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Legume-grass forage mixes for maximizing yield and competitiveness against weeds in early establishment

by

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I dedicate this thesis to Charlie Kaufmann, whose love and supported helped me complete this project.

"A weed is no more than a flower in disguise" – James Russell Lowell

"A flower is an educated weed" – Luther Burbank

"To forget how to dig the earth and to tend the soil is to forget ourselves" – Mohandas K. Gandhi

"*There are two spiritual dangers in not owning a farm. One is the danger of supposing that breakfast comes from the grocery and the other that heat comes from the furnace" – Aldo Leopold*

Abstract

A field experiment from 2003 to 2005 at two sites examined the impacts of forage species and legume proportion on forage sward production. Grasses generally established rapidly and out-yielded swards high in legume content, although legumes did improve forage quality. Alfalfa was retained at greater relative biomass in mixed swards than swards containing clover. Legume persistence also varied depending on neighbouring grass species.

A greenhouse study examined competitive interactions between Canada thistle (a common pasture weed), white clover and Kentucky bluegrass during establishment. Although thistle was most susceptible to intra-specific competition, and strongly affected forage yield, the latter also influenced weed biomass. Competitiveness of forages depended directly on soil medium, emphasizing the importance of abiotic factors on vegetation dynamics in mixed swards.

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List of Abbreviations

ADF – Acid Detergent Fibre
ADSY – Acid Detergent Soluble Yield
CP – Crude Protein
CPY – Crude Protein Yield
E1 – Ellerslie 1 study location (established 2003)
E2 – Ellerslie 2 study location (established 2004)
N - Nitrogen
TCPY – Total Crude Protein Yield
W240 – West 240 study location at the Edmonton Research Station

Chapter 1. Introduction

1.1. Background

Legumes are known to benefit the forage sward, both through a positive contribution to yield and/or improved forage quality (Barnett and Posler 1983, Sleugh *et al*. 2000, Papadopoulos *et al*. 2001). This benefit could be caused by the diversity created by adding legumes into a grass sward, which may allow for the utilization of soil resources not used by neighboring grasses. Overyielding is the phenomena that occurs when the benefits of combining two or more species of plants with complementary growth forms outweighs the cost of increasing competition, and subsequently produces greater yields than each individual species grown alone (Gokkus *et al*. 1999, Posler *et al*. 1993). The addition of legumes however, does not guarantee that overyielding will occur (Sengul 2003).

More importantly, legumes are beneficial due to the symbiotic relationship they have with nitrogen (N) fixing bacteria. Alfalfa, white clover and alsike clover can provide up to 258 (Burity *et al*. 1989), 545 (Elgersma and Hassink 1997) and 86 (Fairey 1986) kg N ha^{-1}, respectively to a forage sward, and can negate the requirement for N addition to maximize productivity, even of the grass component. The addition of atmospheric N reduces the need for the addition of fertilizer, thereby reducing establishment costs, energy consumption, and the potential for N loss (i.e. through leaching or runoff). The addition of legume to swards at seeding is a common practice due to widespread acceptance of its benefits to forage yield and quality (Elgersma and Hassink 1997), and animal production (Bertlisson and Murphy 2003, Dewhurst *et al.* 2003) compared to grass grown alone.

The addition of legumes to a forage stand can provide benefits to the sward (Kunelius *et al*. 2006, Sleugh *et al*. 2000), but the proportion of legume required to optimize these benefits has not been delineated. Plant species and growth forms may also change the positive impacts of legumes on grass growth, as well as overall sward yield. To date, little is known about how mixing different root growth forms of grasses (i.e. bunchgrasses vs. rhizomatous sod-grasses) or legumes (i.e. shallow rooted clovers vs. tap-rooted alfalfa) may impact overall production and quality of a sward. The different root systems of grasses (i.e. fibrous vs. rhizomatous) may influence the ability of a plant to obtain resources when grown along side legumes. This may have a significant impact on their ability to achieve overyielding based on differences in growth and associated forage biomass and/or quality. Finally, information on the optimal proportion of legumes relative to grasses within seeding mixtures is needed to consistently achieve maximum forage production in a single cut hay system. In Alberta, this is particularly important as forage shortages have been increasing in recent years, and represent one of the greatest limitations to expansion of the beef industry.

When weedy species invade an establishing forage sward managers must make decisions regarding their control. The risk of losing beneficial N fixing plants may prevent producers from spraying fields to remove weeds. While spraying herbicides at reduced rates may reduce mortality to legumes, it also results in reduced weed control (Mesbah and Miller 2005). Moreover, while the use of herbicides with residual properties may improve long-term weed control, it also prolongs the period before legume re-establishment can be undertaken.

Invasive weeds lead to production losses in agronomic systems, including range and pasture environments (Masters and Sheley 2001). Canada thistle is a widespread perennial weed impacting both annual and perennial crops, and is found in North America, Europe, and Asia (Donald 1990, Ang et al. 1994, Freidli and Bacher 2001). Across Canada, thistle is highly adaptable to a wide range of growing conditions. In the 1997 Western Canada Weed Survey, Canada thistle was found to be present in 53% of cereal, oilseed and pulse crops surveyed (Thomas *et al*. 1998). However, this report underestimates the weed's impact as it does not include perennial fields. In Alberta, 44 of 47 reporting counties indicated that they had moderate to high Canada thistle infestations (Agriculture and Rural Development 2009).

Intense competition from Canada thistle often leads to a reduction in plant production. Canada thistle is an aggressive plant that has been shown to reduce wheat (Mclennan *et al*. 1991) and canola (O'Sullivan *et al*. 1985) yields by up to 49% and 26%, respectively. In perennial crops such as forages, Canada thistle can lead to reduced seed production (Moyer et al. 1991) and biomass yield losses of up to 2 kg ha^{-1} of Canada thistle biomass present (Grekul and Bork 2004). In addition to reductions in yield the impact in pastures is intensified due to Canada thistle's ability to deter grazing, reducing grazing potential (Seefeldt *et al.* 2005). Recent research has begun to look at the impacts of Canada thistle on pasture and hay systems (Seefeldt *et al*. 2005, Grekul and Bork 2004), as well as the potential for using perennial plants to outcompete the weed (Wilson and Kachman 1999). However, these studies often focus on grasses and seldom address systems that include legumes.

Canadian provinces have legislation requiring the control of specific plants deemed a threat to both agronomic and native plant systems. In Alberta, the Weed Control Act (Government of Alberta,1980) classifies weeds of concern as either restricted, noxious or nuisance. Canada thistle is considered a noxious weed under this legislation, consistent with other western provinces, and as a result, control of this plant by the land owner is required to prevent its spread. Weed control methods available to producers include: chemical methods using herbicides, both selective and non-selective (Hodgson 1970, Bixler 1991, Grekul and Bork 2007), the addition of fertilizer to improve weed suppression through competition from increased forage (Grekul and Bork 2007), biological control methods using insects (Friedli and Bacher 2001) or livestock grazing (De Bruijn and Bork 2006), and mechanical means such as tilling (Lukashyk *et al*. 2008) or mowing (Schreiber 1967, Beck and Sebastian 2000). Several herbicides are approved for use on Canada thistle in both annual and perennial cropping systems (Alberta Agriculture and Rural Development 2009). While the application of herbicides has been shown to be effective for Canada thistle control, these chemicals may remove beneficial legumes from the plant community.

1.2. Research Objectives

By studying the impacts of different forage species, both on each other and on a weed (Canada thistle), we can begin to understand the complex competitive dynamics that regulate forage availability, as well as improve Canada thistle control by using the competitive influences of herbage. This research evaluates the role of legumes in optimizing forage production and quality within newly seeded swards. Additionally, this research assesses the inter-specific dynamics between a common legume (clover), a common pasture grass (Kentucky bluegrass), and Canada thistle during early forage establishment. Better information on the role of legumes in forage mixes, including their relation to Canada thistle abundance, will allow producers to make more informed decisions regarding herbicide application for weed control.

The specific objectives of this research include:

(1) Reviewing the benefits of legumes in forage swards, legume-grass sward dynamics, Canada thistle biology, and the potential interactions between Canada thistle and legume-grass mixed swards (Chapter 2).

(2) Evaluating the relative contribution of various amounts of legumes to total forage yield and quality, when established in mixtures with various perennial grasses (i.e. test of overyielding) (Chapter 3),

(3) Determining the specific competitive and facilitative trade-offs between a noxious weed (Canada thistle), a legume forage (white clover) and a common perennial grass (Kentucky bluegrass) grown in combination with one another in the greenhouse (Chapter 4),

(4) Develop recommendations for newly established mixtures so as to optimize forage availability (biomass and quality), as well as the ability of common forages to compete with Canada thistle (Chapter 5).

1.3. Literature Review

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Chapter 2. Literature Review

2.1. Grass-Legume Interactions

2.1.1. Legumes

2.1.1.1. Clover Biology

White clover (*Trifolium repens* L.) is native to the Mediterranean (Pederson 1995) and is widely used as a legume in grazing systems throughout temperate zones such as Europe, Australia, New Zealand, Japan and North America (Frame 2005). Optimum growing conditions for white clover includes temperatures between 20 and 25°C, minimum annual precipitation of 310 mm and a soil pH range of 5.5 to 6.0 (Frame 2005). White clover grows best in loamy or well drained clay soils with high fertility (Pederson 1995). Sandy soils are often nutrient poor and dry, reducing white clover abundance. This forage does not do well under long periods of soil saturation or acid soils (Frame 2005). While commonly used as a perennial forage species, white clover can also be considered a weed invading roadsides and waste areas (Turkington and Burdon 1983). White clover is often found to invade areas after fertility has improved and the influence of plant shading has been reduced (Turkington and Burdon 1983).

During the initial stages of seedling development white clover produces a tap root that survives for the first two years (Engin and Sprent 1973). Under the right growing conditions seedlings send out stolons from leaf axils that develop into plantlets on bare soil or other available niche locations (Frame 2005). Plantlets subsequently develop shallow fibrous roots systems, with the majority of root mass in the top 10 cm of soil (Turkington and Burdon 1983). As white clover plants develop further, the tap root and primary stolons die, severing connections between plantlets and the original parent (Peterson 1995).

White clover grows best with non-aggressive grasses, although it is often seeded with aggressive grasses such as perennial ryegrass (*Lolium perenne* L.), orchard grass (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb) (Annicchiarico and Piano 1994). Compared to grasses, white clover is a weak competitor for inorganic N (Hogh-Jensen and Schjoerring 1997). Shading from grasses may reduce stolon formation and increase stolon internode length (Frame 2005). White clover exhibits phenotypic plasticity in response to defoliation, producing smaller leaves and shorter internodes (Frame 2005).

Alsike clover (*Trifolium hybridum* L) is indigenous to Europe (Winton 1914) and was introduced to Canada in 1839 (Fairey 1986). It is a short lived perennial that is tolerant of poor soil conditions including alkaline, wet and acidic soils. Alsike clover is a non-creeping perennial that is semi-erect with long thin stems that arise from the crown. Yields of alsike clover are often lower than those of red clover (*Trifolium pratense* L.), but the former is a hardier plant being able to withstand saturated soils (Fairey 1986). Alsike clover is most productive during the year of establishment (4.08 t ha^{-1}), with yield declining in subsequent years (i.e. 0.32 and 0.37 t ha^{-1} in the second and third years, respectively) (Fairey 1986). Alsike clover is most compatible with non-aggressive grass species and provides low levels of competition to weeds. Alsike clover is tolerant of MCPB, 2,4-DB and benazolin herbicides, providing some options for the control of broadleaf weeds during and after establishment (Frame 2005).

2.1.1.2. Alfalfa Biology

Alfalfa is a perennial legume that originated in Iran, but its hardiness to extreme temperatures allows it to grow throughout the world (Barnes and Sheaffer 1995). Alfalfa is a large erect plant with heights averaging between 60 and 90 cm (Barnes and Sheaffer 1995). This legume is deep-rooted, reaching up to 9 m into the soil profile, with branching occurring in the top 15cm. During drought conditions alfalfa is able to extract moisture from deep in the soil profile and enter into a dormant state should moisture deficits become severe (Barnes and Sheaffer 1995). The deep tap root of alfalfa is also able to extract N from deep in the soil profile (Russelle *et al*. 2001). Alfalfa obtains most of its N from symbiotic relationships with *Rhizobium* bacteria, most of which occurs in the nodules of fibrous roots near the soil surface.

Alfalfa is a highly productive legume (Frame 2005), and can produce more protein per hectare than grain or oil crops. Studies have shown that cattle grazing alfalfa can gain up to 0.67 kg ha^{-1} day^{-1}, although grazing alfalfa does increase the risk of bloat to cattle (Marten *et al*. 1987). While alfalfa is capable of withstanding multiple harvests per year in wet climates, it is generally only harvested once or twice annually in Alberta. It is suggested that in northern climates, 4-6 weeks of recovery are needed prior to the first killing frost (-3°C) to ensure survival (Barnes and Sheaffer 1995). Harvesting schedules for alfalfa will influence yield, quality, persistence and plant development. Attempts to harvest alfalfa at the highest protein level (bud stage) may sacrifice yield and stand persistence. Harvesting at the early-flowering stage is a compromise between forage quality and quantity. The greatest protein is found in plant leaves, and as a result, protein values decline after flowering due to leaf loss. In addition to the loss of high protein leaves, quality of alfalfa declines due to an increase in the amount of fibre in the stem. The use of alfalfa in crop rotation can increase soil organic matter (Wu *et al*. 2003) and reduce disease (Speakman *et al*. 1978) Other benefits of using alfalfa include reducing soil erosion and water run off (Barnes and Sheaffer 1995).

2.1.2. Grasses

2.1.2.1. Smooth Brome

Smooth brome (*Bromus inermis* L.) is a sod-forming perennial grass native to Eurasia (Otfinowski *et al*. 2007). It was introduced to Canada as early as 1888 as a source of perennial forage. Smooth brome is a tall (20 – 100 cm) plant capable of shading out lowered statured neighbors (Otfinowski *et al*. 2007). The majority of smooth brome roots reside in the top 10 cm of soil, however some can penetrate as deep as 1.5 m allowing the plant to readily withstand moisture stress (Otfinowski *et al*. 2007).

Smooth brome is a popular grass in both hay and pasture mixes across western Canada. In Alberta it grows best in the Dark Brown and Black Chernozemic soils of central regions, although it is found throughout the province on all soil types. This grass requires 280 – 450 mm precipitation for favorable establishment and growth, and tends to increase in competitive ability with the addition of nutrients (Otfinowski *et al*. 2007). Smooth brome was first reported outside its cultivated range in 1903 (Otfinowski *et al*. 2007). It is now found in native grasslands, ditches, forest edges, shorelines and disturbed areas throughout Canada. Smooth brome can be detrimental to native grasslands, leading to a 70% reduction in native grassland diversity (Otfinowski *et al*. 2007). Under the right conditions smooth brome may invade forage stands where it was not seeded and produce extensive monocultures.

2.1.2.2. Kentucky Bluegrass

Kentucky bluegrass is believed to have originated in Eurasia and is now found throughout the world (Wedin and Huff 1996). Bluegrass has the ability to quickly form a thick sod, which makes it an attractive species for erosion control, lawns and sports turf. It has also been used extensively as pasture forage and has been observed to invade plant communities such as roadside ditches, as well as poor condition pastures with a history of heavy grazing (McCartney and Bittman 1994). This grass grows best in cool, moist and fertile conditions (Wedin and Huff 1996). Hot, dry conditions lead to a reduction in growth and may even force the plant into early senescence.

Bluegrass is a short statured plant (leaf blades 5 – 15 cm long) with narrow leaf blades (2-4 mm wide). It reproduces readily through both sexual and vegetative means. Bluegrass seeds have a 14 day germination time and a long juvenile stage leading to slow establishment (Wedin and Huff 1996). Seedlings of bluegrass initially develop a seminal root that persists only a short time.

Adventitious roots begin to develop on the lower nodes of each side of the axillary bud (Etter 1951). Once established, bluegrass is quick to colonize surrounding areas though the development of rhizomes. After spreading laterally, rhizomes emerge from the soil surface and develop into a new shoot, after which the parent plant no longer supplies the new offshoot with nutrients (Etter 1951). The extensive development of rhizomes under ideal conditions can spread a single bluegrass plant out over an area up to two square meters in two years (Etter 1951).

2.1.2.3. Meadow Brome

Meadow brome also is indigenous to Eurasia and grows in the cooler, moister areas of the range where smooth brome is found (Vogel *et al*. 1996). It is most successful in the Black and Gray Luvisolic soil zones, together with mesic areas of the Dark Brown soil zone (Knowles *et al*.1993). Often used in similar management systems to smooth brome, meadow brome has the advantage of quick regrowth following defoliation (Knowles *et al*. 1993, Lawrence and Ratzlaff 1985). Meadow brome regrowth comes from existing tiller bases, rather than the crowns and rhizomes as it does in the case of smooth brome. Similar to smooth brome, meadow brome propagates through seed and rhizomes (Vogel *et al*. 1996). However, the rhizomes of meadow brome are much shorter and give the plant a distinctly ‘bunched’ appearance. As a forage species, meadow brome is greater in digestibility than smooth brome, but contains lower crude protein levels (Ferdinandez and Coulman 2001).

2.1.3. Benefits of Legumes

The benefits of forage legumes have long been recognized for increasing both sward quality and yield (Kunelius *et al*. 2006, Sleugh *et al*. 2000), improving animal production (Bertlisson and Murphy 2003, Dewhurst *et al.* 2003), and extending the grazing season. Benefits to sward quality have been documented through increases in protein concentration, decreases in the fibre content of feed, and the creation of a more balanced mineral composition of forage (Haynes 1980, Fraser and Kunelius 1995). Forage N and protein levels typically increase in direct proportion to the amount of legume present. Moreover, there can be a positive linear relationship between grass N levels and the proportion of neighboring legume present (Mallarino and Wedin 1990).

There are other benefits to growing forages in mixtures besides the obvious benefit of a continuous N supply. Soil organic matter also improves under legumes (Wu *et al.* 2003). Legumes can enhance the temporal distribution of forage yield and quality throughout the growing season by providing greater relative productivity than grasses later in the season (Sleugh *et al*. 2000), thereby extending the period during which high quality forage is available.

Production from legume-grass sward mixtures can vary depending on the legume species, grass species (Sengul 2003) and environment. Factors such as forage plant stature (upright or prostrate), root morphology (tap vs. fibrous), leaf orientation, growth pattern, and N fixation can all affect the level of benefits provided to neighboring grasses. The rate of N fixation is influenced by the same environmental conditions that influence photosynthesis, and may include soil moisture, fertility and temperature (Hardarson and Atkins 2003). Optimal temperature ranges for most legume nodulation are 20-30^{o}C. Lower temperatures can reduce both the number of nodules and the rate of N fixation (Gibson 1971).

2.1.3.1. Nitrogen Fixation and Transfer

There are three primary mechanisms by which N transfer can occur from legumes to grasses. The first is transfer through mycorrhizal fungi that directly connect the roots of legumes and grasses, or by mycorrhizae depositing N in the soil, from where they can then be extracted by plant roots for uptake (Johansen and Jensen 1996, Rogers *et al*. 2001, Hogh-Jensen and Schjoerring 2001). The second is through the breakdown of both above and below ground plant matter (Ledgard and Steele, 1992), while the third is root exudation directly into the soil profile (Paynel *et al.* 2001).

Older plants are known to transfer more N through biomass degradation (Johansen and Jensen 1996, Hogh-Jensen and Schjoerring 2001). Nitrogen transfer from the breakdown of plant matter provides a long term source to support grass growth (Dubach and Russelle 1994, Tomm *et al.* 1994, Russelle *et al.* 1994, Johansen and Jensen 1996).

On a short term basis N can be transferred to grasses through mycorrhizae or root exudation into the rhizosphere (Paynel *et al*. 2001). While young plants transfer very little N through mycorrhizal fungi (Rogers *et al*. 2001), other plants have been shown to transfer N through root exudation (Paynel *et al.* 2001, Paynel and Cliquet 2003). The majority of N transfer is directed from the legume to the non-legume plant in the mix, with some evidence suggesting that there is also N transfer in the opposite direction (Tomm *et al.* 1994). The importance of each method of N transfer varies among legume species (Ta and Faris 1987). While species such as alfalfa and red clover are seen to excret N through their roots, birdsfoot trefoil relies mainly on root and nodule turnover (Ta and Faris 1987). Thus, the rate of N transfer and legume benefit to overall sward yield is likely to vary depending on the species of legume as well as the responsiveness of neighboring grasses to N made available.

2.1.4. Sward Dynamics

2.1.4.1. Legume Persistence

The ultimate management goal of legume-grass pastures is to maintain the legume population for as long as possible. This goal can be affected by the environment, forage species and management . Competitive ability of a legume can be impacted by moisture, light and nutrients. Some legume species such as white clover are not able to withstand long periods of moisture stress, and thus rapidly decline during drought (Turkington and Burdon 1983). Conversely, the growth pattern of alfalfa allows this species to enter into a dormant period during periods of low moisture, minimizing die-back (Hall *et al.* 1988).

When grown with tall growing species such as smooth brome, many low growing legumes do not receive sufficient light to maintain required rates of photosynthesis, thereby reducing their competitive ability and causing their abundance to decline (Marcuvitz and Turkington 2000). Consequently, management systems that allow grasses to regrow for extended periods cause white clover populations to decline under heavy shading (Haynes 1980). Conversely, white clover is known to be grazing tolerant even under frequent defoliation, and competes well with grasses provided light is adequate (Sheaffer 1989). Similarly, quick regrowth following defoliation of alfalfa allows this species to compete against slower growing grasses such as smooth brome (Sheaffer 1989). When alfalfa and white clover are grown with the same grasses, alfalfa is better able to maintain consistent yields over time, while white clover declines in total yield (Frame and Harkess 1987). Breeders of alfalfa have developed more grazing resistant varieties, and although they maintain higher populations under grazing, they decline with time (Katepa-Mupondwa *et al*. 2002).

High levels of soil N can also be detrimental to the legume component of a sward (Ledgard and Steele 1992). Abundant N suppresses nitrogen fixation forcing legumes to rely on soil available N. As grass roots take up N quickly, they reduce the N available to legumes and decrease legume competitive ability. The addition of N to a pasture can take many forms including animal excreta, which can also lead to an increase in grass growth, that in turn reduces legumes in the sward (Vinther 1998). Within mixtures of white clover and ryegrass, ryegrass was found to obtain a larger fraction of available soil N, thereby out-competing the clover (Haynes 1980).

