing visible buds producing shoots.

However, root pieces 5 cm in length were used in that study. In a related study, the same author incubated root fragments in the dark for 3 wk and found 81% of root fragments 6 cm in length produced root buds, while only 50% of root fragments 2.0 cm in length produced root buds. In further research with Canada thistle, it may be useful to use longer root pieces to increase germination success.

Temperature and photoperiod may also play an important role in shoot emergence from root fragments. Hunter and Smith (1972) found the number of shoot buds formed on roots to be inversely related to temperature and length of photoperiod, with shoot bud formation greatest at 16°C and 21°C (versus 27°C), and at 8 and 12-h photoperiods (versus 14 and 16-h photoperiods). McAllister and Haderlie (1985) found the greatest number of root buds to be formed at 20°C (compared to 10 or 30°C) under a 13-h photoperiod (compared to a 15-h photoperiod). My study was conducted under a 16-h photoperiod with a greenhouse air temperature of 22 ± 5 °C, which may have contributed to poor root shoot emergence.

Soil aeration may also be important for shoot emergence from root fragments. In my study, several more bud-less root pieces resulted in shoot emergence over time, but only those that 'floated' to the soil surface with watering prior to germination. Improved aeration near the soil surface may have contributed to this emergence. The study soils were kept well watered, which may have reduced soil aeration. Reduced aeration in wet growing medium can result in significantly less root shoot emergence in other vegetatively spreading weeds such as leafy spurge (McIntyre 1979). Also, establishment and survival of Canada thistle plants originating from pieces of stem material is higher if the stem source piece is only partially buried in the soil (Donald 1994). Perhaps root pieces respond similarly under certain conditions.

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APPENDIX 2

Data and Analyses for Field Study I

Table A2.1. Eight unique treatment combinations of herbicide application, mowing, and grass seeding applied to Canada thistle (*Cirsium arvense* [L.] Scop.) plots at each of six different field sites across the eastern plains of Colorado. Field sites consisted of three wetland sites (Akron Wet, Denver Wet, Bonny Wet) and three upland sites (Akron Dry, Denver Dry, and Bonny Dry), and plots were harvested two seasons post-treatment.

Treatment Number	Treatment Application
WETLAND SITES	
1	Canada thistle + untilled
2	Canada thistle + tilled
3	Canada thistle + tilled + prairie cordgrass + Regreen
4	Canada thistle + tilled + herbicide
5	Canada thistle + tilled + herbicide + prairie cordgrass
6	Canada thistle + tilled + herbicide + prairie cordgrass +Regreen
7	Canada thistle + tilled + herbicide + prairie cordgrass + mowing
8	Canada thistle + tilled + herbicide + prairie cordgrass + Regreen + mowing
DRY UPLAND SITES	
1	Canada thistle + untilled
2	Canada thistle + tilled
3	Canada thistle + tilled + western wheatgrass + Regreen
4	Canada thistle + tilled + herbicide
5	Canada thistle + tilled + herbicide + western wheatgrass
6	Canada thistle + tilled + herbicide + western wheatgrass +Regreen
7	Canada thistle + tilled + herbicide + western wheatgrass + mowing
8	Canada thistle + tilled + herbicide + western wheatgrass + Regreen + mowing

Table A2.2. Preliminary three-way analysis of variance (ANOVA) summary for Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass grown for two field seasons on the eastern plains of Colorado at six different locations (Akron Wet, Akron Dry, Denver Wet, Denver Dry, Bonny Wet, and Bonny Dry), two different habitats (wet and dry), and Canada thistle at each site location was treated with the same eight unique treatment combinations of herbicide application, mowing, and/or grass seeding. Data were analyzed using a square root transformation.

Source	df	F	P
Location	2	47.08	<0.001
Habitat	1	54.95	<0.001
Treatment	7	78.16	<0.001
Location x Habitat	2	42.74	<0.001
Location x Treatment	14	2.07	0.017
Habitat x Treatment	7	1.88	0.076
Location x Habitat x Treatment	14	6.06	<0.001
Error	144		

Table A2.3. One-way analysis of variance (ANOVA) summary for Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass grown for two field seasons on the eastern plains of Colorado at each site: Akron Wet, Akron Dry, Denver Wet, Denver Dry, Bonny Wet, and Bonny Dry. All site data were analyzed separately and transformed using a square root transformation, with the exception of the Akron Dry site, which was analyzed nonparametrically using Wilcoxon Rank Sum.

Source	Significance at Each Site					
	Akron Wet	Akron Dry	Denver Wet	Denver Dry	Bonny Wet	Bonny Dry
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table A2.4. One-way analysis of variance (ANOVA) summary for grass shoot biomass grown for two field seasons post-treatment on the eastern plains of Colorado. Grass species seeded included prairie cordgrass at the wet sites, western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve) at the dry sites, and Regreen at both wet and dry sites. A log transformation was used for analyses of this data.

Source	Significance at Each Site					
	Akron Wet	Akron Dry	Denver Wet	Denver Dry	Bonny Wet	Bonny Dry
Treatment	NA	NA	NA	0.069	NA	0.506

*NA = indicates grass was not harvestable at this site. Prairie cordgrass failed to establish at any site, and Regreen growth was limited and heavily grazed, preventing harvesting of this grass. Western wheatgrass failed to grow at the Akron Dry site. Drought was likely a significant factor.

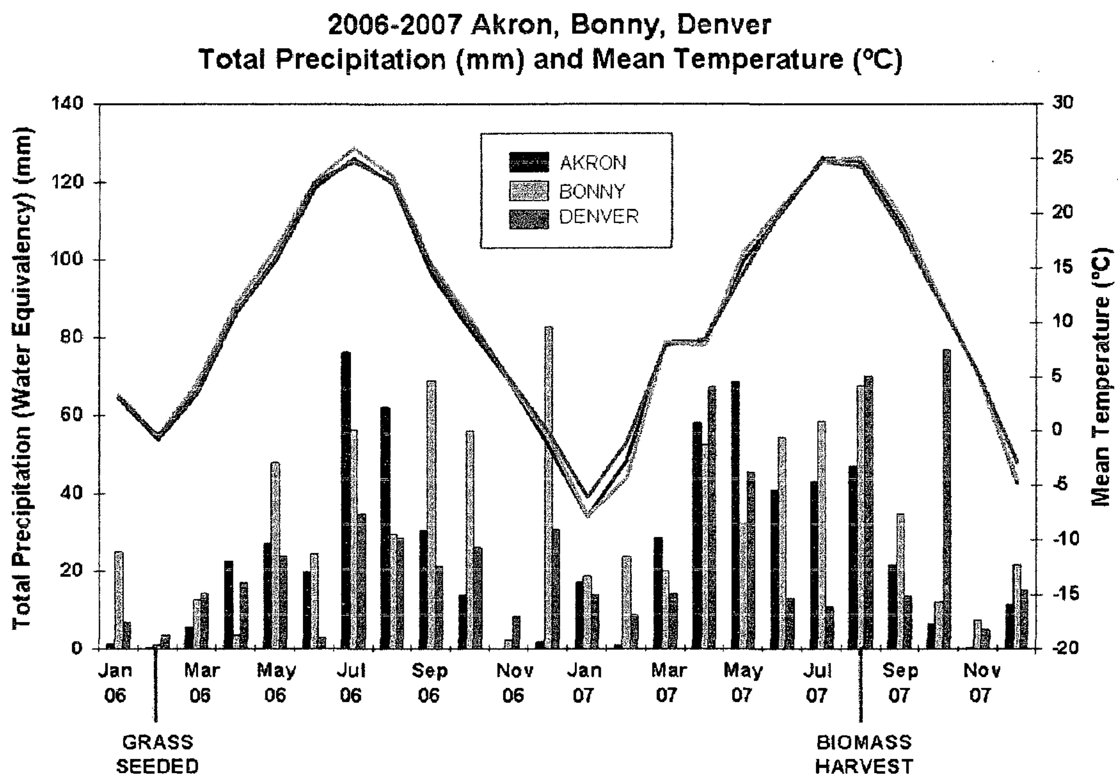

Figure A2.1. Total precipitation (mm) and mean temperature (°C) by month for the years 2006 and 2007 at Akron, Bonny, and Denver. Precipitation data are indicated with bars, and presented as water equivalency. Temperature data are indicated with lines. Timing of grass seeding (February 2006) and final biomass harvest (August 2007) are also indicated. Climatological data were obtained from the National Climatic Data Center, U.S. Department of Commerce, 2009. Data collection stations for Akron, Bonny, and Denver were located at Akron Colorado Plains Regional Airport (24015/AKO), Bonny Reservoir at (BONNY DAM2NE), and Denver International Airport (03017/DEN), respectively.