2.1.4.2. Grass Persistence

The persistence of grasses within a sward is again dependant on environmental conditions, management, and the type of plant species present. Grass species that are not strong competitors tend to have a limited impact on neighboring legumes, and therefore aid in the maintenance of legumes. Grasses such as smooth brome, bluegrass and meadow brome are all competitive species that, depending on the other species in the mixture, may come to dominate the sward, particularly when fertilized with N. This scenario may reduce legumes until soil N levels are depleted and the competitive advantage of legumes has again been restored. When grown with alfalfa, both smooth brome and meadow brome maintain low levels of legumes in the sward (Lawrence and Ratzlaff 1985). The competitive nature of these two grasses makes them equally able to suppress potential weeds (Lawrence and Ratzlaff 1985).

The persistence of smooth brome can be highly dependant on management. When cut for hay this species can maintain vigorous populations. However, when grazed smooth brome populations decline and eventually require pasture rejuvenation (McCartney and Bittman 1994, Lawrence and Ratzlaff 1985). In contrast, meadow brome is more persistent under rotational grazing and is able to maintain a higher proportion in the sward than smooth brome (Lawrence and Ratzlaff 1985). Nevertheless, both smooth brome and meadow brome are equally persistent when subject to intensive grazing in Saskatchewan (McCartney and Bittman 1994). Under heavy or continuous grazing, populations of Kentucky bluegrass are known to increase (Hein and Vinall 1933, Waddington *et al*. 1999).

2.1.5. Contribution of Legumes in Optimizing Forage Yields

Clovers are extensively used in parts of New Zealand, Australia and Europe as an alternative to inorganic fertilizers. The majority of research focuses on white clover and its contribution to the yield and quality of pastures in these areas. In fertile soils, alsike clover has been found to be a stronger competitor against annual weeds than white clover, possibly due to its taller stature (Ross *et al*. 2001). Swards of grass grown with white clover produce lower biomass but higher crude protein compared to the same grasses grown with alsike clover (Kuusela 2004). Mixtures of white and alsike clovers grown with grasses produce intermediate biomass and crude protein (Kuusela 2004). Caution should be used when grazing alsike clover as this species may cause photosensitivity and poisoning in horses (Nation 1989). The amount of biologically fixed N in white clover can vary between 54 and 545 kg N ha^{-1} yr^{-1} (Peoples *et al.* 1995, Elgersma and Hassink, 1997). The amount of N transferred from clover to associated grasses can vary between 0 and 80% of total grass N (Broadbent *et al*. 1982, Brophy *et al.* 1987, Ledgard 1991), and can be influenced by the spatial relationship between the grass and legume (Brophy *et al*. 1987).

In an Alberta study, the addition of 33% alfalfa to a smooth brome sward increased total yields by more than 5000 kg ha^{-1} (Malhi *et al.* 2002). Presence of legume in a sward can increase production at a level equivalent to that produced by the application of 100 kg ha^{-1} of fertilizer N to a pure brome stand (Malhi *et al.* 2002). While the benefits of adding legume to grass swards have been well documented, a pure alfalfa stand may provide the highest productivity both in terms of biomass yield and forage quality (Sleugh *et al*. 2000). Nitrogen fixation in alfalfa ranges from 80 to 258 kg N ha^{-1} (Burity *et al.* 1989, Haby *et al*. 2006) with up to 20 kg N ha^{-1} being transferred to associated grasses (Burity *et al.* 1989). Grasses can obtain up to 77% of their N from legumes (Haby *et al.* 2006). Similar results have been found for other legumes. For example, with every 1% increase in birdsfoot trefoil, grass N levels improved by 0.2 g kg^{-1} (Mallarino and Wedin 1990).

In general, the amount of N transferred from legumes to grasses can range between 29 and 454 kg N ha^{-1} (Elgersma *et al*. 2000, Elgersma and Hassink 1997). As a forage stand matures the rate of N transfer to grass increases (Elgersma *et al*. 2000), provided legumes are retained within the mixture and do not decline due to grazing, drought, fertilization, or intense competition from neighboring grasses. Many grasses are known to cause declines in the proportion of legumes over time (e.g. Kuusela 2004). As a result, the retention of legumes in mixed forage swards is considered a priority in forage production systems. This in turn, has led to many questions about the ideal forage mix to both 1) optimize forage yield and quality, and 2) optimize resistance to weed invasion and/or weed suppression through interspecific competition.

2.2. Canada Thistle

Canada thistle [*Cirsium arvense* (L.) Scop.] is a perennial weed native to Europe, northern Africa and western Asia (Hayden 1934, Hodgson 1968, Donald 1990). Canada thistle is a problematic weed throughout the world including Canada, the United States, Europe, western Asia, North Africa, South America, New Zealand and Australia (Freidli and Bacher 2001, Ang *et al*. 1994, Donald 1990). This species is known by several common names including creeping thistle, California thistle, perennial thistle and cursed thistle (Holm *et al*. 1977). There are four recognized varieties of Canada thistle (var *vestitum* Wimm. & Grab., var *integrifolium* Wimm. & Grab., var *arvense* and var *horridum* Wimm. & Grab.) that are identifiable by differences in leaf structure (Hodgson 1964, Moore and Frankton 1974).

2.2.1. Canada Thistle Biology

Canada thistle is a slender plant ranging between 30 and 150 cm in height (Moore 1975). The stem is branched with leaves that are alternate, sessile and clasping. The leaves are irregularly lobed with spiny margins and an oblong outline. Stems are terminated by 1-5 sessile flower heads. Canada thistle is dioecious with individual plants either male or female (Heimann and Cussans 1996).

Canada thistle can reproduce via both sexual and vegetative means. Seed production is not considered to be a major source of weed infestation (Hamdoun 1972). In order for insect pollination to occur male and female plants must be within 390 m of each other (Amor and Harris 1974). Viable achenes are produced approximately nine days after the flower head opens (Derscheid and Schultz 1960). While CT has the potential to produce large amounts of seed, few are viable (Bakker 1960) and they have limited potential to spread great distances (Wallace *et al*. 2005). Seeds on the soil surface have little to no germination success, although seeds buried at shallow depths have increased germination (Amor and Harris 1975). Seedling establishment in pastures may be low due to high temperature requirements for germination (Heimann and Cussans 1996, Wilson 1979) and high light requirements for growth (Wilson 1979). Seedling survival of those that do establish depends on environmental conditions. Seedlings subject to intense shading have low survival, as do those subjected to wet soil conditions with poor aeration (Bakker 1960). Once seedlings have established the plant's main source of propagation is through an extensive network of creeping roots.

Vegetative spread is the main method of propagation for Canada thistle. Creeping roots can run both horizontally and vertically within the soil profile. Roots have been known to spread up to 12.2 m per year depending on conditions (Amor and Harris 1975). Canada thistle roots can be 1.8 m deep, with more than 50% within the top 40 cm (Nadeau and Vanden Born 1989). As many as eight Canada thistle shoots have been found for every meter of root below the soil surface (Nadeau and Vanden Born 1989). Shoots can emerge from two main locations. Non-adventitious shoots arise from nodes on the shoot, and are often stimulated by damage to the main stem. Adventitious root buds form along the roots, and under the right conditions can elongate and form daughter shoots (Donald 1994). Root bud initiation has been found to decline at moderate to high N fertilizer levels (210 – 420 ppm), although the size of the shoots developing from initiated root buds increases compared to low fertilizer levels (5.25 – 21.0 ppm) (McIntyre and Hunter 1975). High levels of N fertilizer (420 ppm) can also be toxic to Canada thistle, reducing the number of root buds initiated and associated shoot number, root dry weight, and the number of Canada thistle leaves (McIntyre and Hunter 1975, Hamdoun 1970). Root fragments as small as 10 mm in length and 1 mm width are capable of producing a viable shoot (Hamdoun 1972).

2.2.2. Canada Thistle Management

2.2.2.1. Impact of Canada Thistle

Canada thistle can reduce production of annual and perennial crops. Canada thistle has been shown to reduce winter wheat, spring wheat, barley, and canola yields (Donald and Khan 1996, McLennan *et al*. 1991, Mamolos and Kalburtji 2001, O'Sullivan *et al.* 1982, 1985). Densities of 13 to 20 Canada thistle shoots m^{-2} have led to yield losses of 30% in winter wheat, 29 to 60% in spring wheat, 40% in barley and 62% in oats (Hodgson 1955, 1968). The presence of Canada thistle at an average density of 21 plants m^{-2} reduced alfalfa biomass production by 4600 kg ha^{-1} per year over a four year period (Schreiber 1967). In alfalfa fields grown for seed, thistle densities of 10 and 20 shoots m^{-2}

caused an estimated reduction in seed yield of 34% and 48%, respectively (Moyer *et al*. 1991).

In the Parkland of central Alberta, perennial grassland yield losses can be as high as 2 kg ha^{-1} of forage for every kg of Canada thistle biomass present (Grekul and Bork 2004). Similarly, increases in Canada thistle stem density of one stem per square meter decreases pasture forage by 4.3 kg ha^{-1} (Grekul and Bork 2004). Canada thistle stem densities just over 20 per m^{-2} can reduce consumption of neighboring forage by 5200 kg ha^{-1} (Schreiber 1967). Research has begun to look at the impacts of Canada thistle on pasture and hay systems, including the potential for using perennial plants to outcompete the weed. However, these studies often focus on grasses or legumes separately and seldom address agro-ecosystems that include both.

2.2.2.2. Canada Thistle Control Methods

The growth form of Canada thistle makes this weed difficult to control. The creeping root system allows the plant to spread over a large area. The ability to produce shoots along the entire root system enables Canada thistle to establish clonal plants outside the treatment area. Canada thistle shoots have been seen to survive and spread at the edge of spray zones and mowing areas allowing them to continue to flourish under control treatments. In order to successfully control Canada thistle, methods must target the root system, either through a reduction in their carbohydrate reserves or death. Root carbohydrates reserves of Canada thistle fluctuate throughout the growing season (Wilson *et al*. 2006). When new shoots develop in the spring and early summer there is a marked reduction in root carbohydrates, which are then replenished in late summer and fall. Timing the application of control methods to when root carbohydrates are low may increase control of the weed (McAllister and Haderlie 1985). Control measures that have been studied in the past include herbicides, cultivation, mowing, biological control, grazing, fertilization and crop competition (Donald 1990).

There are several herbicides approved for the treatment of Canada thistle in Canada with varying levels of control or suppression (Alberta Agriculture and Rural Development 2009). Timing of herbicide application affects control success. Fall application of herbicide can increase Canada thistle control by 59% (Wilson *et al*. 2006). Fall applied herbicide acts directly on Canada thistle by killing the shoot, but also reduces the plant's ability to replenish fall carbohydrate stores, reducing spring survival (Wilson *et al*. 2006). Annual pre-harvest applications in barley crops with glyphosate reduce Canada thistle populations by 98% (Darwent *et al*. 1994). A combination of spring tillage and late summer spraying during the rosette stage will reduce Canada thistle density by 99% (Hunter 1996).

The use of herbicides for Canada thistle control in perennial systems containing legumes is often avoided due to the risk of legume loss. The use of MCPB applied at 1.12 kg ai ha^{-1} resulted in only 27% Canada thistle control while causing 40% injury to alfalfa (Mesbah and Miller 2005). Imazamox or imazethapyr applied at 0.05 kg ai ha^{-1} and 0.07 kg ai ha^{-1}, respectively, resulted in 29 to 35% Canada thistle control, but did not cause measurable damage to alfalfa

(Mesbah and Miller 2005). Benzone has been found to cause low levels of alfalfa injury (less than 13%) and still achieve high Canada thistle control (greater than 80%) (Meshbah and Miller 2005). Picloram at 1.12 kg ai ha^{-1} gave 100% control of Canada thistle in a timothy-red clover sward, but reduced red clover by 87% (Peterson and Parochetti 1978). Four treatments of Bentazon at a rate of 0.28 kg ai ha^{-1} led to 100% control of Canada thistle but also caused injury to 96% of birdsfoot trefoil in the stand (Boerboom and Wyse 1988). By increasing the rate of Bentazon to 0.42 kg ai ha^{-1} and reducing the application frequency to two times, injury to birdsfoot trefoil was reduced to 52% while Canada thistle control remained 92%. Despite the ability of herbicides to provide timely control of Canada thistle, management decisions regarding this weed must weigh the relative cost of losing legumes against the benefit of the forage gained by weed elimination.

The use of mechanical controls such as ploughing or mowing are not considered effective options for Canada thistle control. Ploughing will often produce unsuccessful results due to the depths to which roots grow (Nadeau and Vanden Born 1989). Root fragments created by ploughing may not be small enough to prevent shoot establishment, and may lead to a flush of newly initiated shoots. Ploughing can increase field populations by spreading root fragments beyond the initial infestation area. While mowing can prevent the production of seed, the timing of mowing is important, with frequent and timely mowing treatments over several years needed for successful CT control (Schreiber 1967, Beck and Sebastian 2000).

The use of competition from neighboring forage is another option for Canada thistle control (Masters and Sheley 2001), and may enable legumes to be retained within the sward. Perennial grass can control up to 90% of Canada thistle over a three year period (Wilson and Kachman 1999). This control is equal to yearly applications of clopyralid at 0.55 kg ha^{-1} (Wilson and Kachman 1999). Other research shows that by leaving infested swards ungrazed, Canada thistle shoot densities per plant can be reduced up to 80% with recovery of neighboring vegetation (Eerens *et al*. 2002). In perennial forage systems plants that are not injured or removed during treatment can aid in controlling the weed through interspecific competition. The addition of fertilizer to a pasture sward in conjunction with herbicide application increases Canada thistle control by up to 65 kg ha^{-1} (Grekul and Bork 2007), although this is not always the case (Reece and Wilson 1983).

Where access to pastures by spraying or mowing equipment may be difficult or impossible, biological control methods may provide a means of reducing Canada thistle infestations. Introducing invertebrate predators from the plant's native habitat may lead to long term control. Pathogens such as *Sclerotinia sclerotiorum* (Lib.) de Bary and *Puccinia punctiformis* (F. Strauss) Rhol have been shown to reduce Canada thistle growth (Bosten and Sands 1986, Demers *et al*. 2006). Similar to other methods of Canada thistle control however, most biological methods require several treatments (Bourdot *et al*. 2006). Success of using pathogens and invertebrates for biological control can be enhanced when combined with other weed control methods. For example, mowing increases the rate of pathogen infestation, resulting in increased weed control success with the latter (Demers *et al*. 2006). Large herbivores have also proven successful for Canada thistle control. Grazing of Canada thistle by goats reduced the total number of shoots by 30% after two years (Booth and Skelton 2009). Using intensive grazing applied twice annually, cattle can reduce Canada thistle growth in pasture through both utilization and trampling (De Bruijn and Bork 2006).

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Chapter 3. Interspecific Relationships Between White Clover, Bluegrass and Canada Thistle: A Greenhouse Study

3.1. Introduction

Invasive weeds are a problem throughout the world, leading to losses in biodiversity, forage production and livestock profitability (Masters and Sheley 2001). Attempts have been made to control pasture weeds with many strategies, including the use of herbicides (Beeler *et al.* 2004, Hein and Miller 1991), fertilization to enhance crop competitiveness (Thomson and Saunders 1986, Cole et al. 1999), prescribed fire (MacDonald *et al.* 2007, Emery and Gross 2005), mechanical means such as mowing (Rinella *et al*. 2001, Wilson and Clark 2001), biological control (Seastedt *et al*. 2003) or a combination of these treatments. While individual weed control methods are variable in their effectiveness, integrated approaches often increase weed suppression in range and pasture systems (Hodgson 1958, Masters and Sheley 2001, Bork *et al.* 2007).

Canada thistle [*Cirsium arvense* (L.) Scop.] is a widespread noxious weed in pastures across the prairies of western Canada (Skinner *et al.* 2000), where it has been shown to cause forage yield losses as high as 2 kg ha^{-1} for each kg of CT biomass present (Grekul and Bork 2004). The competitiveness of Canada thistle is attributed to its extensive creeping root system, which allows it to capture soil resources and colonize large areas (Donald 1994). Methods to control Canada thistle in pasture include mowing (Schreiber 1967, Beck and Sebastian 2000), herbicides, either broadcast sprayed (Enloe *et al.* 2007; Grekul and Bork 2007) or applied with a weed wiper (Grekul et al. 2004), and controlled livestock grazing

(DeBruijn and Bork 2006). Additionally, fertilization can augment the effect of herbicides, reducing Canada thistle abundance and prolonging the benefits of herbicide application (Grekul and Bork 2007).

Pasture productivity is often greater within more diverse forage stands, particularly where grasses are combined with nitrogen (N)-fixing legumes (Chestnutt *et al.* 1980). Legumes have the benefit of increasing forage quantity and quality of the sward (Sleugh *et al*. 2000, Papadopoulos 2001). These benefits occur in part, due to the transfer of N from legumes to neighboring grasses during the decomposition of legume roots (Ledgard and Steele 1992, Paynel *et al.* 2001).

Where moisture is relatively abundant across western Canada, many pastures contain abundant clover (*Trifolium* spp.), particularly white clover [*Trifolium repens* (L.)]. This species readily establishes from seed commonly found in the soil seed-bank (Sanderson *et al*. 2007). Volunteer establishment of white clover leads to many communities in the Aspen Parkland and Boreal natural sub-regions to contain a significant white clover component (Aarssen and Turkington 1985). Despite being a relatively shallow rooted species, white clover is able to maintain itself under grazing (Williams *et al*. 2000, Deak *et al*. 2007), largely due to its abundant creeping root system and associated vegetative reproduction (Burdon 1983). Under these conditions, white clover is frequently found together with Kentucky bluegrass [*Poa pratensis* (L.)] (KBG) (Aarssen and Turkington 1985). White clover is known to contribute as much as 545 kg ha^{-1} of N to forage swards (Elgersma and Hassink 1997) and is therefore a valued forage species for livestock producers.

As pasture swards containing volunteer white clover are those with a mesic moisture regime, they are also typically prone to invasion by weeds, including Canada thistle. Within this context, many herbicides are effective for controlling Canada thistle, including glyphosate (Darwent *et al.*1994), 2,4-D (Hodgson 1970, Carson and Bandeen 1975), dicamba (Donald 1992), clopyralid (Bixler 1991, Enloe *et al*. 2007), and picloram (Hunter and Smith 1972). However, these same compounds have been shown to reduce the abundance of legumes, including white clover (Grekul *et al*. 2005, Bork *et al*. 2007). As a result, the benefits of broadleaf weed control using herbicides may lead to the undesirable loss of legumes within mixed forage swards containing legumes (Peterson and Parochetti 1978, Grekul *et al*. 2005, Mesbah and Miller 2005).

Little information exists on the 3-way interactions present within recently seeded mixed forage swards, particularly those containing both an undesirable noxious weed and beneficial legume (i.e. grass-legume-weed mixes). Many producers are reluctant to control broadleaf weeds in newly seeded pasture using herbicides for fear of legume loss, resulting in persistent Canada thistle problems in untreated fields. Information on the interspecific relations between all 3 components will improve our understanding of the impacts that species mixtures and density can have, including the extent to which Canada thistle and white clover may be detrimental and beneficial, respectively.

3.2. Study Objectives

The goal of this study was to quantify the interspecific relationships between 2 common forage species, white clover and bluegrass, and the pasture weed, Canada thistle. Three complementary greenhouse experiments were performed under controlled conditions in order to meet the following specific objectives:

(1) Assess the competitive ability of forage species such as white clover and bluegrass against the noxious weed Canada thistle during forage establishment.

(2) Quantify the relative impact of Canada thistle on herbage yield losses when grown with neighbors consisting of white clover, bluegrass or mixtures of the two during establishment.

(3) Isolate the potential facilitative effect of the legume, white clover, on the growth of bluegrass and Canada thistle.

3.3. Materials and Methods

A greenhouse environment was used to determine the inter-specific relationships between white clover, bluegrass and Canada thistle during early forage establishment. Although a greenhouse environment does not reflect the full range of variation in growing conditions associated with field studies, greenhouse studies do allow isolation of inter-specific relationships among plant species, which are often problematic to assess in the field under 'noisey' conditions. Moreover, because forage establishment is often conducted in fallow fields following intensive site preparation (i.e. cultivation), the examination of these relationships among species in a greenhouse environment is comparable to early forage establishment in the field.

3.3.1. Experimental Design

Three separate greenhouse trials were conducted between October 2005 and February 2007. Trial 1 was conducted between November 2004 and February 2005 as a preliminary investigation to evaluate the experimental design. During trial 1, each treatment was done using densities of 3, 6 and 9 plants per pot, while maintaining each of the planting ratios. A total of 5 reps of each treatment (N=150 pots total) were examined. The potting medium utilized during the first trial was Metromix^{TM}, which is predominantly a peat-based media. Results of trial 1 are shown in Appendix A1.

Trial 2 was conducted between October 2005 and January 2006, while trial 3 was conducted between May 2006 and August 2006. During trials 2 and 3, only 2 planting densities (3 and 9 plants per pot) were examined to assess density effects. Initial analysis of aboveground biomass in trial 1 indicated no significant differences in total shoot biomass between the moderate and high densities of 6 and 9 plants per pot. However, the number of reps of each treatment was also increased to 10 in trials 2 and 3 (N=200 pots per trial). Additionally, the potting medium used was changed to a mixture of 30% topsoil and 70% sand to facilitate the harvest of roots.

Each trial compared 10 treatments intended to assess the performance of all 3 plant species (white clover, bluegrass, or Canada thistle), grown either in monoculture, or in 1:2 or 2:1 binary mixtures, as well as a 1:1:1 mixture of the three. The ten treatments included:

1. Monoculture of Canada thistle
2. Monoculture of white clover
3. Monoculture of bluegrass
4. 2:1 ratio of Canada thistle : white clover
5. 1:2 ratio of Canada thistle : white clover
6. 2:1 ratio of Canada thistle : bluegrass
7. 1:2 ratio of Canada thistle : bluegrass
8. 2:1 ratio of white clover : bluegrass
9. 1:2 ratio of white clover : bluegrass
10. 1:1:1 ratio of Canada thistle : white clover : bluegrass

Each of the above 10 treatments was also conducted at several planting densities, depending on the trial.

3.3.2. Plant Propagation and Establishment

Canada thistle plants were established vegetatively from root cuttings, while both bluegrass and white clover were grown from seed. The former was done because Canada thistle is difficult to germinate due to specific temperature and light requirements (Heimann and Cussans 1996). Moreover, this scenario (thistle cutting and forage seed) represents those conditions likely to occur where

Canada thistle infested land is seeded down to tame forages. White clover seeds were inoculated with *Rhizobium* bacteria. Troy Kentucky bluegrass was the variety used for this study.

Prior to each trial, Canada thistle roots were harvested from a densely infested lowland (Black Chernozemic soil) at the Parkland Conservation Farm located 19 km west of Vegreville, Alberta (86 km east of Edmonton). Thus, in trials 1, 2 and 3, respectively, roots were removed in October 2004, July 2005, and November 2005. Roots were kept moist, initially washed, and immediately placed in a growth medium (Turface™ during trial 1; Metromix™ in trials 2 and 3), and stored at 5˚C to slow development prior to planting. At the time of installation into pots, the identity of all Canada thistle roots were verified based on root characteristics and emerging root buds. Canada thistle roots had a diameter ranging between 2.5 – 3.5 mm and were cut to 3 cm centered on a viable root bud. This size root cutting has previously been shown to consistently produce viable Canada thistle plants (Hamdoun, 1972). At the time of installation, all secondary Canada thistle shoots were removed and the ends of each root cutting coated in paraffin wax to prevent disease entry.

White clover and bluegrass were propagated from seed. Both species were over-seeded directly into 12.5 cm x 12.5 cm square pots (13 cm deep) at the same time as Canada thistle installation, and promptly thinned after emergence to the required density and planting ratio. Pots were seeded (and planted in the case of Canada thistle) in a design that allowed for maximum interactions among plants of different species within a mixture, regardless of planting density (see

Figure 3.1). To prevent drying out of seeds and seedlings during the initial stages of each trial, a thin layer of Metromix™ was placed on top of each pot. Canada thistle root cuttings or seedlings of white clover or bluegrass that had not emerged and/or survived the first 3 weeks after seeding/planting were replaced from nursery stock. However, plant losses after that point were considered mortality indicative of treatment impacts.

After establishment, all pots were placed in a greenhouse for 90 days with a day/night cycle of 16:8 hrs, and temperature of 23 ^{o}C, respectively. Plants were watered as needed to prevent drying but avoid saturation. A slow release fertilizer of 13-13-13 at 2.5 kg m^{-3} was added to the soil mix during preparation. Following the first month, experimental pots received a complete fertilizer of 15-30-15 biweekly to avoid nutrient deficiencies. Pots were randomized every two weeks for the first six weeks and then weekly for the duration of the experiment.

3.3.3. Harvest and Vegetation Measures

Pots were harvested 90 days after initial planting. Aboveground biomass was removed at the soil surface and separated into Canada thistle, white clover and bluegrass. Root biomass for trial 1 was separated into Canada thistle and white clover/bluegrass combined. Roots of white clover and bluegrass were separated for trials 2 and 3. All biomass was dried at 60˚C and dry weights recorded.

3.3.4. Statistical Analysis

Prior to analysis, all data were checked for normality using plots of the residuals and a Shapiro-Wilkes test. In trial 1, data on Canada thistle root biomass per plant and Canada thistle mortality were transformed using a square root transformation. In trials 2 and 3, only Canada thistle shoot biomass per pot and Canada thistle average shoot height were normal. All other parameters underwent a square root transformation.

Due to differences in experimental design (i.e. number of planting densities) and planting media, trial 1 was analyzed separately from trials 2 and 3. Additionally, the results of trial 1 were considered preliminary, and as a result, presentation of these data is limited to Appendix A1.

All data were assessed with an ANOVA for a completely randomized factorial design using a subset of the 10 treatments and the two or three density treatments as fixed factors. Data from trials 2 and 3 were pooled for analysis, with 'trial' included as an additional fixed effect in the analysis. Trial was included as a fixed effect because of differences in the timing of Canada thistle removal of roots from the field to facilitate the planting studies, as well as the time of year during which these trials were conducted (e.g. trial 2 = summer; trial 3 = fall/winter).

Data analysis was conducted in three steps. In order to assess the effect of Canada thistle abundance on neighboring forage (i.e. biomass of white clover or bluegrass), the latter were run as dependent variables in response to the 5 treatments containing mixtures of forage with Canada thistle (i.e. Canada thistle with either white clover at 1:2 or 2:1 ratios, Canada thistle with bluegrass at 1:2 or 2:1 ratios, and Canada thistle with both white clover and bluegrass at a 1:1:1 ratio). Responses were assessed both at the pot level and on a per plant basis, although the latter is emphasized in Chapter 3, with pot level responses largely provided in Appendix A1.

Second, the suppressive affect of white clover and/or bluegrass on neighboring Canada thistle was assessed through measures of Canada thistle shoot and root biomass, shoot height and stem density as the response variable, again on a pot and plant basis. This analysis utilized the same 5 treatments listed above, but also included monocultures of Canada thistle to evaluate the intra-specific competitive effect of the weed. Only individual plant-based responses are reviewed in detail in Chapter 3.

Third, forage production responses in relation to varying mixtures of white clover and bluegrass, either alone or in combination, were assessed on a pot and plant basis (with only the pot data reviewed in Chapter 3). This analysis used 4 treatments, including monocultures of white clover and bluegrass, as well as the 1:2 and 2:1 mixtures containing these species as neighbors.

All analysis was completed using LSmeans in Proc Mixed in SAS (SAS Institute Inc. 1988), with significant main effects and interactions at $p<0.05$. Post-hoc mean comparisons were conducted on all significant effects with a significance of 5%. Where treatment by density effects existed, comparisons between neighbor treatments within a planting density were emphasized.

3.4. Results

Vegetation responses in trial 1 were considered preliminary, particularly as this trial utilized a planting media (potting soil – largely an organic medium) that was inconsistent with most field conditions (i.e. mineral soils) in central Alberta. As a result, these results are provided in Appendix A1 and are not discussed further here. Similarly, all Canada thistle responses at the pot level are shown in Appendix A1, as are aggregate forage responses to the presence of Canada thistle. Hence, subsequent results and discussion described in Chapter 3 are limited to trials 2 and 3, with an emphasis on individual plant responses.

3.4.1. Canada Thistle Response to Neighbors

Individual Canada thistle plant responses to the presence and abundance of neighboring vegetation assessed in the 2 trials included the density of 'new' shoots, shoot height and biomass, as well as root biomass (Table 3.1). Root:shoot ratios were also assessed (Table 3.2). In trials 2 and 3, the identity of neighboring plant species, planting density, and trial all demonstrated significant ($p \leq 0.001$) effects on Canada thistle shoot biomass per plant, Canada thistle root biomass per plant and the number of Canada thistle shoots per plant. The sole interaction involving different plant species occurred for shoot height, which was affected by combinations of different species and planting density ($p<0.001$). There was an additional interaction ($p<0.05$) between trial and planting density on Canada thistle root biomass per plant.

Levels of Canada thistle shoot biomass per plant in trials 2 and 3 were lowest within the Canada thistle monoculture and greatest when Canada thistle was established with bluegrass as 2/3 of its neighbors (Figure 3.2, A). In comparison, Canada thistle grown with 2/3 white clover as neighbors resulted in intermediate shoot biomass for the weed. While a mix of clover and bluegrass resulted in intermediate Canada thistle shoot biomass compared to single-species neighbors of either forage, this level did not differ ($p>0.05$) from the treatment containing only bluegrass as a neighbor.

Canada thistle shoot biomass per plant at low density (3 plants/pot: 6.47±0.23 g $plant^{-1}$) was greater ($p<0.01$) than when Canada thistle was established at high densities (9 plants/pot: 3.10±0.23 g $plant^{-1}$). Shoot biomass levels were also greater ($p<0.01$) in trial 3 (5.88±0.22 g $plant^{-1}$) than trial 2 (3.69±0.25 g $plant^{-1}$).

Similar to Canada thistle shoot biomass, root biomass per plant was greatest when Canada thistle was installed with bluegrass as 2/3 of the neighboring vegetation in trials 2 and 3 (Figure 3.2, B). Canada thistle root biomass was generally low when Canada thistle was grown with neighbors that included itself (i.e. where 2 or more Canada thistle plants were present), or when grown alone with 2 white clover plants as neighbors. The trial x density effect on root biomass revealed that root biomass was overall lower ($p<0.05$) in trial 3 (approximately half), with additional differences between density levels within each trial ($p<0.05$): root biomass per Canada thistle plant was 45-56% lower when planted at the greater density (data not shown).

The density of Canada thistle shoots per installed plant followed a trend similar to that of shoot biomass (Figure 3.2, C). Once again, Canada thistle plants installed with only bluegrass as neighbors led to the greatest number of shoots, although this did not differ from Canada thistle grown with white clover, or a mix of white clover and bluegrass ($p>0.05$). Canada thistle grown with neighbors that included itself and a forage species led to moderate shoot densities, with the Canada thistle monoculture resulting in the fewest thistle shoots per plant (Figure 3.2, C).

Canada thistle shoot densities were also greater ($p<0.05$) in trial 3 (3.54 ± 0.16 shoots plant^{-1}) than trial 2 (1.04 ± 0.18 shoots plant^{-1}), and greater ($p<0.05$) when planted at low densities (3.00 ± 0.17 shoots plant^{-1}) than at high densities (1.58 ± 0.17 shoots plant^{-1}).

Within trials 2 and 3, Canada thistle root:shoot ratios were affected by neighbor plant species, density, and planting trial ($p<0.001$), as well as interactions of trial by species and trial by density ($p<0.001$) (Table 3.2). Canada thistle root:shoot ratios were low in trial 3, ranging from 0.18 ± 0.05 to 0.24 ± 0.05, with no difference among treatments (Figure 3.3). During trial 2, however, marked differences in Canada thistle root:shoot ratio were evident. Canada thistle root:shoot ratios were greatest when thistle was installed with both clover and bluegrass, which then declined, reaching a minimum for thistle plants installed with any number of other Canada thistle plants. Generally, thistle grown in the presence of clover led to lower root:shoot ratios of the weed than those grown with bluegrass, although these differences were not significant ($p>0.05$).

Canada thistle root:shoot ratios were similar ($p>0.05$) among densities in trial 3 (data not shown), but differed in trial 2. In the latter, Canada thistle root:shoot ratios were greater when installed at low densities (0.48 ± 0.03) than at high densities (0.13 ± 0.03).

3.4.2. Individual Forage Responses to Weed Presence

3.4.2.1. White Clover Response to Neighbors

Specific responses of white clover examined at the pot level were limited to those treatments containing similar numbers of white clover plants at seeding. White clover shoot biomass responses per pot in trials 2 and 3 showed similar responses at both densities (Tables 3.3 and 3.4). Pots containing white clover grown with bluegrass generally had greater ($p<0.05$) shoot biomass than those grown with Canada thistle for all comparable treatments, the lone exception being pots containing white clover as the major component at low density in trial 3 (Table 3.4).

White clover root biomass trends paralleled those of the shoot data, generally being greater when grown in a mixture with bluegrass instead of Canada thistle (Table 3.3 and 3.4). However, these differences were not consistent among trials or planting densities, with more white clover root biomass differences evident in trial 2 (Table 3.3) than trial 3 (Table 3.4). Moreover, differences between the impact of Canada thistle and bluegrass on white clover biomass responses per pot were only evident at the high planting density in trial 3.

White clover was also assessed for shoot and root production on a per plant basis for both trials. Shoot biomass per plant in trials 2 and 3 was influenced by the three-way interaction between species, density and trial (Table 3.5), although patterns among treatments were similar between densities and trials (Figure 3.4A). White clover plants generally increased in individual shoot biomass when grown with increasing amounts of bluegrass instead of in monoculture, with the exception of low density plantings in trial 3 (Figure 3.4A). Conversely, white clover plants were smaller when grown with neighboring Canada thistle of any amount. Absolute variation in shoot size among treatments was expressed to the greatest extent at low density plantings in trial 2, with the least separation at high density plantings (Figure 3.4A). Nevertheless, differences among treatments remained present in all situations.

Similar to the shoot data, white clover root biomass per plant was influenced by the interaction of species, density and trial (Table 3.5). While no differences in white clover root biomass were evident among treatments of trial 2 grown at high density, prominent effects were apparent in low density pots (Figure 3.4B). Trends in white clover growth paralleled those of the shoot data in this situation, with white clover root biomass increasing when grown with neighboring bluegrass, and decreasing with Canada thistle. White clover root biomass per plant remained similar between pots containing all 3 species and those containing only white clover (Figure 3.4B).

In trial 3, limited differences were apparent in clover root biomass grown at low densities: monoculture clover and pots with clover grown with a major bluegrass component remained greater than pots with a major Canada thistle component. Under high density conditions, differences among treatments were similar to those in the shoot data, with bluegrass as a neighbor leading to greater clover root biomass, and Canada thistle reducing clover root biomass (Figure 3.4B).

3.4.2.2. Bluegrass Response to Neighbors

Comparison of bluegrass responses at the pot level were limited to those treatments containing similar numbers of bluegrass plants. In trial 2 pots containing a majority of bluegrass in the mixture had greater biomass of this species when grown with white clover as a neighbor (i.e. compared to Canada thistle), regardless of planting density (Table 3.6). There were no differences in total bluegrass shoot biomass per pot in trial 3 (Table 3.7).

Patterns of bluegrass root biomass per pot remained similar to those of shoot biomass, with differences apparent only in trial 2, and only when bluegrass was established as the dominant component of the forage mix. At low density plantings, bluegrass root biomass was greater with clover as a neighbor instead of Canada thistle (Table 3.6). However, this pattern reversed in the high density planting.

The interaction between species, density and trial influenced the size of individual bluegrass plants (Table 3.5). Pots containing only bluegrass consistently produced the greatest shoot biomass compared to the mixtures, a trend consistent across all trial–density combinations (Figure 3.5A). Additionally, differences among the various treatments were expressed to a greater extent within trial 2. Under both the high and low density conditions of trial 2, bluegrass shoot biomass progressively declined when grown with increasing amounts of white clover (Figure 3.5A). In the same trial, even small amounts of Canada thistle led to a large reduction in bluegrass shoot biomass. In trial 3, shoot biomass of individual bluegrass plants declined similarly with exposure to either Canada thistle or white clover as neighbors, regardless of their abundance.

Root biomass responses of bluegrass per plant followed a pattern similar to those described for shoots (Figure 3.5B). That is, large reductions in bluegrass root biomass occurred when this species was grown with increasing amounts of white clover or Canada thistle. Individual root responses of bluegrass were also more limited in trial 3 than trial 2. At low planting densities, monocultures of bluegrass were greater in root biomass than bluegrass grown with 2 Canada thistle plants (Figure 3.5B). With high density conditions, monocultures of bluegrass had greater root biomass compared to any treatment containing Canada thistle.

3.4.3. Forage Responses in the Absence of Canada Thistle

Interactions occurring specifically among the two forage species, white clover and bluegrass, were assessed through the examination of treatments containing only these species. Forage yields per pot in trials 2 and 3 were effected by species, planting density and trial ($p<0.001$). Additionally, there was an interaction of species mix by trial ($p<0.01$). Closer examination of forage yields in each trial revealed similar patterns within each trial, with a distinct trend for increasing forage yields as the species mix shifted from bluegrass towards that of white clover (Figure 3.6). Mixtures of white clover and bluegrass were consistently intermediate in forage yield.

3.5. Discussion

3.5.1. Suppression of Canada Thistle by Neighbors

Data for the greenhouse experiment were evaluated primarily at the individual (ramet) level to assess species responses, with aggregate forage responses also assessed at the pot level. Vegetation responses at the pot level can be considered to represent simplified forage ‘communities’ during initial sward establishment, and therefore represent the net effect of species interactions. In contrast, responses at the plant level indicate the relative performance of individuals within each species. Notably, Canada thistle responses assessed at the pot and plant level paralleled one another quite closely, with the greatest overall variation in responses associated with the individual trial.

In both trials, Canada thistle exhibited the greatest reduction in performance, including shoot and root biomass, as well as shoot number, when grown with other thistle plants, highlighting the susceptibility of this species to intra-specific competition. Although increases in planting density reduced plant sizes in all trials and species mixes, evidence for intra-specific competition in Canada thistle remained apparent across variable planting densities. The superior competitive influence of Canada thistle on itself may be partly explained by the fact that Canada thistle plants were established from root cuttings rather than seed, which may have led to more rapid development of larger plants, which then had the potential to more strongly influence con-specifics (Hamdoun 1970). This is supported by average biomass data on the Canada thistle, white clover and bluegrass monoculture treatments, which were 12.62 (range 7.87±0.5 to 17.37±0.5 among trials), 3.94 (range 3.68±0.3 to 4.12±0.2) and 2.78 (6.41±0.3 to 0.33±0.07) g/pot, respectively. Another form of competition that plants in this study were subjected to is the competition for light. Canada thistle plants were observed to have a height advantage over forage species giving it an competitive advantage for light. When grown with itself it would be direct competition for light when other Canada thistle plants (personal observation, data not shown). Canada thistle plants may also have been susceptible to more light competition when grown with itself.

The ability of Canada thistle to produce large amounts of biomass both above and below ground appeared to give this species a competitive advantage against neighboring plants, and may account for the ability of this weed to cause distinct forage yield losses (e.g. Grekul and Bork, 2004). Canada thistle roots are estimated to grow up to 1.50 m in a growing season (Amor and Harris 1975). This extensive root system creates a competitive advantage over forage species but also is likely to lead to intra-specific competition when grown in a restricted root volume such as that used in this study. As the boundaries of the pot would have prevented roots of individual Canada thistle plants from expanding outward, this would assist with initiating competition both with adjacent forage species as well as con-specifics within the confines of the pot.

The greater relative growth of Canada thistle observed here may make this species a superior competitor against surrounding plants including itself. Observed reductions in Canada thistle in this study agree with findings by Leathwick et al. (2006) where the impact of increasing thistle shoot density coincided with a reduction in root growth, leading to less shoot recruitment per plant at higher densities, in essence imposing density-dependent restrictions on vegetative reproduction. A smaller root mass, in turn, would likely provide fewer adventitious root buds, leading to fewer new thistle shoots being produced. While the combined use of root cuttings and seed for plant propagation in this study of thistle and forage plants, respectively, may confer a distinct advantage to the weed, this comparison nevertheless effectively captures important agro-ecological interactions among these species. While initial infestations of Canada thistle develop from seed, the vast majority of infestations arise from surviving roots and root fragments (Donald 1994), such as those that might arise following cultivation during soil preparation prior to forage seeding. Moreover, cultural practices such as tillage, particularly discing, is likely to fragment Canada thistle roots into small pieces that then have the potential to survive and produce new plants, even when as small as 5 mm (Hamdoun 1972). Subsequent seeding of forages such as white clover and/or bluegrass into these conditions would therefore result in direct competition between Canada thistle plants establishing from root fragments, and much smaller forage seedlings.

Aside from the strong influence of Canada thistle on itself, secondary influences were observed from forage species as a neighbor, although these responses differed between study trials. In both trials 2 and 3, white clover reduced the shoot biomass of Canada thistle plants. Unlike white clover, bluegrass seedlings appeared to be an inferior competitor against thistle, with Canada thistle shoots reaching their largest size under these conditions. Similar trends were observed for Canada thistle shoot density in both trials.

Although not reviewed in detail, the results of trials 2 and 3 differed sharply from those results observed in trial 1 (see Appendix A1), likely due to a change in growth medium. The former 2 trials were conducted using a mix of top soil and sand, conditions which appeared to either favor white clover growth, or conversely hinder Canada thistle growth. As the use of a mineral soil-sand mix is more likely to approximate field conditions found in central Alberta, this is the outcome more likely to occur involving young establishing swards of white clover and bluegrass infested with Canada thistle.

Previous studies have found that the ranking of plant traits among various species remains consistent between field and potting studies (Mokany and Ash 2008). However, growing conditions (i.e. fertility) are also known to alter relative plant trait performance between field and potting studies (Mokany and Ash 2008), which indeed appears to be the case in the current study. Nevertheless, as potting studies are one of the few methods available to isolate and quantify inter-specific relationships, the distinct trends in rank competitiveness among all 3 species suggest some degree of applicability to the field, depending on soil conditions.

The superior competitiveness of white clover rather than Kentucky bluegrass against Canada thistle in trials 2 and 3 may be explained by several factors. For example, many perennial grasses including bluegrass are known to establish relatively slowly (Wedin and Huff 1996), which despite the favorable greenhouse environment in this investigation, may have rendered bluegrass a poor competitor against rapidly establishing thistle. Conversely, white clover is known to volunteer extensively in mesic pastures (Turkington and Burdon 1983), and the favorable growing conditions maintained by greenhouse conditions may have maximized its establishment, particularly in the soil-sand media, and subsequent competitive influence on Canada thistle. Finally, white clover also has the potential advantage over bluegrass of fixing atmospheric nitrogen, which could confer a competitive advantage to this species in competing against Canada thistle. Fixation of N would reduce white clover dependence on mineralized nitrogen that may be limiting when using a growth medium with a high proportion of sand, thereby leading to superior competitive suppression of Canada thistle by white clover. However, at the time of root harvest root nodules were not evident and therefore may not have been a factor in the competitive dynamics between plants. This conclusion is furthered by the fact that pots were fertilized prior to and during the study, which would reduce any competitive advantage obtained by the clover through N fixation.

There has also been evidence that white clover may be more competitive in its second year of growth following establishment (Chapter 4), conditions beyond the scope of this study. In fact, conclusions about the competitiveness of white clover and bluegrass against Canada thistle must be tempered by the short-term dynamics of initial establishment examined here. Nevertheless, the three months of growth in the greenhouse are likely at least somewhat comparable to the first growing season following forage seeding in pastures of western Canada, and therefore provide useful insight into the potential role of clover and bluegrass in competing against Canada thistle.

3.5.2. Forage Yield Loss Due to Canada Thistle

In both trials 2 and 3, white clover exhibited superior growth relative to bluegrass in all situations regardless of Canada thistle presence. Additionally, differences among treatments containing different plant mixes (i.e. competitive differentiation) tended to decrease at high planting densities. Although not known for certain, one explanation for the reduced differentiation among treatments at high planting densities is that these pots may have reached a conditions after 3 months where plants had approached (or reached) saturation relative to the use of available soil resources.

3.5.2.1 Bluegrass Responses to Canada Thistle

Within trials 2 and 3 where plants were grown in a soil-sand media, bluegrass demonstrated greater susceptibility to competitive suppression from Canada thistle. In fact, mean plant size of bluegrass declined more than for white clover in relation to increasing presence of Canada thistle as a neighbor. Observations that bluegrass shoot and root biomass decreased when grown with any neighbor, but particularly Canada thistle, indicates that bluegrass may generally be a poor competitor during early sward establishment, and is highly susceptible to suppression by competing vegetation. Similar to other perennial grasses, bluegrass is known to be a relatively slow establishing species (Wedin and Huff 1996). This characteristic renders bluegrass susceptible to competition from neighboring vegetation, and will inevitably reduce its performance relative to when grown in monocultures.