APPENDIX 3

Data and Analyses for Field Study II

Table A3.1. Original 21 unique treatment combinations applied to Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass grown for two field seasons at the Platteville, Colorado site.

Treatment Letter	Treatment Application
A	Canada thistle + untilled (untilled control)
B	Canada thistle + untilled + herbicide
C	Canada thistle + tilled (tilled control)
D	Canada thistle + tilled + herbicide
E	Canada thistle + tilled + OM (organic matter)
F	Canada thistle + tilled + Mn
*S	Canada thistle + tilled + western wheatgrass
*T	Canada thistle + tilled + intermediate wheatgrass
G	Canada thistle + tilled + herbicide + OM
H	Canada thistle + tilled + herbicide + Mn
I	Canada thistle + tilled + herbicide + western wheatgrass
J	Canada thistle + tilled + OM + western wheatgrass
K	Canada thistle + tilled + Mn + western wheatgrass
L	Canada thistle + tilled + herbicide + OM + western wheatgrass
M	Canada thistle + tilled + herbicide + Mn + western wheatgrass
N	Canada thistle + tilled + herbicide + intermediate wheatgrass
O	Canada thistle + tilled + OM + intermediate wheatgrass
P	Canada thistle + tilled + Mn + intermediate wheatgrass
Q	Canada thistle + tilled + herbicide + OM + intermediate wheatgrass
R	Canada thistle + tilled + herbicide + Mn + intermediate wheatgrass
*U	Canada thistle + untilled + herbicide + mowing

Table A3.2. Preliminary one-way analysis of variance (ANOVA) summary for Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass treated with 21 unique treatment combinations and grown for two field seasons at the Platteville, Colorado site. Data were analyzed using a square root transformation.

Source	df	F	P
Treatment	20	14.95	<0.001
Error	58		

Table A3.3. The influence of tilling alone, tilling plus herbicide application, and mowing plus herbicide application on Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass grown for two field seasons post-treatment. Data were analyzed using a square root transformation.

Treatment	Canada Thistle Mean Shoot Biomass (grams per m^2)
Untilled control (A)	237.0*a*
Tilled control (C)	298.2*a*
Untilled + herbicide (B)	194.1*a*
Tilled + herbicide (D)	15.97*b*
Untilled + herbicide + mowing (U)	221.4*a*

*Means with letters in common are not significantly different using Tukey's HSD ($\alpha = 0.05$).

Table A3.4. Three-way analysis of variance (ANOVA) summary for Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass that was tilled and treated with unique combinations of grass seeding, soil amendment addition, and herbicide application. Plants were grown for two field seasons post-treatment at the Platteville, Colorado site. Data were analyzed using a square root transformation.

Source	df	F	P
Grass Seeding	2	1.992	0.147
Soil Amendment	2	4.281	0.019
Herbicide	1	217.4	<0.001
Grass x Amendment	4	0.479	0.751
Grass x Herbicide	2	0.346	0.709
Amendment x Herbicide	2	12.92	<0.001
Grass x Amendment x Herbicide	4	1.680	0.170
Error	49		

Table A3.5. Two-way analysis of variance (ANOVA) summary for Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass that was tilled and treated with unique combinations of grass seeding, soil amendment addition, and herbicide application. Plants were grown for two field seasons post-treatment at the Platteville, Colorado site. Data were analyzed using a square root transformation.

Source	df	F	P
Grass Seeding	2	1.992	0.147
Treatments	5	49.89	<0.001
Grass x Treatments	10	0.911	0.531
Error	49		

Table A3.6. Grouping of tilled treatments resulting from the two-way analysis of variance (ANOVA) for Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass grown for two field seasons post-treatment at the Platteville, Colorado site. The analyses averaged across grass seeding treatments, since grass seeding was not found to be significant.

Treatment Letter	Treatment Application
C+S+T	Canada thistle + tilled
D+I+N	Canada thistle + tilled + herbicide
E+J+O	Canada thistle + tilled + OM (organic matter)
F+K+P	Canada thistle + tilled + Mn (manganese)
G+L+Q	Canada thistle + tilled + herbicide + OM
H+M+R	Canada thistle + tilled + herbicide + Mn

Table A3.7. The influence of herbicide application and soil amendment addition (organic matter or manganese) on tilled Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass grown for two field seasons post-treatment at the Platteville, Colorado site. Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron (88 g per ha). Data were analyzed using a square root transformation.