As a result, it appears that successful establishment of bluegrass in pasture swards may depend heavily on minimizing the influence of competing vegetation, particularly Canada thistle, at least during the early stages of development. Similarly, where competing vegetation is likely to be abundant, prompt action may have to be taken to control unwanted vegetation, such as through the use of selective broadleaf herbicides (Beck and Sebastian 2000, Bork *et al*. 2007). Unfortunately, this action typically precludes the simultaneous inclusion of a legume such as white clover into the forage mix, as most herbicides effective on Canada thistle will also remove legumes (Peterson and Parochetti 1978, Grekul *et al*. 2005, Mesbah and Miller 2005).

3.5.2.2. White Clover Responses to Canada Thistle

White clover responses to Canada thistle in trials 2 and 3 were similar. White clover plants declined in shoot and root size when grown with Canada thistle, but not to the same extent as bluegrass, thereby demonstrating superior resistance in withstanding competitive influences from the weed when grown in a soil-sand mix, regardless of the planting ratio (i.e. 1:2 and 2:1). Moreover, differential responses between trials 2 and 3, and those in trial 1 (see Appendix

A1) highlight the potential ability of the growth media to change the competitiveness of white clover, both relative to bluegrass as well as the noxious weed Canada thistle, and reinforce the need for pasture managers to consider soil conditions during forage establishment in areas where weeds such as thistle may be a problem. For example, forage species seeded into areas where Canada thistle is likely to establish should be well adapted to the soil conditions present in order to maximize resistance to competitive influences from neighboring weeds.

Consistent suppression of white clover by Canada thistle is not surprising given the rapid establishment of thistle from root cuttings in this investigation, and the large proportion of total biomass consisting of thistle in mixes involving these species (e.g. 80 to 90%, and 88 to 95% in trials 2 and 3, respectively). Nevertheless, the more favorable resistance of white clover than bluegrass to competitive suppression by Canada thistle, specifically in the soil-sand media (i.e. conditions closer to field conditions), may stem in part from the potential ability of white clover to fix its own N. White clover is known to fix as much as 545 kg/ha of N (Elgersma and Hassink 1997), which would increase its growth and performance relative to neighboring species. While it is known that shading can impact the growth of clover (Frame 2005) the ability of white clover to reduce the impact of shading from Canada thistle through leaf arrangement may also give it a competitive advantage over Kentucky bluegrass.

3.5.3. Aggregate Forage (White Clover + Bluegrass) Growth Responses

Predictably, forage responses at the pot level in the absence of Canada thistle were largely a function of planting density. This observation suggests that pots ultimately did not constrain the size of white clover and/or bluegrass plants when grown alone.

Trials 2 and 3 utilized a mix of soil and sand to facilitate root harvest. High sand conditions would result in a lower water and nutrient holding capacity, which in turn, may have favored white clover over bluegrass, thereby accounting for the more favourable response of the former. This appears to be supported by the observation that bluegrass plants grown with clover as a neighbor had a lower root:shoot ratio than in other mixtures, even compared to bluegrass grown with Canada thistle. Nevertheless, unlike the current study, work done by Badra *et al*. (2005) resulted in bluegrass being more productive both above and below grown when grown in sand compared to a loam soil. That field study was conducted by seeding bluegrass into an existing sod, and suggests that soil medium alone may not account for the relatively poor growth and competitiveness of bluegrass in trials 2 and 3.

White clover plants grown with bluegrass generally had a much larger root biomass, which may account for the apparent suppression of bluegrass root growth. Although both clover and bluegrass are known to have relatively well developed fibrous root systems (Sullivan *et al*.2000, Turkington and Burdon 1983), the current study suggests that white clover is the more rapidly establishing species of the 2, including belowground. Furthermore, the large observed root system of white clover may have had the capacity to fix N, reducing its dependence on mineralizable derived N, and this possibility may further explain its greater competitive ability against bluegrass. Similarly, white clover seedlings may be more tolerant of the drier soil moisture conditions likely to be present in a sand dominated media where water infiltration and free drainage are high. White clover has been shown to have longer stolon internode lengths and a lower frequency of branching of the roots when grown in sand compared to a potting mixture contained peat, perlite and soil (Welham *et al*. 2002). However, a study by Welham *et al*. (2002) also showed that although white clover roots were more elongated when grown in sand, they had visibly less biomass than those grown in soil. Thus, further studies appear necessary to understand the root responses of white clover to planting media and soil environmental conditions.

Studies have shown that the development of an extensive root system is important for the survival of bluegrass in conditions of heat and drought stress (Bonos & Murphy 1999). Kentucky bluegrass cultivars with deep fibrous root systems are able to obtain water held deeper in the soil profile and maintain root activity for periods longer than those with a shallow root system. Bluegrass root development was minimal throughout trials 2 and 3 (pers. observation), potentially indicating that these plants had reduced ability to grow in the greenhouse. Although the soil-sand mix used in these trials consisted of a mineral soil, this planting media is not representative of typical ‘loamy’ soil conditions found on glacial till soils across central Alberta. As soil texture is known to regulate forage plant establishment and growth (Evers and Parsons 2003, Nuttall

1985), this in turn may change the growth, and presumably competitive ability, of different plant species, including white clover and bluegrass.

3.6. Conclusions

Relationships between Canada thistle and neighboring forage plants of white clover and bluegrass during establishment were strongly dependent on growing conditions, particularly soil media. Abiotic conditions such as soil and potentially moisture, temperature and light, may all have played an important part in regulating the competitive ability of plants in this study. When grown in an organic rich environment in the preliminary study (reported in Appendix A1), bluegrass was the greater forage producer but was not a superior competitor against Canada thistle. In this growth medium, only planting density had an affect on Canada thistle. When the growth medium was changed to a mineral soil mix of top soil/sand mixture, competitive plant dynamics changed markedly, with white clover out-producing bluegrass and the former becoming the superior competitor against Canada thistle. This situation is more representative of field conditions in western Canada.

Despite the limitations of a greenhouse study such as that reported here, these findings have implications for the strategic management of Canada thistle through interspecific competition. White clover appears to enhance suppression (i.e. biomass) of Canada thistle compared to bluegrass in mineral sandy-based soils. The superior competitive ability of white clover provides additional support to the benefits of including legumes in newly established forage swards. By including clover, producers may create a sward that is better able to suppress regenerating Canada thistle.

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Table 3.1. Summary of ANOVA F-value results from PROC MIXED analysis of Canada thistle shoot density and height, as well as shoot and root biomass per plant, for trials 2 and 3.

Factor	df	Canada thistle shoots per plant	Canada thistle root biomass per plant	Canada thistle shoots per root	Canada thistle average shoot height
Species Treatment (Spp)	5	9.38***	4.05**	5.41***	1.15
Density (Den)	1	105.89***	45.03***	49.70***	0.07
Trial	1	51.23***	37.15***	179.87***	9.89***
Spp*Den	5	.84	1.56	1.24	2.09
Spp*Trial	5	.61	0.27	0.66	1.43
Trial*Den	1	.07	6.17*	2.43	3.20
Trial*Spp*Den	5	.26	0.56	1.04	1.29

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table 3.2. Summary of ANOVA F-value results from the PROC MIXED analysis of root:shoot ratio per plant for Canada thistle, white clover and bluegrass, averaged across trials 2 and 3.

Factor	df	Canada thistle root:shoot ratio	White clover root:shoot ratio	Bluegrass root:shoot ratio
Species Treatment (Spp)	5	4.03***	1.62	2.37*
Density (Den)	1	10.74***	2.36	0.21
Trial	1	25.14***	17.44***	132.57***
Spp*Den	4	1.80	1.65	2.05
Trial*Spp	4	5.26***	0.85	5.23***
Trial*Den	1	15.62***	1.10	0.15
Trial*Spp*Den	4	1.80	1.54	2.69***

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively

Table 3.3. Summary of LSmeans (±SE) of white clover shoot biomass and root biomass in trial 2. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# WC plants	Neighbour	WC shoot biomass (g pot^{-1})	WC root biomass (g pot^{-1})
3	1	1CT+1KBG	1.25 (0.57) b	0.55 (0.18) b
		2 CT	0.69 (0.54) b	0.16 (0.17) c
		2 KBG	4.36 (0.76) a[1]	0.95 (0.27) a
	2	1 CT	1.99 (0.60) b	0.36 (0.19) b
		1 KBG	5.41 (0.60) a	1.48 (0.19) a
9	3	3CT+3KBG	1.52 (0.64) b	0.48 (0.20) b
		6 CT	1.14 (0.64) b	0.57 (0.20) b
		6 KBG	4.63 (0.64) a	1.11 (0.20) a
	6	3 CT	3.31 (0.57) b	1.15 (0.47) a
		3 KBG	5.98 (0.64) a	1.49 (0.20) a

[1] Within a variable, planting density and number of white clover plants, means with different letters differ, $p<0.05$.

Table 3.4. Summary of LSmeans (±SE) of white clover shoot biomass and root biomass in trial 3. CT, WC, and KBG are Canada thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# WC plants	Neighbour	WC Shoot biomass (g pot^{-1})	WC Root biomass (g pot^{-1})
3	1	1CT+1KBG	1.25 (0.54) b	0.26 (0.17) a
		2 CT	1.41 (0.54) b	0.13 (0.18) a
		2 KBG	2.29 (0.54) a[1]	0.37 (0.17) a
	2	1 CT	2.20 (0.54) a	0.58 (0.18) a
		1 KBG	2.89 (0.54) a	0.43 (0.17) a
9	3	3CT+3KBG	0.41 (0.54) b	0.077 (0.17) b
		6 CT	0.92 (0.54) b	0.053 (0.18) b
		6 KBG	4.80 (0.54) a	0.89 (0.17) a
	6	3 CT	1.86 (0.54) b	0.42 (0.17) b
		3 KBG	6.72 (0.54) a	1.55 (0.17) a

[1] Within a variable, planting density and number of clover plants, means with different letters differ, $p<0.05$.

Table 3.5. Summary of ANOVA F-value results from PROC MIXED analysis of white clover and bluegrass shoot and root biomass per plant, for trials 2 and 3.

Factor	df	White clover shoot biomass per plant	White clover root biomass per plant	Bluegrass shoot biomass per plant	Bluegrass root biomass per plant
Species Treatment (Spp)	5	38.45***	15.31***	50.48***	17.46***
Density (Den)	1	108.55***	40.54***	27.51***	12.63***
Trial	1	12.97***	34.39***	300.38***	454.44***
Spp*Den	5	1.34	1.44	2.92*	1.99
Spp*Trial	5	0.82	1.22	14.62***	6.71***
Trial*Den	1	0.97	0.06	2.79	4.30*
Trial*Spp*Den	5	3.38***	5.01***	2.80*	0.0078**

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table 3.6. Summary of LSmeans (±SE) of bluegrass shoot biomass and root biomass in trial 2. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# KBG plants	Neighbour	Bluegrass shoot biomass (g pot^{-1})	Bluegrass root biomass (g pot^{-1})
3	1	1CT+1WC	0.20 (0.18) a[1]	0.19 (0.20) a
		2 CT	0.14 (0.18) a	0.25 (0.20) a
		2 WC	0.35 (0.18) a	0.33 (0.19) a
	2	1 CT	0.43 (0.26) a	0.56 (0.24) b
		1 WC	1.05 (0.23) b	1.48 (0.22) a
9	3	3CT+3WC	0.33 (0.19)a	0.36 (0.20) a
		6 CT	0.55 (0.19) a	0.64 (0.19) a
		6 WC	0.44 (0.19) a	0.29 (0.20) a
	6	3 CT	0.94 (0.17) a	2.14 (0.18) a
		3 WC	1.91 (0.19) b	1.42 (0.20) b

[1] Within a variable, planting density and number of bluegrass plants, means with different letters differ, $p<0.05$.

Table 3.7. Summary of LSmeans (±SE) of bluegrass shoot biomass and root biomass in trial 3. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# KBG plants	Neighbour	Bluegrass Shoot biomass (g pot^{-1})	Bluegrass Root biomass (g pot^{-1})
3	1	1CT+1WC	0.075 (0.17) a[1]	0.021 (0.17)a
		2 CT	0.036 (0.16) a	0.0079 (0.17)a
		2 WC	0.084 (0.16) a	0.032 (0.17) a
	2	1 CT	0.16 (0.16) a	0.049 (0.17) a
		1 WC	0.16 (0.16) a	0.042 (0.17) a
9	3	3CT+3WC	0.054 (0.16) a	0.012 (0.18) a
		6 CT	0.069 (0.16) a	0.017 (0.17) a
		6 WC	0.14 (0.16) a	0.053 (0.17) a
	6	3 CT	0.30 (016) a	0.051 (0.17) a
		3 KBG	0.31 (0.16) a	0.10 (0.17) a

[1] Within a variable, planting density and number of bluegrass plants, means with different letters differ, $p<0.05$.

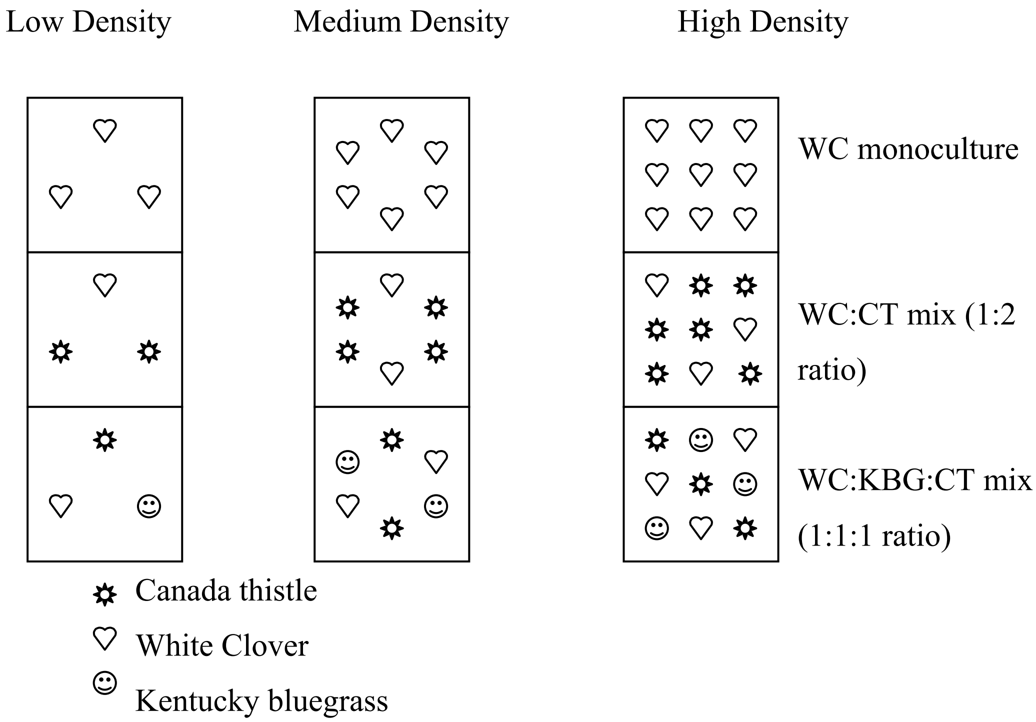


Figure 3.1. Example of 3 treatments containing either 3 (low density), 6 (medium density) or 9 (high density) plants per pot. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

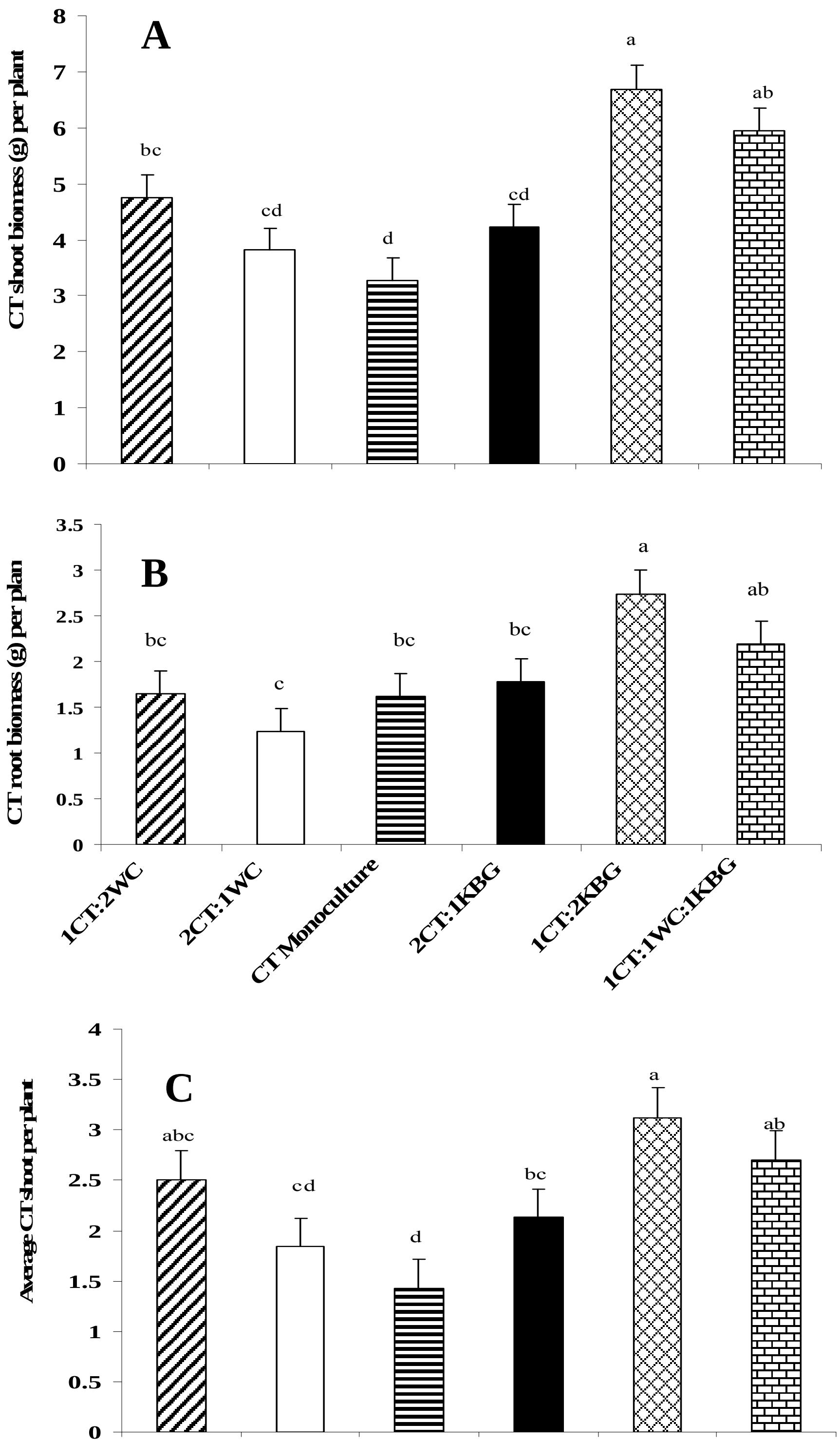


Figure 3.2. Comparison of mean (±SE) Canada thistle shoot biomass (A), root biomass (B), and number of shoots (C) per plant, averaged across trials 2 and 3. Means with different letters differ *p*<0.05. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

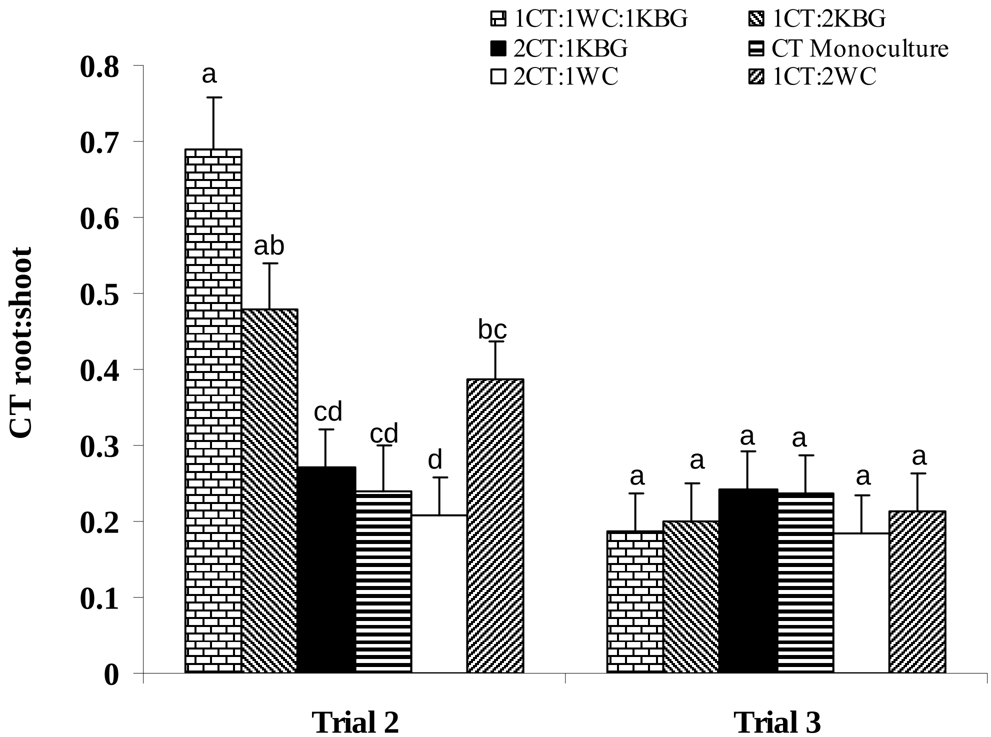
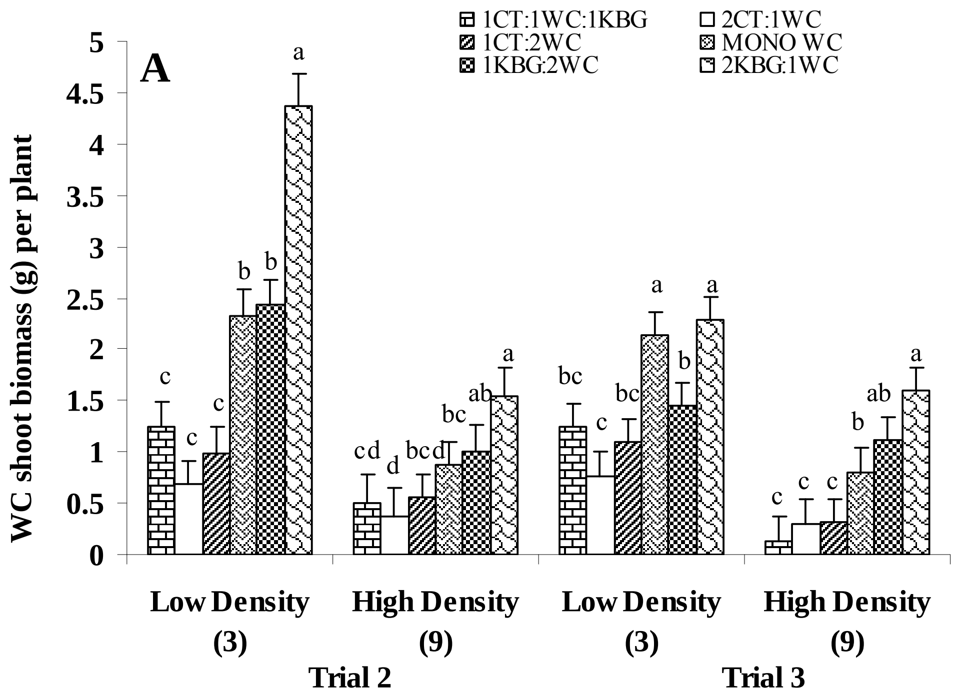


Figure 3.3. Mean (±SE) root:shoot ratio of Canada thistle among various planting treatments, within trials 2 and 3. Within each trial, means with different letters differ, $p<0.05$. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.



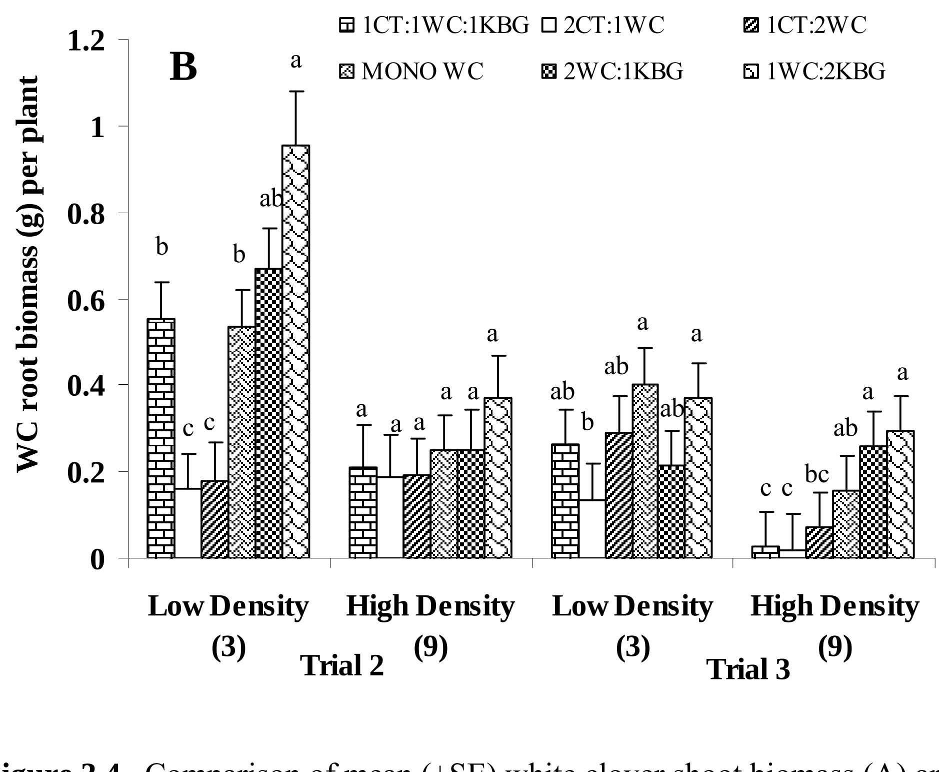
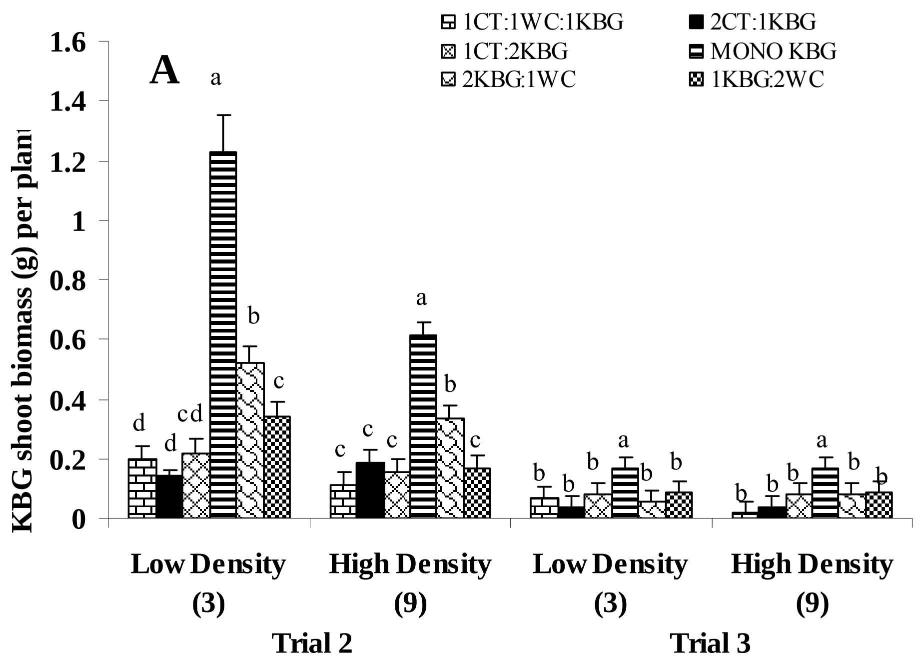


Figure 3.4. Comparison of mean (±SE) white clover shoot biomass (A) and root biomass (B) per plant, averaged across trials 2 and 3. Within a trial and density, means with different letters differ $p<0.05$. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.



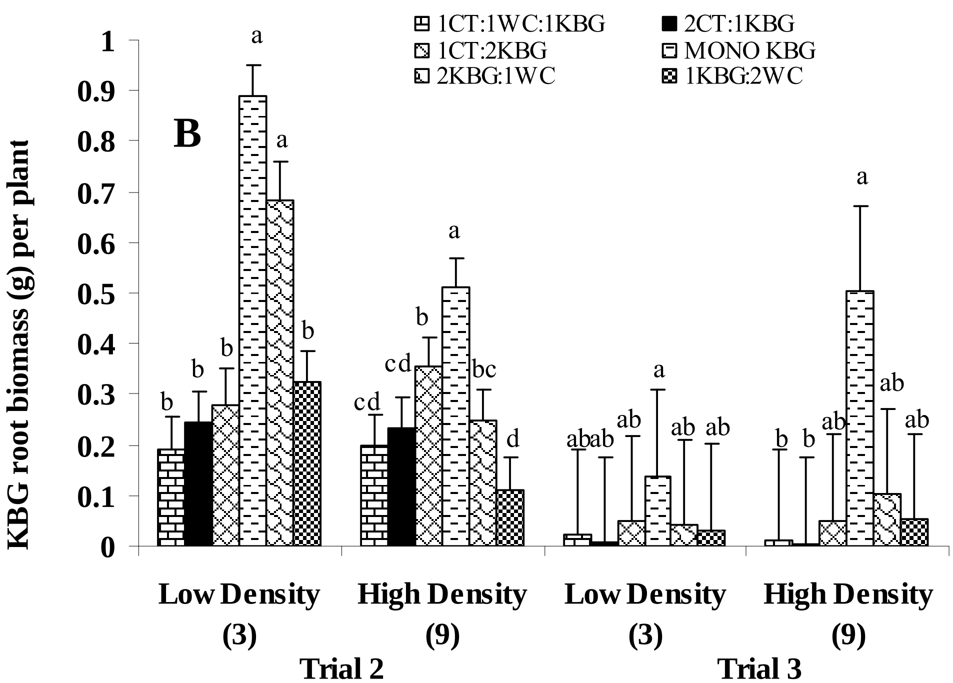


Figure 3.5. Comparison of mean (±SE) bluegrass shoot biomass (A) and root biomass (B) per plant, averaged across trials 2 and 3. Within a trial and density, means with different letters differ $p<0.05$. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

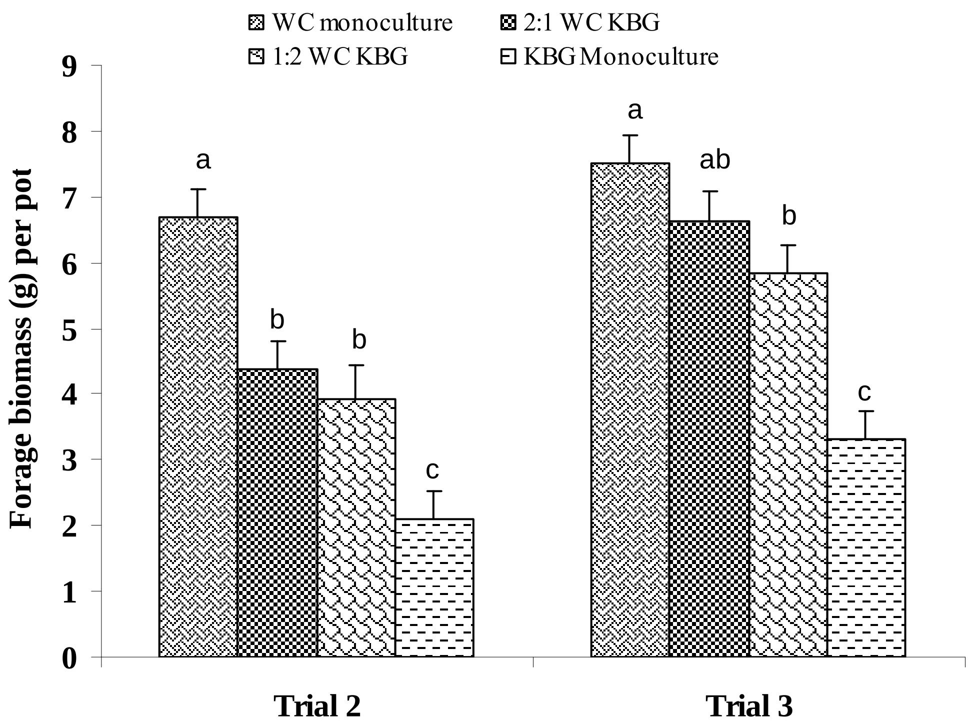


Figure 3.6. Mean (±SE) forage biomass (white clover and bluegrass combined) per pot grown in the absence of Canada thistle, and averaged across trials 2 and 3. Within a planting density, species treatments with different letters differ $p<0.05$. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

Chapter 4. Clarifying Legume Contributions to Forage Yield in Mixed Forage Swards: Establishment Dynamics

4.1. Introduction

Plant growth is generally a function of genetics and environmental factors. Within diverse plant communities, overall growth (i.e. community biomass) is a function of the aggregate interactions among plant species (Buck 1986, Callaway and Walker 1997). While negative interactions (i.e. competition) are considered detrimental to plant growth, positive interactions (i.e. facilitation) may enhance the growth of individual species.

Competition arises when reductions in available light, water or nutrients due to the presence of neighboring vegetation, reduces the ability of plants to maximize their growth based on genetic potential (Hill 1990). Competitive influences vary with resource availability and the ability of neighboring plants to usurp resources. In contrast, the presence of a specific plant species may enhance the growth of neighboring vegetation, either through the amelioration of microclimate such that growing conditions are closer to the optimum (i.e. reducing frost, increasing the growing season length, or reducing herbivory - see Powell and Bork 2004) or by increasing resource availability. While some plants are known to increase nutrient levels near the soil surface through litter deposition (i.e. the 'Asart' nutrient pumping effect), symbiotic relationships between legumes and N-fixing bacteria more commonly provide N to both the legume and surrounding plants (Carlsson and Huss-Danell 2003). The latter benefit through subsequent legume root turnover and decomposition, which releases N into the soil for uptake by neighboring vegetation

Overyielding is the ability of mixtures of complementary plant species to contribute positive increases in biomass relative to those same species when grown alone. In the case of grass-legume mixtures, legumes are thought to exploit the deeper soil profile relative to grasses, minimizing competition (Buxton and Wedin 1970). In addition, mixed swards can enhance protection of susceptible plant species to severe weather (Jackobs 1967). Within forage production systems, the presence of legumes in forage swards has long been known to increase both total forage yield and/or enhance the nutritional quality of the sward compared to grass alone (Barnett and Posler 1983, Sleugh *et al*. 2000, Papadopoulos *et al*. 2001), although evidence for overyielding from mixtures has not typically been found (Sleugh *et al*. 2000).

Forage mixtures typically combine the benefit of N-fixing legumes with perennial grasses that are highly responsive to N addition. During two growing seasons, white clover (*Trifolium repens* L.) is reported to have contributed from 545 to 710 kg/ha of N, with 84 and 92% of N in pure and mixed stands derived from biological fixation (Elgersma and Hassink 1997, Hogh-Jensen and Schjoerring 2001). However, improvements in quality arise from not only the presence of the legume itself, which are typically greater in quality to begin with (Sleugh *et al*. 2000), but also improvements in soil quality (Su 2007) and the indirect transfer of N from leguminous to non-leguminous species (Elgersma and Hassink 1997, Gylfadottir *et al*. 2007). Dairy heifers grazing on legume-grass mixes are able to reduce their feed intake while maintaining similar digestible nutrient removal (Rutter *et al*. 2002), and even increase their productivity (Wu *et al*. 2001).

In western Canada, legumes are an important component of forage swards, both hay crops and pasture used for cattle grazing (McCartney 1993, Walton 1983). The legume of choice in most newly established pastures in western Canada is alfalfa (*Medicago sativa* L.), a tap-rooted perennial that has been shown to fix from 80 to 258 kg N ha^{-1} (Burity *et al.* 1989, Haby *et al*. 2006). Although persistence has often been an issue with alfalfa, and pure swards of legumes are utilized for both hay and pasture production (Sheaffer *et al*. 1990, Van Keuren and Matches 1988), many producers are unwilling to graze pure alfalfa due to the potential of this practice to lead to bloat (Popp *et al*. 2000). In addition, pure alfalfa stands are susceptible to weed encroachment over time (Moyer *et al.* 1999). The addition of highly palatable grasses to alfalfa-based pastures has been shown to reduce the incidence of bloat, as well as increase overall biomass yield relative to monocultures (Chamblee and Collins 1988). However, the presence of highly competitive grasses may reduce alfalfa abundance and associated forage yield and quality. As a result, alfalfa is often combined with species such as meadow brome (*Bromus riparius* Rohm), which provides a favorable long-lived and early growing companion grass beneficial for pasture due to its favorable recovery following defoliation (Casler and Carlson 1995).

In contrast to newly established pastures, older pastures often contain white clover and alsike clover (*Trifolium hybridum* L), which are common to the soil seed bank of temperate pastures (Tracy and Sanderson 2000). These species together with grasses such as Kentucky bluegrass (*Poa pratensis* L.) and smooth brome (*Bromus inermis* L.), are grazing tolerant and often increase with heavy grazing (Waddington *et al*. 1999). Although clovers are particularly common in areas where moisture is less limiting for plant growth (Pederson 1995), and are thought to be an aggressive competitor in mixtures (Blaser *et al*. 1956), white clover can be susceptible to competition from species like bluegrass (McKenzie *et al*. 2005). Early maturing grasses have been shown to favour white clover establishment and are more compatible with white clover during establishment relative to late maturing species (Sanderson and Elwinger 1999).

While the general benefits of including legumes in a forage sward have been well-documented (e.g. Papadopoulos 2001), and some studies have examined the effect of mixing different grasses in various combinations with a companion legume (e.g. McKenzie *et al*. 2005), little is known of the specific proportion of legume needed to optimize forage yield and quality, including how this may depend on the identity of the legume and grass component. Moreover, little is known about the optimal amount of legume in mixed swards necessary to result in overyielding (if present), thereby promoting the agronomic production efficiency of these stands.

4.2. Study Objectives

The objectives of this study were to assess the benefits of including legumes in mixed forage swards in the Aspen Parkland region of central Alberta, including to:

1. Identify the specific agronomic benefits [i.e. changes in forage yield (biomass and crude protein yield) and quality (crude protein and digestibility)] associated with planting legumes at different initial levels within mixed swards,
2. Establish how these agronomic benefits differ based on the identity of different legumes (alfalfa vs clover) and perennial forage grasses (the bunchgrass meadow brome vs a rhizomatous smooth brome/bluegrass mix), and
3. Document early sward dynamics (i.e. changes in composition) following the establishment of forage mixes containing variable amounts of legumes and grasses.

Information on the above objectives would assist producers with deciding which species to seed in order to maximize agronomic returns, as well as to understand the limitations associated with seeding these legume-grass mixtures in various combinations.

4.3. Materials and Methods

4.3.1. Study Site

This research was conducted at the University of Alberta's Ellerslie Research Station (53° 25' N, 113° 33' W) and Edmonton Research Station (53º 31' N, 113º 32' W) in Edmonton, Alberta, Canada (Figure 4.1). Edmonton is located in the Aspen Parkland natural subregion of Alberta and has a continental climate with long, cold winters and short, warm summers. Average annual precipitation is 460 mm with 70% falling during the growing season from May through September (Figure 4.2 and 4.3). The Ellerslie and Edmonton Research Stations both have Black Chernozemic soils that have historically been used for agronomic research. Soil analysis for each site was conducted prior to seeding to determine fertilizer requirements (Table 4.1). A fertilizer mix of 18-20-10-15 was applied at a rate of 127 kg ha^{-1} to both sites at Ellerslie, and 11-52-0 was applied to the W240 site at a rate of 56 kg ha^{-1} at seeding.

4.3.2. Experimental Design and Treatments

This research was designed as a split-plot randomized complete block with four replications at each of three sites. The sites included Ellerslie 1 (E1) at the Ellerslie Research Station, which was established during 2003. In 2004, a second site (E2) was established at the Ellerslie Research Station, and an additional site was established at the Edmonton Research Station (W240). Each whole plot measured 18 x 6 m representing a forage mixture was divided into six subplots (3 x 6 m each) containing the varying legume proportions (Figure 4.4). Additionally, there was a 10 m buffer surrounding each site and a 4 m buffer between whole plots.

Main plot treatments consisted of forage species mixes combining two grass growth types with two legume types in a 2 x 2 factorial design, giving four mixtures in total. A blend of smooth brome and bluegrass (60:40) was used to represent rhizomatous grasses commonly found in old pastures of the region, while meadow brome is a highly productive grass frequently seeded into new pastures. Similarly, alfalfa is a productive legume often found in Alberta forage stands, while clovers are common volunteers in older pastures. The clovers used in this study were a 50:50 blend of white clover and alsike clover. Ratios were calculated using gross seed weight but were adjusted based on levels of pure live seed for each species.

Each of the four species combinations was further divided into six treatments of varying percent legume at seeding. The percent legume in the seeded mix varied from 0% (i.e. a grass monoculture) to 11%, 22%, 33%, 67% or 100% (i.e. a pure legume stand). Germination tests were conducted for each species just prior to seeding. Each plot was replicated four times at each site in blocks. Seeding rates were set according to Alberta Agriculture's Forage Manual (1988) (meadow brome 15kg ha^{-1}, smooth brome 8.4kg ha^{-1}, Kentucky bluegrass 5.6 ha^{-1}, alfalfa 9 ha^{-1}, clover 6kg ha^{-1}) and adjusted for levels of pure live seed of each forage species. Plots at W240 were hand weeded to reduce impact from non-focal species. High weed populations at both the E1 and E2 sites prevented similar control methods and thus, no control measures were undertaken.

4.3.3. Vegetation Sampling

Plant community composition was determined for each plot using four, 0.5 m^2 (1m x 0.5m) randomly placed quadrats. Composition was determined by percent canopy cover for grass, legume and weed components. Measurements were taken in July at E1 in 2003 and 2004, as well as at E2 and W240 during 2004.

Sward biomass for each subplot was measured by combining the biomass harvested within four randomly placed 0.25 m^2 (50 x 50 cm) clip plots. All current annual growth was removed 2.5 cm aboveground and separated into legume, grass and weed components. Biomass was collected during each year at peak production, coincident with the period when alfalfa was blooming in July. Samples were subsequently dried to constant mass, weighed and converted to kg ha^{-1} for analysis.

Grass and legume samples were ground separately to 1mm size in a Wiley Mill™ for the subsequent determination of crude protein (CP) and acid detergent fiber (ADF) concentrations. Estimates of nitrogen were determined using a LECO model FP-428 auto-analyzer, which were subsequently converted to CP concentration using equation (1).

$$\%CP\ concentration = \%N * 6.25 \quad (1)$$

Values of crude protein yield (CPY) may be a superior indicator of forage value compared to annual peak production or CP concentration alone, as CPY is weighted by measures of both forage quantity and quality. As a result, total CPY for each component was calculated by multiplying the proportion of CP by biomass (kg ha^{-1}) [see equation (2)]. Finally, total sward CPY was determined by summing CPY estimates from the grass and legume components harvested from each plot (3).

$$CPY\ (kg\ ha^{-1}) = (\%CP / 100) * biomass\ (kg\ ha^{-1})$$

(2)

$$CPY\ total\ forage\ (kg\ ha^{-1}) = CPY\ of\ legume + CPY\ of\ grass$$

(3)

The ANKOM™ filter bag technique, as described by Komarek (1993), was used to determine ADF concentrations. This process quantifies the proportion of nondigestible fibrous material in harvested samples. Similar to CPY, values of ADF were combined with biomass to quantify acid detergent soluble yield (ADSY) using equations (4) and (5), with the hypothesis that greater legume abundance would increase CPY and ADSY.

$$ADSY\ (kg\ ha^{-1}) = [1- \%ADF / 100] * biomass\ (kg\ ha^{-1})$$

(4)

$$ADSY\ total\ forage\ (kg\ ha^{-1}) = ADSY\ of\ legume + ADSY\ of\ grass$$

(5)

4.3.4. Data Analysis

Prior to analysis all data were tested for normality and homogeneity of variances. Plots of residuals and a combination of Shapiro-Wilkes and Levene's tests indicated that data at E1 and E2 were normally distributed. Legume biomass and legume CPY were normalized using a square root function. Data were then analyzed using Proc MIXED in SAS for a split-plot analysis of variance (ANOVA) (SAS Institute Inc. 1988), with forage mix, legume ratio at seeding, and year of sampling as fixed factors, and replicate within site as the random factor. Year of measurement was included as a split plot factor during analysis. Due to differences among sites, including in the year of initiation of the study, all three sites were analyzed separately. Where significant main effects and interactions were found ($p<0.05$), LSmeans were compared using a Tukey test (minimum significance value of 5%), with emphasis placed on comparing forage mixes and/or legume ratios within years.