Treatment	Canada Thistle Mean Shoot Biomass (grams per m^2)
Tilled control (C+S+T)	256.8*a*
Tilled + herbicide (herbicide alone) (D+I+N)	26.59*c*
Tilled + OM (organic matter alone) (E+J+O)	229.2*a*
Tilled + Mn (manganese alone) (F+K+P)	194.0*a*
Tilled + herbicide + OM (G+L+Q)	20.69*c*
Tilled + herbicide + Mn (H+M+R)	88.72*b*

*Means with letters in common are not significantly different using Tukey's HSD ($\alpha = 0.05$).

Table A3.8. Response of tilled Canada thistle (*Cirsium arvense* [L.] Scop.) shoot biomass to herbicide application and/or soil amendment addition (organic matter or manganese) compared to the untilled control. Plants were grown for two field seasons post-treatment at the Platteville, Colorado site. Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. Data were analyzed using a square root transformation.

Treatment	Canada Thistle Mean Shoot Biomass (grams per m^2)
Untilled control (A)	237.0*a*
Tilled control (C+S+T)	256.8*a*
Tilled + herbicide (herbicide alone) (D+I+N)	26.59*b*
Tilled + OM (organic matter alone) (E+J+O)	229.2*a*
Tilled + Mn (manganese alone) (F+K+P)	194.0*a*
Tilled + herbicide + OM (G+L+Q)	20.69*b*
Tilled + herbicide + Mn (H+M+R)	88.72*b*

*Means with letters in common are not significantly different from the untilled control using contrasts ($\alpha = 0.05$).

Table A3.9. Three-way analysis of variance (ANOVA) summary for grass shoot biomass grown for two field seasons in the presence of Canada thistle plants treated with herbicide or soil amendments (organic matter or manganese) and grown for two field seasons post-treatment at the Platteville, Colorado site. Grass species seeded in treatment plots was either western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve) or intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey). Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All plots were tilled prior to grass seeding. Data were analyzed using log transformation.

Source	df	F	P
Grass Seeding	1	260.2	<0.001
Herbicide	1	32.11	<0.001
Soil Amendment	2	5.518	0.009
Grass x Herbicide	1	2.450	0.127
Grass x Amendment	2	3.036	0.062
Herbicide x Amendment	2	1.610	0.215
Grass x Herbicide x Amendment	2	5.116	0.012
Error	33		

Table A3.10. Influence of Canada thistle presence, herbicide application, and soil amendment addition (organic matter or manganese) on grass shoot biomass grown for two field seasons post-treatment at the Platteville, Colorado site. Grass species seeded in treatment plots was either western wheatgrass (WW) (*Pascopyrum smithii* [Rydb.] A. Löve) or intermediate wheatgrass (INT) (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey). Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. Data were analyzed using a log transformation.

Treatment	Grass Mean Shoot Biomass (grams per m^2)
Tilled + WW (S)	3.750 *b*
Tilled + herbicide + WW (I)	6.092 *b*
Tilled + OM + WW (J)	3.025 *b*
Tilled + Mn + WW (K)	3.213 *b*
Tilled + herbicide + OM + WW (L)	7.308 *b*
Tilled + herbicide + Mn + WW (M)	10.14 *b*
Tilled + INT (T)	12.47 *b*
Tilled + herbicide + INT (N)	178.2 *a*
Tilled + OM + INT (O)	79.79 *a*
Tilled + Mn + INT (P)	105.0 *a*
Tilled + herbicide + OM + INT (Q)	195.0 *a*
Tilled + herbicide + Mn + INT (R)	168.6 *a*

*Means with letters in common are not significantly different using Tukey's HSD (α = 0.05).

Table A3.11. Two-way analysis of variance (ANOVA) summary for soil manganese content in Canada thistle plots two years post-treatment at the Platteville, Colorado site. Plots were treated with herbicide application and/or soil amendments (organic matter or manganese). Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. No transformation was used for data analyses.

Source	df	F	P
Grass Seeding	2	0.087	0.917
Treatments	5	8.797	<0.001
Grass x Treatments	10	2.092	0.042
Error	51		

Table A3.12. Difference in soil manganese content (ppm) between the tilled control, herbicide alone, manganese alone, or manganese plus herbicide treatments. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. No transformation was used for data analyses.