4.4. Results

Data from E1 were consider preliminary in this investigation, and are provided solely in Appendix A2. Similarly, few responses were found in ADF and ADSY. As a result, results for the latter are shown in Appendix A2, and are not reviewed in detail in Chapter 4.

Summary results arising from the ANOVA for response variables at E2 and W240 are provided in Tables 4.2 and 4.3, respectively.

4.4.1. Total Forage Biomass

Total forage biomass was affected by both species mix and the proportion of legume at E2 (Table 4.2). At W240, legume proportion but not species mix affected forage yield (Table 4.3), with an additional interaction of species mix with legume. In addition to strong year effects at each site, year interacted with legume at E2 (Table 4.2) and W240 (Table 4.3).

Differences in total forage biomass among mixes at E2 were consistent across years, with plots containing smooth brome producing greater total forage compared to meadow brome grown with the same legumes (Figure 4.5). Total biomass yields at this site consisted mainly of grass, with differences among mixes largely due to increased grass contributions to forage yields within plots containing smooth brome and bluegrass, and lower legume yields in plots containing clover rather than alfalfa (Figure 4.5). Within the smooth brome-bluegrass plots, the vast majority (i.e. 95%) of biomass was smooth bromegrass.

Both sites demonstrated strong legume by year interactions, with few to no differences in total forage yield among plots containing variable legume in the first year of establishment (Figures 4.6 and 4.7). Legume effects in the year of establishment were only apparent at W240 (Figure 4.6), where plots with pure or nearly pure legume were lower in yield, primarily due to smaller amounts of legume biomass relative to the grass component. Notably, this trend for reduced yield in high legume plots remained evident at W240 (Figure 4.6) and E2 (Figure 4.7) during year 2 of establishment. High legume plots remained chronically low in overall yields due to a much lower contribution of legumes relative to grasses in low legume plots.

Although no significant increases in total forage were evident at intermediate legume seeding ratios, total forage yields in the second year at E2 (Figure 4.7) and W240 (Figure 4.6) had the highest numerical yield within plots containing 11-22% legume at seeding. The lone forage mix by legume ratio effect observed at W240 revealed little new information (Figure 4.8): meadow brome-alfalfa mixes at this location showed the greatest potential for forage overyielding at intermediate mixtures of 11-22% legume, but once again remained non-significant. Moreover, closer examination of these data indicated that the favorable yields under these conditions were largely due to the ability of alfalfa to remain at moderate levels in swards seeded to predominantly grass mixtures (Figure 4.8). Conversely, plots containing clover had low legume yields: clover remained similar in yield to alfalfa only when seeded with little to no grass (Figure 4.8).

4.4.2. Total Crude Protein Yield

Total crude protein yield (TCPY) was affected by species mix at E2 (Table 4.2). Levels of TCPY were also affected by the proportion of legume seeded at W240 (Table 4.3), with an additional legume by year interaction (Table 4.3). Strong overall year affects were evident on TCPY at both sites (Table 4.2 and 4.3).

Total crude protein yield values among species mixes at E2 were consistent across years. Treatments containing clover produced lower TCPY compared to those containing alfalfa (Figure 4.9), primarily due to poor yields associated with clover relative to alfalfa. Grass contributed the greatest CPY in all mixtures except meadow brome-alfalfa, which had the greatest contribution from legume among all treatments. When grown with the same companion legume species, meadow brome provided 87.9 to 103.4 kg ha^{-1} less CPY compared to smooth brome.

Patterns in TCPY among legume treatments at W240 varied markedly among the 2 years for which they were assessed (Figure 4.10). While there were no differences in TCPY among legume treatments in 2005, plots seeded to a majority of legume had lower TCPY during 2004, the first year of forage establishment. Changes in TCPY during the first 2 years may be attributed to temporal variation in legume and grass crude protein concentrations, combined with biomass responses.

During 2004, TCPY levels among legume treatments at W240 (Figure 4.10) paralleled those of biomass (Figure 4.6), suggesting little role of variation in protein concentrations. Indeed, no differences ($p>0.10$) in protein were evident among treatments in either the grass (ranging from 12.9±0.4% to 13.4±0.4%) or legume (16.5±0.5% to 17.2±0.5%) component of these stands during that year. The lack of differences in TCPY during 2005 contrasts the biomass patterns at W240 (Figure 4.6), and suggests crude protein concentration differences stabilized this parameter in 2005 (Figure 4.10).

Average legume and grass protein values also differed between 2004 and 2005 ($p<0.001$). Legume protein values ranged from 16.3± 0.4% to 17.4±0.4%, while grass values ranged from 16.9±0.4 % to 9.3±0.4% in 2004 and 2005 respectively. The large decline in grass protein values between 2004 and 2005 may account for the lower TCPY values of treatments high in grass biomass during the second year.

4.5. Discussion

4.5.1. Comparison among Forage Mixes

Forage yields were overall greater in mixes containing alfalfa than clover, although these results were inconsistent among sites. At W240, forage mixes were generally similar in total biomass and CPY through the first 2 years. Removal of weedy species in year one at W240 may have reduced the influence that species mixes played in altering forage biomass. Mean production at W240 was 9545 kg ha^{-1} by the second year, which was generally greater than that at E2 (8330 kg ha^{-1}) during the same period. A reduction in competition against seeded forage at W240 may have allowed both seeded grasses and legumes to reach their full growth potential more quickly, with grasses and legumes subsequently trading off in abundance through direct competition with one another. In contrast, large weed populations were evident at Ellerslie, which could have resulted in seeded forages struggling to maintain a competitive advantage over not only their seeded neighbors, but also volunteer weeds.

At E2 alfalfa remained greater in relative abundance (both biomass and CPY) when grown with meadow brome as a companion species rather than smooth brome. E2 was seeded in 2004 when conditions were near the long term precipitation average but still below average during the month of seeding (June). These drier conditions tend to favor smooth brome over meadow brome (Knowles *et al.* 1993), which in turn could have suppressed alfalfa within the smooth brome-alfalfa mix.

Regardless of the reason, these results provide strong evidence for the importance of alfalfa in increasing overall biomass and CPY levels in forage swards, and provide some indication that swards of meadow brome-alfalfa may be more desirable than smooth brome-alfalfa due to the formers' greater ability to retain alfalfa over time. Being a tap rooted species, alfalfa may also minimize below ground competition with shallower rooted neighboring grasses by reaching deeper into the soil profile for both water and nutrients (Brun and Worcester 1975, Russelle *et al*. 2001). Finally, the full advantage of mixing alfalfa with forage grasses possessing complementary root systems could manifest itself 3 years or later after establishment as these swards continue to undergo changes.

Unlike alfalfa, clover contributed limited forage biomass and CPY at E2 in both years of monitoring, particularly when grown with smooth brome, suggesting the latter resulted in greater competitive suppression of clover. Differences in the suppressive affect of neighboring plants can be influenced by morphological differences between species (Lamba *et al.* 1949). The rhizomatous root system of smooth brome allows this species to effectively seek out nutrients and water in the shallow soil layer (Otfinowski and Kenkel 2008), in turn potentially increasing competition with surrounding plants. In contrast, meadow brome has a deep fibrous root system (Jacobs and Siddoway 2007) and therefore could be less likely to compete with shallow rooted clover that is less able to access deep soil resources (Turkington and Burdon 1983). We hypothesize that smooth brome and clover may be exploiting similar areas of the soil profile, thereby maximizing competition among them and potentially contributing to heightened clover suppression in the smooth brome-clover mixture at E2.

Despite the low clover abundance observed, values of total biomass in the smooth brome-clover mixture remained similar to those in the smooth brome-alfalfa mix through the first 2 years of establishment in the current study. If our hypothesis is correct, long-term forage production in this sward may be expected to sharply decline. This is a particular concern given that smooth brome has been known to undergo marked declines in production after several years of production in hayland (Lardner *et al*. 2001).

At E2, clover mixes tended to have lowest legume contribution relative to those mixes containing alfalfa. Clover is known to be a relatively early seral opportunistic plant species adapted to frequent disturbance, and is relatively shade intolerant (Frame 2005). As a result, the frequency of harvest and timing of harvest can strongly affect the ability of plants like clover to compete. Berdahl *et al*. (2004) found that a single cut system favored grass dominance compared to a multi-cut system. As swards in the current study were harvested once at peak growth in mid-season, this could account for the decrease in clover. Moreover, because white clover is known to be susceptible to competition from taller grasses (Marcuvitz and Turkington 2000) and frequent defoliation is beneficial for growth (Yu *et al* 2008), more frequent defoliation may have been necessary to maintain clover in these swards. Had our plots been cut or grazed earlier in the growing season, this could have maintained greater light and opportunity for clover retention. This likely explains the increase in the presence of clover in older long-term pastures (Aarssen and Turkington 1985). Similar to more frequent harvesting, earlier harvesting may also have altered the sward dynamics by returning the competitive advantage to those species such as clover that can easily recover from the removal of top growth. Infrequent harvesting, such as that done here, would favor highly competitive legumes such as alfalfa that are known to persist under low disturbance regimes such as a one or two cut hay system.

Clover decline may also have been exacerbated by increased soil fertility. When provided N fertilizer the clover component of forage swards has been found to decline in mixtures with ryegrass (Camlin 1981).

In contrast, the extensive rhizomatous root systems of smooth brome and bluegrass allows these species to obtain greater resources from the soil during the short time remaining in the growing season (Power 1986, 1988). Rhizomatous roots allow for efficient nutrient and water extraction from throughout the shallow soil layer, which in turn may not be accessed as readily by deep penetrating fibrous root systems such as those found under alfalfa and meadow brome. Rhizomatous species are also able to access nutrients released by N fixing microbes in the shallow soil. Burity *et al*. (1989) observed a distinct difference in nitrogen yields among grass growth habits, with rhizomatous growth forms obtaining a greater amount of total N through root transfer compared to bunchgrasses.

Finally, it is possible that extensive clover mortality may have increased the potential for grass growth, particularly with the high potential for N fixation by clover during the first 2 years of establishment. Root mortality and turnover would increase N availability for neighboring grasses. While removal of the legume competitor improved grass growth, the absence of an improvement in crude protein suggests that either 1) clover had not yet created a large N store in its root system due to below average precipitation and shading by grasses (Serraj *et al*. 1999, Carlsson *et al.* 2009), or 2) that clover roots had not yet been broken down and the immobilized N made available to grasses (Mohr *et al*. 1999).

4.5.2 Role of Legumes in Yield Contribution.

In this study the role of legumes in contributing to the yield of biomass and CPY during the establishment year varied among study sites. At W240, swards seeded to predominantly legumes led to lower biomass during both the first and second year of establishment, with little evidence that legumes were increasing in relative abundance from year 1 to 2. However, even within the W240 site, the meadow brome-alfalfa treatment exhibited the greatest contribution of legume to overall forage yields, with particularly poor contributions from clover when seeded at low to moderate seeding rates (i.e. 11 to 33%). In contrast to the W240 site, seeding more legumes at E2 resulted in no differences in forage yield during the establishment year. Moreover, by the second year of sampling at each of these sites, swards seeded to high legume (67% or greater) were generally lower in forage yield than those plots containing nearly pure grass monocultures.

Although the reason for the poor legume growth relative to grasses is unknown, all sites had high soil fertility, which may have favored grasses during establishment, in turn suppressing legumes within all binary forage mixtures (Vinther 1998). The immediate and more marked suppression of legumes over time at W240 may reflect the particularly high soil N levels found there (Table 4.1). In addition, the W240 site had relatively low weed populations, which may have increased the ability of grasses to survive and grow relative to the legumes. Perennial grass seedlings are known to be relatively slow to establish and are poor competitors, with high susceptibility to damage under heavy weed pressure (Leath *et al*. 1996). Should this be the case here, high weed pressure may actually have favored the establishment and retention of legumes within mixed forage swards by serving to slow grass establishment.

The addition of moderate amounts of legume to forage mixtures did not lead to the development of sward overyielding, where total biomass was expected to increase above levels seen in monocultures of legumes or grasses. Only within meadow brome-alfalfa swards did moderate amounts of legume exhibit a trend towards greater peak biomass. While unable to rule out the possibility that future sward dynamics past 2 years (i.e. the age of the swards examined here) may lead to more pronounced overyielding, the current results suggest that grasses and legumes traded off in roughly a 1:1 ratio, thereby stabilizing forage yields across most legume seeding levels. As a result, the primary benefit of including legumes within forage mixes appeared to be through the maintenance of total CPY.

The absence of over yielding in smooth brome-alfalfa parallels observations by Sengul (2003), where pure legume swards had the lowest yields in three years. However, our results contrast those of McCloud and Mott (1953) in Indiana, USA, who observed that smooth brome-alfalfa mixes outyielded both alfalfa and smooth brome monocultures, with the same trends evident for mixtures of smooth brome and white clover.

The lack of evidence for overyielding among any treatments containing variable legumes during the first 2 years of sward establishment at E2 and W240 may be caused by ample amounts of soil nutrients, particularly N, which could well have minimized the ecological benefits of legumes to collective sward production. Initial N levels were as high as 33 kg ha^{-1} at the time of seeding, and coupled with one-time fertilization at seeding, would have increased the ability of nitrophilic grasses to increase growth (Malhi *et al*. 2008). Pronounced grass growth, in turn, would have reduced the competitiveness of legume seedlings during the first 2 years, thereby inhibiting overyielding. Nuttall *et al*. (1980) demonstrated that the addition of fertilizer to a mixture of grass and legume leads to a reduction of legume in the mix.

As soil N availability changes in a forage sward, the competitive advantage is likely to switch between legumes and other non-N fixing plants (Ledgard and Steele 1992). When soil N levels are high and the N fixing capability of legumes is no longer an advantage in the forage mix, grass plants may be more capable of obtaining the necessary amounts of N, in turn allowing them to out-compete legumes. This is supported by other studies that have shown sites with high fertility often have lower levels of N fixation (Ledgard and Steele 1992, Vinther 1998), potentially undercutting the importance of legumes to the stand. Conversely, as soil N levels decline a legume's ability to fix its own N gives this species a competitive advantage, which then may be more likely to lead to overyielding.

Haby *et al*. (2006) found that N transfer from legume to grass did not peak until the middle of the growing season. Moreover, transfer of N to grass can take up to two years to reach optimal levels (Ta and Faris 1987, Burity *et al.* 1989, Heichel and Henjum 1991). As a result, both high soil N levels and poor precipitation may have delayed the establishment of any beneficial legume effects on yield relationships at these study sites. Furthermore, the use of poorer soils such as Grey Luvisols or Dark Gray Chernozems may have provided better opportunities for the detection of any beneficial effects associated with the inclusion of legumes on overall forage yields in grass pastures.

4.6. Conclusion

Optimal yields were obtained when meadow or smooth bromegrass were grown with alfalfa rather than clover, as clover rapidly disappeared from the swards. While no strong and consistent patterns of overyielding occurred in this study, mixtures seeded to greater grass were quicker to reach increased levels of production. Additionally the primary benefit of legume presence appeared to be in the improvement to forage quality (i.e. total crude protein CPY) rather than forage biomass availability. Between grass species, smooth brome was more detrimental to alfalfa, reducing its proportion in the sward. When grown in mixture with alfalfa, meadow brome appeared to be a more compatible species leading to more stable legume contributions compared to mixtures of alfalfa and smooth brome-bluegrass.

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Table 4.1. Summary of soil characteristics at each of the study sites.

Study Site	Soil Type	Surface Texture	pH	EC	NO_3-N	P	K	SO_4-S
				DS m^{-1}	---------------- (ppm) ----------------			
Edmonton Res. Station (W240)	Orthic Black Chernozem	Clay Loam	6.0	0.45	33	13	223	8
Ellerslie (E1 & E2)	Orthic Black Chernozem	Loam	6.2	0.31	15	26	170	12

Table 4.2. Summary of ANOVA F-value results from PROC MIXED analysis of forage biomass, grass biomass, legume biomass, total crude protein yield (CPY), grass CPY, and legume CPY within Ellerslie 2 over 2 years.

	df	Forage Biomass	Grass Biomass	Legume Biomass	Total CPY	Grass CPY	Legume CPY
Species Mix (Spp)	3	5.78***	23.81***	28.97***	4.18**	11.78***	20.13***
Legume (Leg)	5	10.05***	47.46***	93.39***	0.70	53.86***	70.69***
Spp*Leg	15	1.41	4.06***	1.46	0.81	4.08***	1.33
Year	1	643.42***	564.58***	62.43***	78.43***	80.60***	10.95***
Spp*Year	3	0.67	2.27	7.71***	1.59	0.15	2.46
Leg*Year	5	4.65***	3.30**	19.07***	0.29	0.45	4.76***
Spp*Leg*Year	15	1.00	1.89*	1.74	0.67	1.46	1.40

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table 4.3. Summary of ANOVA F-value results from PROC MIXED analysis of forage biomass, grass biomass, legume biomass, total crude protein yield (CPY), grass CPY, and legume CPY within the West 240 site.

	df	Forage Biomass	Grass Biomass	Legume Biomass	Total CPY	Grass CPY	Legume CPY
Species Mix (Spp)	3	0.45	4.08**	9.29***	0.67	2.59	6.19***
Legume (Leg)	5	31.67***	68.40***	182.55***	2.73*	55.65***	175.20***
Spp*Leg	15	1.81*	2.40**	2.51**	1.30	2.17*	1.19*
Year	1	424.01***	552.29***	4.14*	13.16***	30.44***	9.37**
Spp*Year	3	0.27	0.69	3.38*	1.14	0.46	4.63**
Leg*Year	5	6.20***	4.79***	11.76***	2.60*	2.70*	9.25***
Spp*Leg*Year	15	0.47	0.76	0.50	0.71	0.39	0.56

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

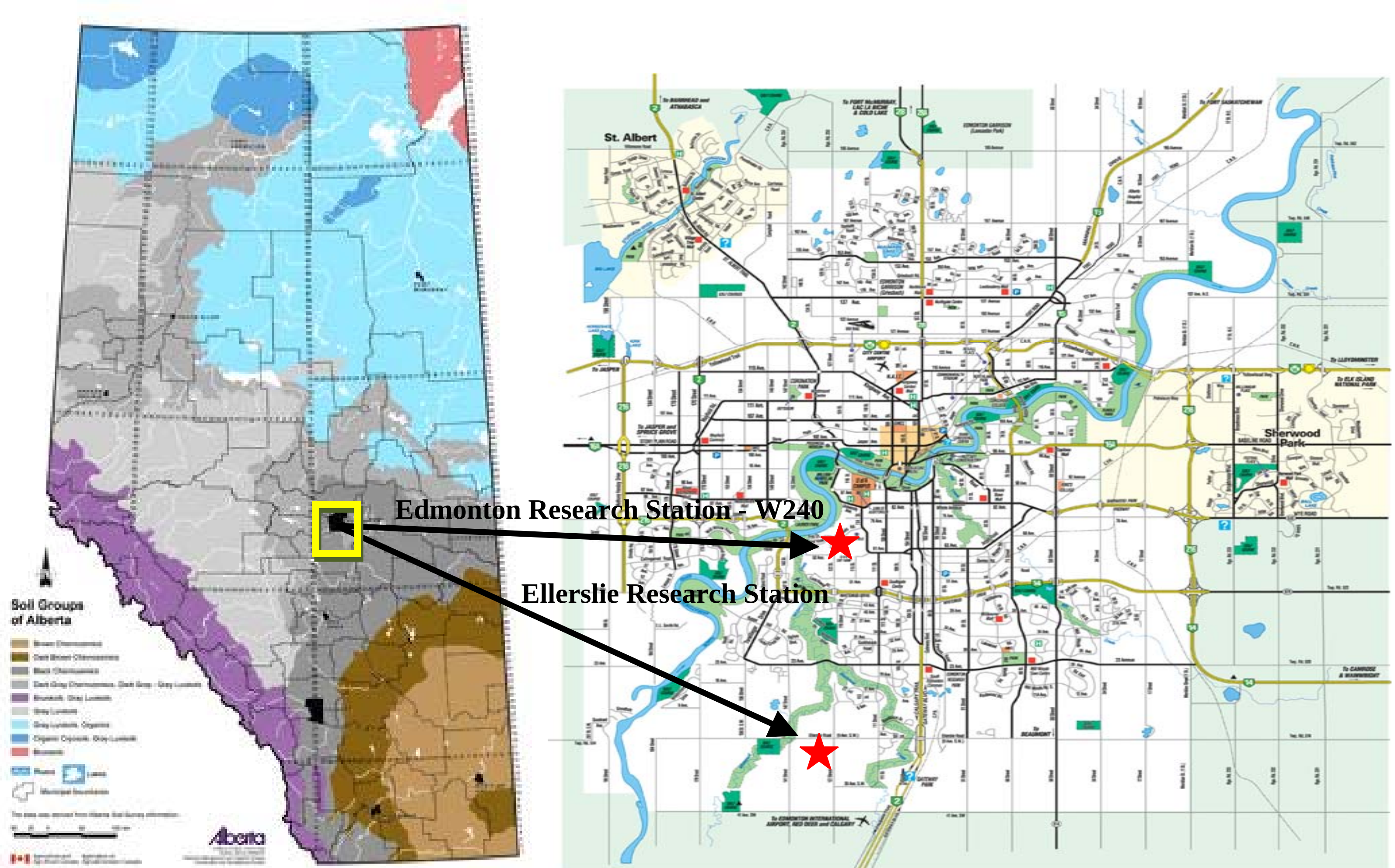

Figure 4.1. Locations of the Ellerslie Research Station (E2) and Edmonton Research Station (W240).

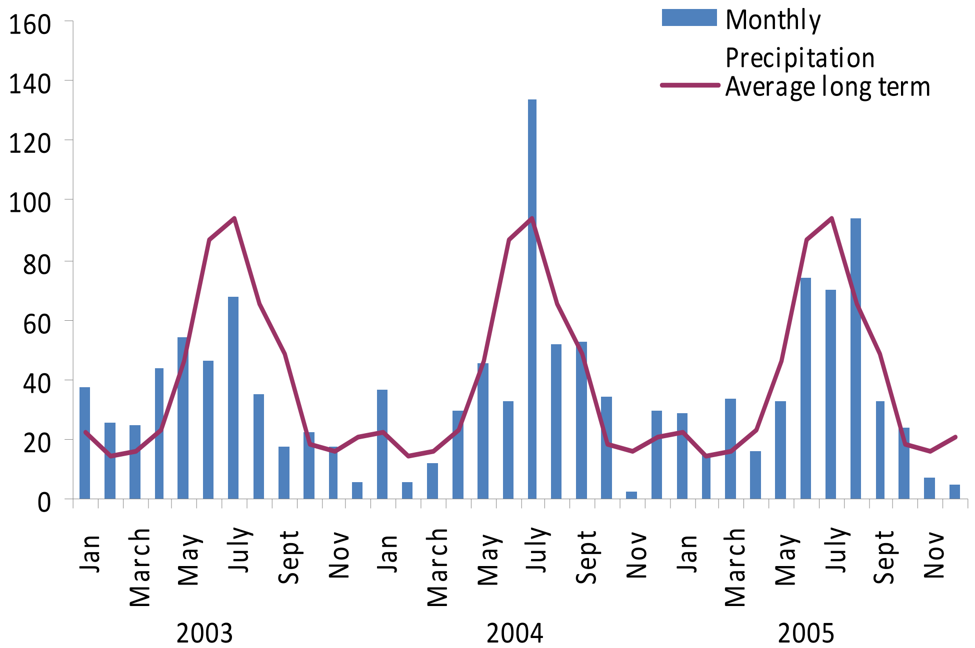

Figure 4.2. Mean monthly precipitation for 2003 to 2005, and the 30 year average precipitation at the Ellerslie Research Station (Alberta Environment 2009).

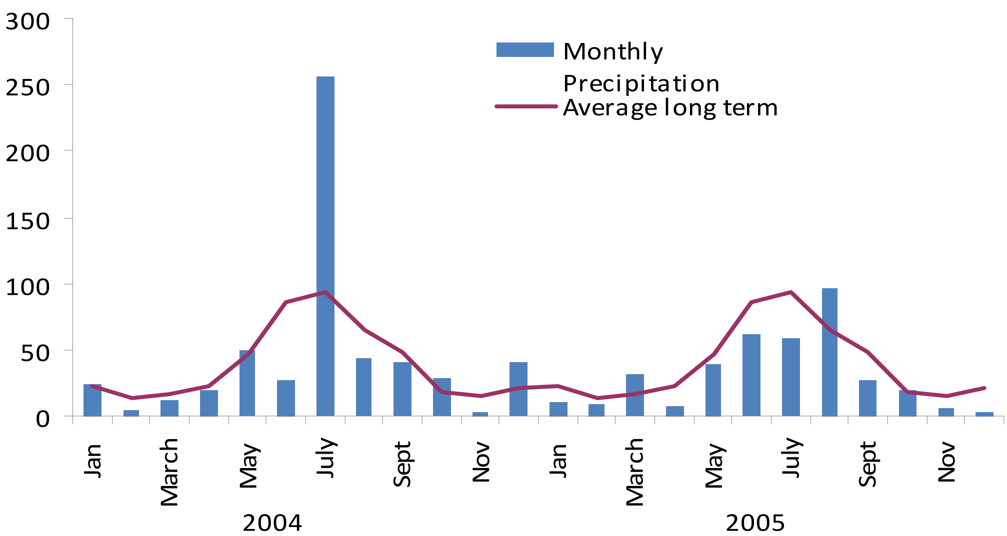

Figure 4.3. Mean monthly precipitation for 2003 to 2005, and the 30 year average precipitation at the Edmonton Research Station (Alberta Environment 2009).

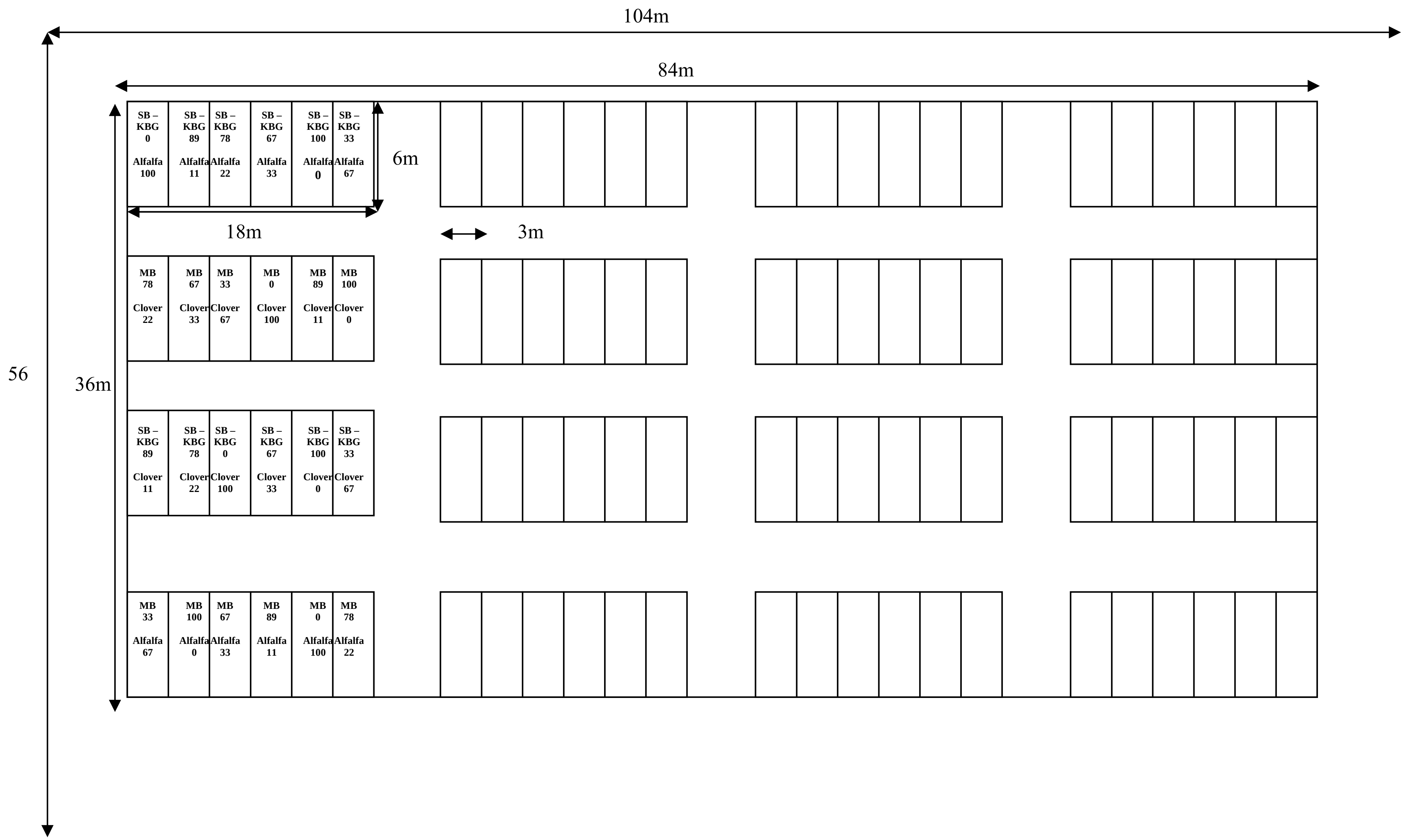

Figure 4.4. Sample site map used in the cross seeding study.

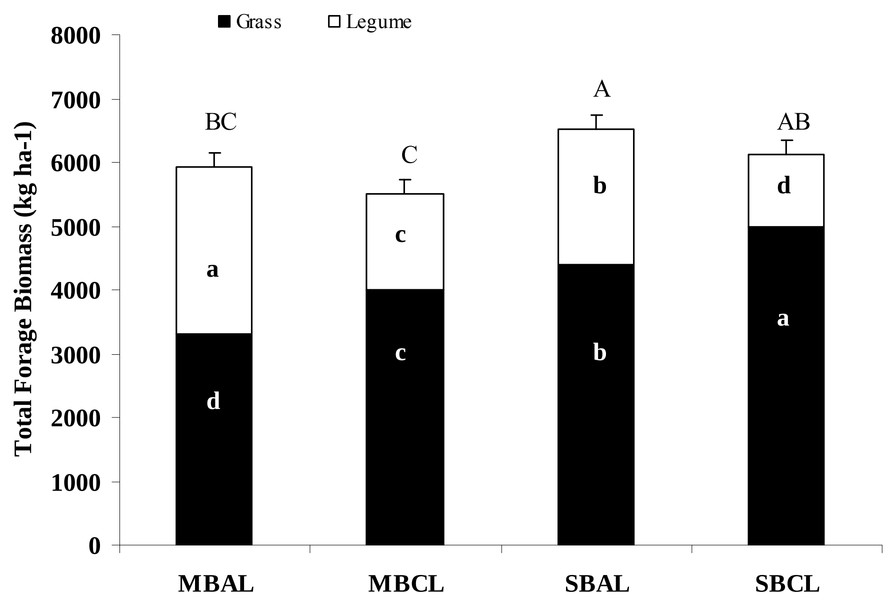

Figure 4.5. Comparison of total forage biomass among species mixtures averaged across two years at the E2 site. Within a vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

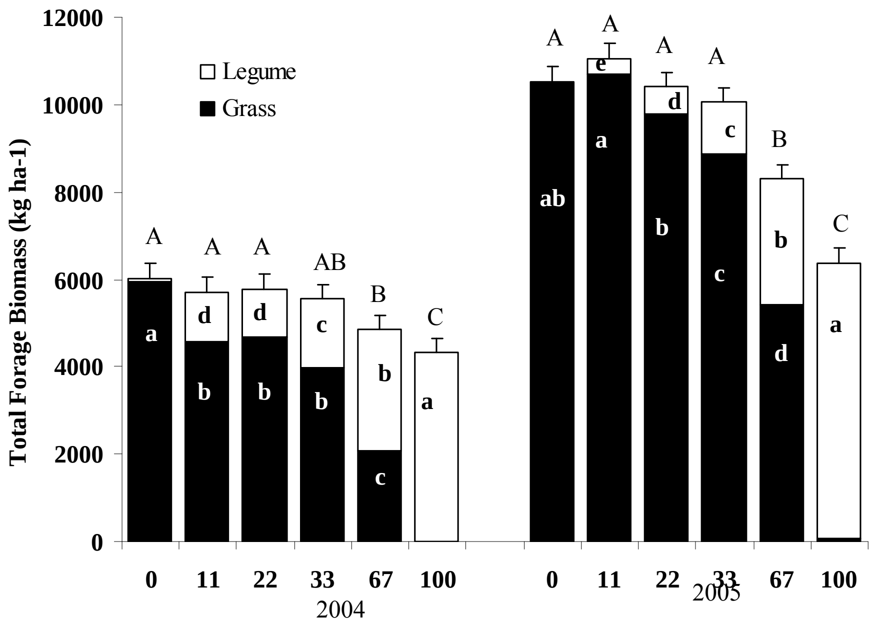

Figure 4.6. Comparison of total forage biomass among varying legume proportions and years at the W240 site. Within each year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means.

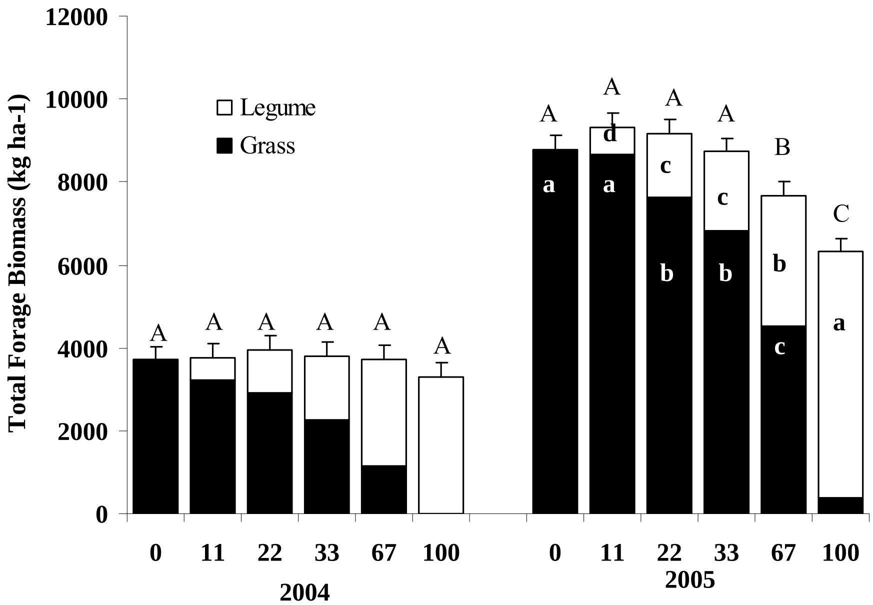

Figure 4.7. Comparison of total forage biomass among varying legume proportions and years at site E2. Within a year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means.

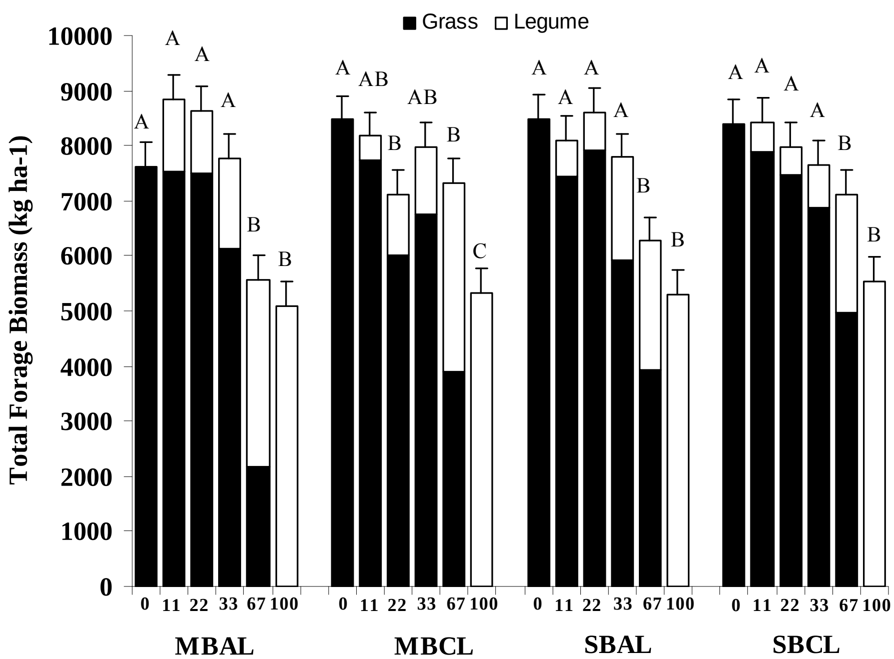

Figure 4.8. Comparison of total forage biomass among legume proportions and species mixtures averaged across two years at the W240 site. Within a species mixture, means with different letters differ, $p<0.05$. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

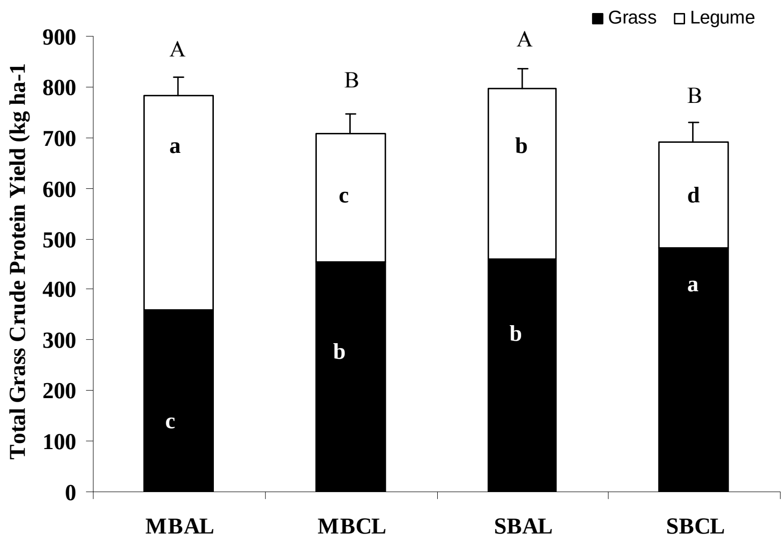

Figure 4.9. Comparison of total CPY among species mixtures averaged across two years at site E2. Within a vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

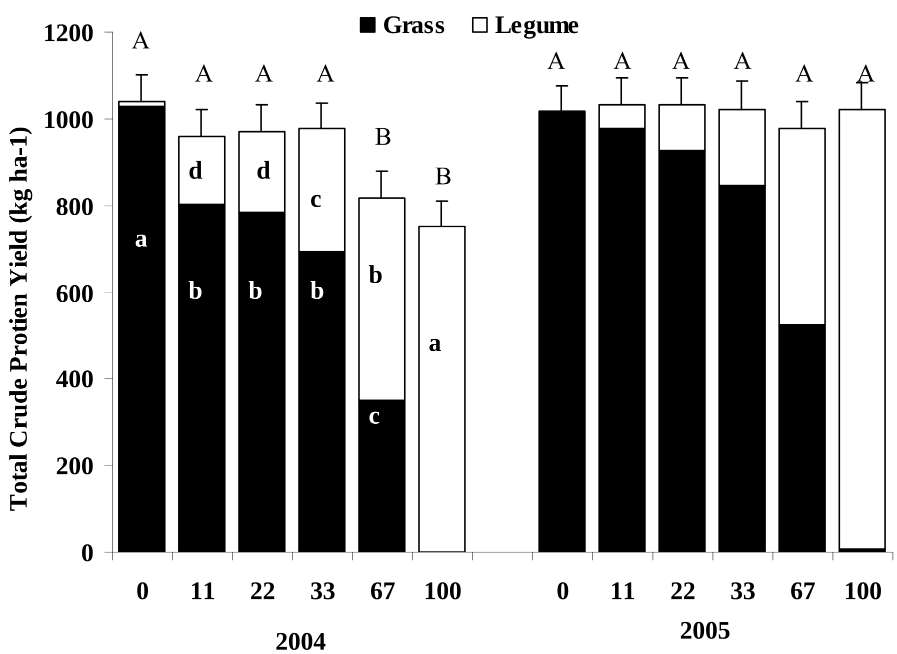

Figure 4.10. Comparison of total CPY among legume proportions and years at the W240 site. Within a year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means.

Chapter 5. Summary and Conclusion

The need to develop and maintain high yielding and high quality forage stands in Alberta is becoming more imperative with the increase in forage demand and decline in available land for forage production. With high costs of production livestock producers require information that will allow them to make decisions to improve their production systems, including those that lead to greater economic viability. Having a better understanding of the role of different forage mixes and the contribution of each species in the mix will assist when making decisions on what to seed prior to forage establishment.

Weeds can reduce the production potential of an establishing legume sward. It is important to understand the potential interactions between plants in agronomic production systems. In the case of a forage sward this includes the three-way interaction between legume, grass and weed. Canada thistle [*Cirsium arvense* (L.) Scop.] is a common weed found in abundance in many agricultural systems. It has been shown to reduce forage yields (Grekul and Bork 2004) and alter animal behaviour (Schreiber 1967). However, the competitiveness of forage swards and the associated impact of neighboring Canada thistle plants on forage may be altered by pasture species composition. While legumes contribute directly to yields and may provide a nitrogen source for plants in the surrounding area, they can also provide a competitive environment that reduces the spread of undesirable species.

The cross-seeding study reported on in this thesis (Chapter 4) was designed to evaluate the relative contribution of legumes in species mixes to overall forage yield and quality. Although total production at all sites increased during the second year of establishment, the addition of legumes to predominantly grass swards did not lead to an increase in total biomass relative to pure grass stands. Moreover, swards containing monocultures of alfalfa [*Medicago sativa* (L.)] had the lowest biomass yields compared to all other mixes containing legume. As a result, it appears that grasses provided the greatest contribution to overall forage production at all swards with low to moderate legume at seeding. Additionally, the benefit of adding legumes to a forage mix was mainly evident through increases in forage quality. Legumes were high in crude protein, which compensated for lower biomass yields and raised sward total crude protein yield (TCPY) values. These results highlight the importance of assessing forage species based on quality as well as yield data.

As swards matured mixtures containing alfalfa typically maintained greater levels of both forage yield and quality relative to those containing white clover [(*Trifolium repens* (L.)]. Forage mix responses also varied among locations. While forage mixes had no effect on forage yield and quality at W240, at the Ellerslie sites mixtures containing white clover had both lower yield and quality. Alfalfa also maintained itself more effectively when grown with meadow brome [*Bromus riparius* (Rohm)] regardless of the site. The competitive ability of the smooth brome [*Bromus inermis* (L.)] – Kentucky bluegrass [*Poa pratensis* (L.)] mix appeared more detrimental to alfalfa retention over time. Contributions by white clover to overall yield and quality of swards were poor, reflective of the rapid loss of this species under high apparent competition by grasses when grown in a mixture.

The potential for mixtures to generate forage overyielding, as seen in other studies (McCloud and Mott 1953), did not occur in this study. However, the advantages to using legumes in forage mixes may not have been optimized in the Black Chernozemic soils tested here. High nutrient levels could have prevented the optimization of N fixation by legumes (Ledgard and Steele 1992). As a result, different results may have occurred had the study in Chapter 4 been conducted in less fertile Grey Luvisolic soils. Finally, the specific mechanism responsible for the rapid loss of white clover in the forage mixes examined here is unknown, and further studies into the specific mechanisms regulating the dynamics of clover in stands of Alberta are needed.

The greenhouse study conducted in Chapter 3 assessed the comparative benefits of planting different species (a legume VS a grass) for the suppression of a common pasture weed (Canada thistle – *Cirsium arvense* L.). Though preliminary, vegetation dynamics in 2 or 3-way mixtures appeared to be influenced by growing medium, highlighting the importance of abiotic factors on plant competition and presumably, associated sward composition. Changing the growth media from a highly organic substrate in trial 1 in the preliminary greenhouse study (Appendix A1) to a mineral soil mix of sand and top soil (Chapter 3), changed the competitive ability (and associated competitive rank) of the forage species tested. While neighboring forage grown in Metromix^{TM} had minimal impact on Canada thistle growth, clover proved to be a more competitive forage species when grown in mineral soil. Notably, Canada thistle growth was also reduced the most when grown in competition with itself, highlighting the fact that thistle is the most competitive of all 3 species during early establishment. While planting density had a minimal effect on overall Canada thistle growth, it did lead to a reduction in individual forage biomass.