Treatment Number	Treatment	Mean Soil Mn Content (ppm)	Treatment Comparison	P-value
1	Tilled control (C+S+T)	5.09	1,4	0.001*
2	Tilled + herbicide (herbicide alone) (D+I+N)	4.21	2,4	<0.001*
3	Tilled + Mn (manganese alone) (F+K+P)	6.68	3,1	0.003*
4	Tilled + herbicide + Mn (H+M+R)	6.83		

*Indicates significantly different using contrasts and a Bonferroni adjustment ($P \geq 0.017$).

Table A3.13. Two-way analysis of variance (ANOVA) summary for soil organic matter content (%) in Canada thistle plots two years post-treatment at the Platteville, Colorado site. Plots were treated with herbicide application and/or soil amendments (organic matter or manganese). Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. Data were analyzed using a log transformation.

Source	df	F	P
Grass Seeding	2	1.992	0.035
Treatments	5	49.89	0.179
Grass x Treatments	10	0.911	0.181
Error	51		

Table A3.14. Differences in soil organic matter content (%) between the tilled control, herbicide alone, organic matter alone, or organic matter plus herbicide treatments. Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. A log transformation was used for data analyses.

Treatment Number	Treatment	Soil Organic Matter (%)	Treatment Comparison	P-value
1	Tilled control (C+S+T)	6.02	1,4	0.577
2	Tilled + herbicide (herbicide alone) (D+I+N)	5.70	2,4	0.179
3	Tilled + OM (organic matter alone) (E+J+O)	7.59	3,1	0.106
4	Tilled + herbicide + OM (G+L+Q)	6.39		

*Indicates significantly different using contrasts and a Bonferroni adjustment ($P \geq 0.017$).

Table A3.15. Two-way analysis of variance (ANOVA) summary for soil pH in Canada thistle plots two years post-treatment at the Platteville, Colorado site. Plots were treated with herbicide application and/or soil amendments (organic matter or manganese). Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. No transformation was used for these analyses.

Source	df	F	P
Grass Seeding	2	1.019	0.368
Treatments	5	37.18	<0.001
Grass x Treatments	10	1.010	0.448
Error	51		

Table A3.16. Differences in soil pH between the tilled control, herbicide alone, manganese alone, organic matter alone, manganese plus herbicide or organic matter plus herbicide treatments. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. No transformation was used for these analyses.

Treatment Number	Treatment	Soil pH	Treatment Comparison	P-value
1	Tilled control (C+S+T)	7.08	1,5	0.803
2	Tilled + herbicide (herbicide alone) (D+I+N)	7.06	2,1	0.620
3	Tilled + Mn (manganese alone) (F+K+P)	7.15	3,1	0.172
4	Tilled + OM (organic matter alone) (E+J+O)	7.43	4,1	<0.001*
5	Tilled + herbicide + Mn (H+M+R)	7.07	5,2	0.799
6	Tilled + herbicide + OM (G+L+Q)	7.42	6,1	<0.001*

*Indicates significantly different using contrasts and a Bonferroni adjustment ($P \geq 0.008$).

Table A3.17. One-way analysis of variance (ANOVA) summary for soil sulfate (SO_{4-}) in Canada thistle plots two years post-treatment at the Platteville, Colorado site. Plots were treated with herbicide application and/or soil amendments (organic matter or manganese). Organic matter (OM) was applied as compost at a rate of 20 tons per acre. Manganese (Mn) was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. No transformation was used for these analyses.

Source	df	F	P
Treatment	13	13.53	<0.001
Error	39		

Table A3.18. Differences in soil sulfate (SO_{4-}) content (mg per kg) between the tilled control, herbicide alone, manganese alone, or manganese plus herbicide treatments. Sulfate was applied as part of the manganese (Mn) soil amendment treatment. Manganese was applied at a rate of 25 lbs Mn per acre as MnSO4. Herbicide was applied as chlorsulfuron at a rate of 88 g per ha. All treatments presented here were tilled. No transformation was used for data analyses.

Treatment Number	Treatment	Mean Soil Sulfate (mg per kg)	Treatment Comparison	P-value
1	Tilled control (C+S+T)	82.72	1,4	<0.001*
2	Tilled + herbicide (herbicide alone) (D+I+N)	87.97	2,4	<0.001*
3	Tilled + Mn (manganese alone) (F+K+P)	27.28	3,1	<0.001*
4	Tilled + herbicide + Mn (H+M+R)	17.93		

*Indicates significantly different using contrasts and a Bonferroni adjustment ($P \geq 0.017$).