The competitive advantage of legumes is thought to stem from their ability to obtain N through a symbiotic relationship with nitrogen fixing bacteria. Under conditions of high soil N the process of N fixation is suppressed (Ledgard and Steele 1992). Throughout the greenhouse experiment, nutrients were provided both through a slow release fertilizer and the application of liquid fertilizer. The addition of fertilizer may have reduced the competitive advantage that white clover had over other species, and provided Canada thistle with a continual source of nutrients to it's large root system. As a consistent availability of mineral nutrients is not representative of most natural systems, further research is required to determine how each plant species would compete under a more natural low nutrient (i.e. low N) system.

The results of Chapter 3 also established differences in competitiveness between bluegrass and white clover relative to Canada thistle. Clover proved to be a more competitive species than bluegrass against Canada thistle in mineral soils. Under field conditions, clover's competitive advantage comes about through the symbiotic relationship with N fixing bacteria. Studies have shown that levels of biologically fixed N tend to decline under high soil N (Ledgard and Steele 1992). In this experiment pots were fertilized regularly to prevent nutrients from being a limiting factor in growth, suggesting that the superior competitiveness of clover against thistle is reflective of factors other than N fixation. In fact, clover competitiveness may be further enhanced under low soil N environments, and is worth exploring in future investigations.

Similarly, Canada thistle produces lower shoot and root biomass when grown in a low N environment (Hamdoun 1970). Thus, a reduction in available N could force both Canada thistle and bluegrass to seek out N made available from white clover. Further studies regarding the influence of abiotic factors (i.e. soil conditions) and disturbance regimes (i.e. grazing frequency and intensity) on the competitive dynamics among grasses, legumes and Canada thistle is warranted.

Soil media in the greenhouse experiment also did not fully represent dominant soil conditions in central Alberta. The high sand content made root extraction possible, but created a micro-environment that made extrapolation to the field scale difficult. The use of a soil media more representative of the loam texture often found in Alberta may provide better insight into the competitive dynamics of forage mixes with Canada thistle.

The information obtained through this research strengthens the rationale for including legumes in a forage mix. Legumes provide high quality forage for livestock and aid in the suppression of weeds. Alfalfa has superior persistence relative to white clover and is more likely to be maintained when grown with meadow brome than smooth brome, resulting in greater retention of the benefits of the legume to improvements in forage quality. While an optimal proportion of legume to achieve overyielding was not specifically identified during the first two years of forage establishment, producers did benefit from high forage quality, which offset lower biomass levels and thereby stabilized total crude protein yield. The importance of abiotic factors in regulating forage sward dynamics cannot be overstated. Soil type played a large role in regulating the competitive hierarchy among the forage and weed plants examined here, and should be considered when choosing a forage mix in order to optimize forage establishment, forage yield and quality, as well as weed suppression.

5.1. Literature Cited

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APPENDIX A1: Supplementary Results from the Greenhouse Study (Chapter 3)

Table A1.1. Summary of LSmeans (±SE) of Canada thistle shoot density, shoot biomass and root biomass, as well as forage (white clover and bluegrass combined) biomass per pot in trial 1. CT, WC and KBG are Canada thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# CT Plants	Neighbor	CT Shoot density (stems pot^{-1})	CT Shoot biomass (g pot^{-1})	CT Root biomass (g pot^{-1})	Forage biomass (g pot^{-1})
3	1	1WC+1KBG	1.67 (0.79) a[1]	3.76 (0.81) a	1.12 (0.58) a	3.42 (0.42) a
		2 WC	1.67 (0.79) a	3.05 (0.81) a	1.38 (0.58) a	1.28 (0.42) b
		2 KBG	2.00 (0.86) a	2.46 (0.89) a	0.60 (0.58) a	2.98 (0.42) a
	2	1 WC	2.80 (0.86) a	4.94 (0.89) a	2.38 (0.58) a	2.54 (0.46) a
		1 KBG	2.20 (0.86) a	4.94 (0.89) a	2.58 (0.58) a	1.30 (0.46) a[3]
6	2	2WC+2KBG	2.60 (0.86) a	3.20 (0.89) a	1.48 (0.58) a	4.61 (0.46) b
		4 WC	1.20 (0.86) a	2.80 (0.89) a	1.24 (0.58) a	1.37 (0.46) c
		4 KBG	1.60 (0.86) a	2.66 (0.89) a	1.58 (0.58) a	7.04 (0.46) a
	4	2 WC	3.40 (0.86) a	6.08 (1.00) a	1.52 (0.58) b	2.94 (0.46) a
		2 KBG	2.80 (0.86) a	6.14 (0.89) a	3.60 (0.58) a	2.17 (0.46) a?
9	3	3WC+3KBG	1.17 (0.79) a2	3.40 (0.89) a	1.54 (0.58) a	5.62 (0.42) b
		6 WC	2.40 (0.86) a	3.26 (0.89) a	1.44 (0.58) a	0.88 (0.46) c
		6 KBG	3.20 (0.86) a	3.50 (0.89) a	1.60 (0.58) a	7.68 (0.46) a
	6	3 WC	4.00 (0.86) a	5.84 (0.89) a	3.88 (0.58) a	3.44 (0.46) a
		3 KBG	4.20 (0.86) a	6.88 (1.00) a	3.10 (0.58) a	0.81 (0.46) b

[1] Within a variable, planting density and number of thistle plants, means with different letters differ, $p<0.05$.
2 – different from 6 bluegrass at $p=0.08$.
3 – different at $p=0.06$

Table A1.2. Summary of LSmeans (±SE) of forage (white clover and bluegrass combined) biomass per pot in trials 2 and 3. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

			Trial 2	Trial 3
Planting Density Per Pot	# CT plants	Neighbour	Forage biomass (g pot^{-1})	Forage biomass (g pot^{-1})
3	1	1WC+1KBG	1.54 (0.39) a	1.32 (0.35) a
		2 WC	1.99 (0.39) a	2.20 (0.35) a
		2 KBG	0.43 (0.56) b	0.16 (0.35) b
	2	1 WC	0.69 (0.35) a	1.27 (0.35) a
		1 KBG	0.14 (0.39) b	0.04 (0.35) b
9	3	3WC+3KBG	1.85 (0.42) b	0.47 (0.35) b
		6 WC	3.31 (0.37) a	1.86 (0.35) a
		6 KBG	0.94 (0.37) b	0.30 (0.35) b
	6	3 WC	1.14 (0.42) a	0.92 (0.35) a
		3 KBG	0.55 (0.42) a	0.07 (0.35) b

[1] Within a variable, planting density and number of thistle plants, means with different letters differ, p<0.05.

Table A1.3. Summary of LSmeans (±SE) of Canada thistle shoot density, shoot biomass and root biomass, as well as forage (white clover and bluegrass combined) biomass per pot in trial 2. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# CT plants	Neighbour	CT Shoot density (stems pot^{-1})	CT Shoot biomass (g pot^{-1})	CT Root biomass (g pot^{-1})
3	1	1WC+1KBG	1.78 (1.19) a	4.87 (1.22) a[1]	3.30 (0.99) ab
		2 WC	1.38 (1.26) a	5.58 (1.29) a	2.88 (1.05) b
		2 KBG	2.60 (1.60) a	7.55 (1.63) a	5.65 (1.33) a
	2	1 WC	2.80 (1.13) a	9.01 (1.16) a	5.05 (0.94) a
		1 KBG	4.00 (1.26) a	7.26 (1.29) a	4.26 (1.12) a
9	3	3WC+3KBG	2.71 (1.35) a	5.70 (1.38) a	3.68 (1.12) a
		6 WC	0.89 (1.19) a	6.78 (1.22) a	4.53 (0.99) a
		6 KBG	1.50 (1.26) a	9.00 (1.22) a	5.57 (0.99) a
	6	3 WC	2.13 (1.26) a	7.50 (1.29) a	4.18 (1.05) a
		3 KBG	1.63 (1.26) a	9.61 (1.29) a	6.57 (1.05) a

[1] Within a variable, planting density and number of thistle plants, means with different letters differ, $p<0.05$.

Table A1.4. Summary of LSmeans (±SE) of Canada thistle shoot density, shoot biomass and root biomass, as well as forage (white clover and bluegrass combined) biomass per pot in trial 3. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# CT plants	Neighbour	CT Shoot density (stems pot^{-1})	CT Shoot biomass (g pot^{-1})	CT Root biomass (g pot^{-1})
3	1	1WC+1KBG	4.80 (1.13) a	9.81 (1.16) ab[1]	1.84 (0.72) a
		2 WC	4.70 (1.13) a	7.40 (1.16) b	1.38 (0.72) a
		2 KBG	5.80 (1.13) a	10.67 (1.16) a	2.28 (0.72) a
	2	1 WC	7.10 (1.13) a	11.71 (1.16) a	1.80 (0.72) a
		1 KBG	8.20 (1.13) a	14.01 (1.16) a	3.21 (0.72) a
9	3	3WC+3KBG	10.00 (1.13) a	17.34 (1.16) a	3.48 (0.72) a
		6 WC	11.00 (1.13) a	11.78 (1.16) b	2.89 (0.72) a
		6 KBG	10.70 (1.13) a	16.49 (1.16) a	3.47 (0.72) a
	6	3 WC	12.40 (1.13) a	19.38 (1.16) a	3.83 (0.72) a
		3 KBG	12.90 (1.13) a	18.04 (1.16) a	3.98 (0.76) a

[1] Within a variable, planting density and number of thistle plants, means with different letters differ, *p*<0.05.

Table A1.5. Summary of ANOVA F-value results from PROC MIXED analysis of Canada thistle (CT) shoot and root biomass per plant, shoots produced per root segment and the average shoot height within trial 1.

	df	CT shoot biomass per plant	CT root biomass per plant	CT shoots per root	CT average shoot height	CT root:shoot ratio
Species Treatment (Spp)	5	0.82	0.75	0.48	0.72	1.03
Density (Den)	2	6.34**1	0.24	3.22*2	1.08	1.89
Spp*Den	10	0.96	1.18	0.35	0.44	1.10

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table A1.6. Summary of ANOVA F-value results from PROC MIXED analysis of total forage (white clover and bluegrass combined) per plant grown with Canada thistle within trial 1, and trials 2 and 3.

Factor	df	Forage yield per plant grown with Canada thistle neighbors (Trial 1)	df	Forage yield per plant grown with Canada thistle neighbors (Trials 2 and 3)
Species Treatment (Spp)	4	15.49***	4	44.88***
Density (Den)	2	14.22***	1	69.44***
Trial	--	-------	1	35.58***
Spp*Den	8	1.58	4	6.09***
Trial*Spp	--	--------	4	3.92***
Trial*Den	--	--------	1	2.09
Trial*Spp*Den	--	--------	4	1.11

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively

Table. A1.7. Summary of LSmeans (±SE) of white clover shoot biomass per pot in trial 1. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# WC Plants	Neighbor	WC shoot biomass (g pot^{-1})
3	1	1CT+1KBG	1.63 (0.52) a[1]
		2 CT	1.30 (0.57) a
		2 KBG	1.63 (0.52) a
	2	1 CT	2.55 (0.52) a
		1 KBG	3.42 (0.52) a
6	2	2CT+2KBG	3.02 (0.57) a
		4 CT	2.42 (0.57) a
		4 KBG	1.87 (0.52) a
	4	2 CT	5.48 (0.57) a
		2 KBG	3.57 (0.52) b
9	3	3CT+3KBG	2.97 (0.52) a
		6 CT	2.88 (0.57) a
		6 KBG	2.18 (0.52) a
	6	3 WC	4.24 (0.57) a
		3 KBG	5.02 (0.52) a

[1] Within a variable, planting density and number of clover plants, means with different letters differ, *p*<0.05.

Table A1.8. Summary of ANOVA F-value results from PROC MIXED analysis of white clover and bluegrass shoot biomass per plant within trial 1.

	df	Clover shoot biomass per plant	Bluegrass shoot biomass per plant
Species Treatment (Spp)	5	1.26	2.59*
Density (Den)	2	7.99***	3.65*
Spp*Den	10	0.27	0.61

*, **, *** Indicate significance at *p*≤0.05, *p*≤0.01, and *p*≤0.001, respectively.

Table A1.9. Summary of LSmeans (±SE) of bluegrass shoot biomass in trial 1. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

Planting Density Per Pot	# KBG plants	Neighbor	Bluegrass shoot biomass (g pot^{-1})
3	1	1CT+1WC	1.78 (0.68) a[1]
		2 CT	2.54 (0.75) a
		2 WC	1.42 (0.68) a
	2	1 CT	2.98 (0.68) b
		1 WC	4.93 (0.68) a
6	2	2CT+2WC	3.10 (0.75) a
		4 CT	2.94 (0.75) a
		4 WC	3.47 (0.68) a
	4	2 CT	7.04 (0.75) a
		2 WC	6.87 (0.68) a
9	3	3CT+3WC	4.63 (0.68) ab
		6 CT	3.44 (0.75) b
		6 WC	6.03 (0.68) a
	6	3 CT	7.68 (0.75) a
		3 WC	7.37 (0.68) a

[1] Within a variable, planting density and number of bluegrass plants, means with different letters differ, $p<0.05$.

Table A1.10. Summary of ANOVA F-value results from PROC MIXED analysis of total forage (white clover and bluegrass combined) per pot and per plant grown in the absence of Canada thistle within trial 1.

Factor	df	Forage yield per pot	Forage yield per plant
Species Treatment (Spp)	3	55.26***	44.21***
Density (Den)	2	4.90*	36.49***
Spp*Den	6	1.58	5.27**

*, **, *** Indicate significance at $p\leq0.05$, $p\leq0.01$, and $p\leq0.0001$, respectively.

Table A1.11. Summary of ANOVA F-value results from PROC MIXED analysis of total forage (white clover and bluegrass combined) per pot and per plant grown in the absence of Canada thistle within trials 2 and 3.

Factor	df	Forage yield per pot	Forage yield per plant
Species Treatment (Spp)	3	35.09***	34.07***
Density (Den)	1	24.11***	46.58***
Trial	1	35.95***	83.71***
Spp*Den	3	1.11	6.56**
Spp*Trial	3	5.53**	3.75*
Trial*Den	1	2.26	16.84***
Trial*Spp*Den	3	2.25	3.63*

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.0001$, respectively.

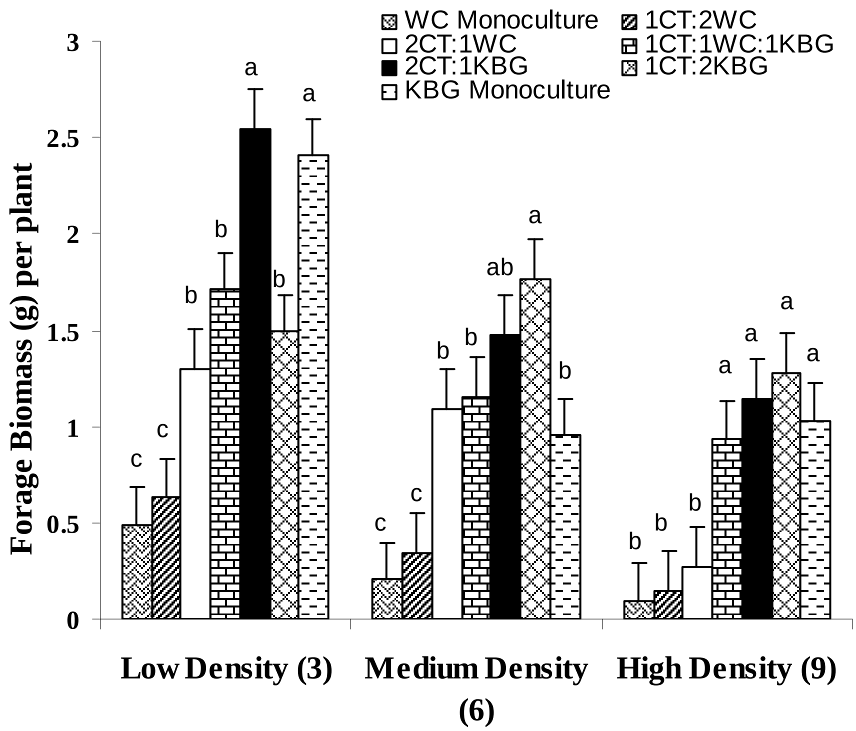

Figure A1.1. Mean (SE) total forage (white clover and bluegrass combined) biomass per plant grown with Canada thistle and in monoculture during trial 1. Within each density, means with different letters differ *p*<0.05. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

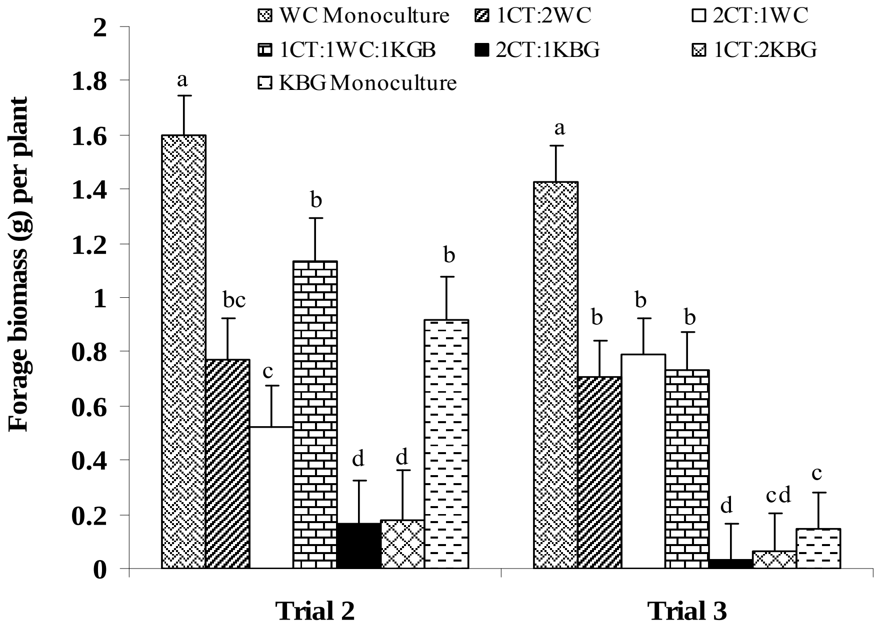

Figure A1.2. Figure 3.4. Mean (SE) total forage (white clover and bluegrass combined) biomass per plant grown with Canada thistle and in monoculture, averaged across trials 2 and 3. Within a planting density, means with different letters differ *p*<0.05. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

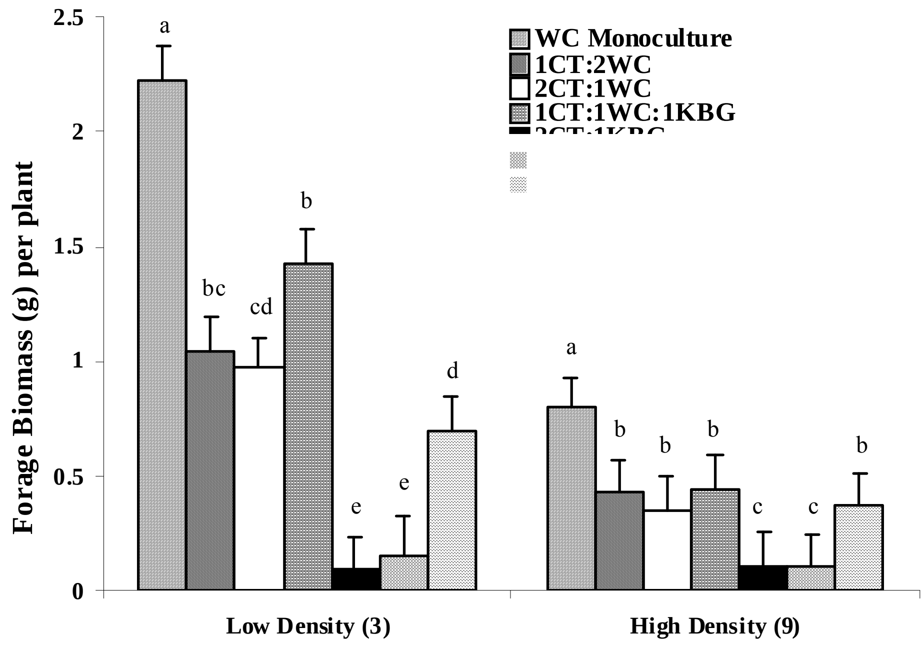

Figure A1.3. Mean (SE) total forage (white clover and bluegrass combined) biomass per plant grown with Canada thistle and averaged across trials 2 and 3. Within a planting density, means with different letters differ *p*<0.05. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

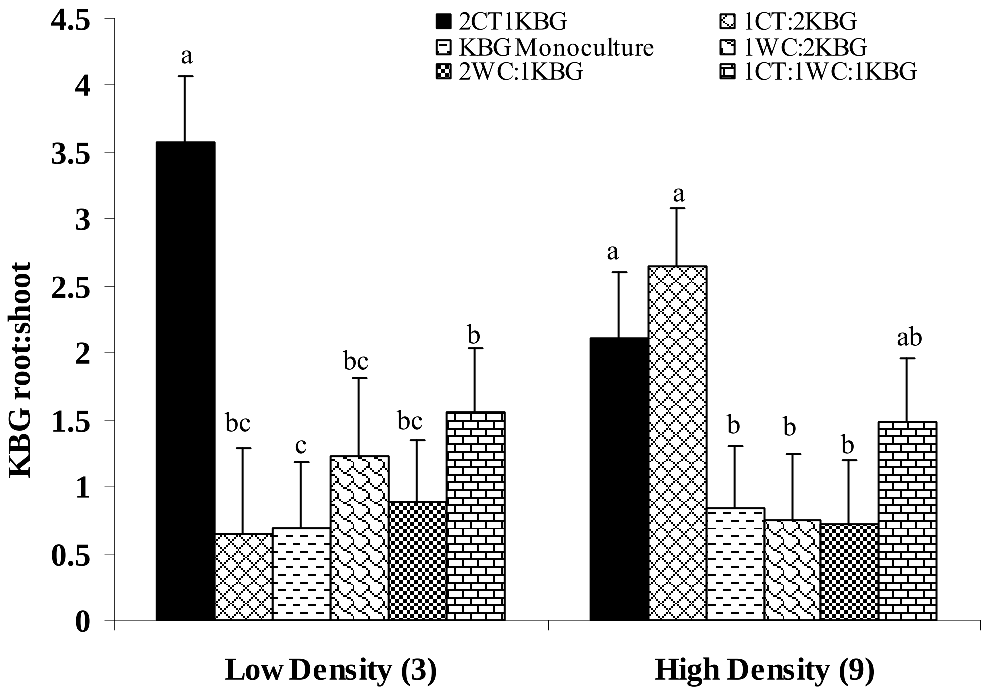

Figure A1.4. Mean (SE) root:shoot responses of bluegrass within trial 2. Within a planting density, means with different letters differ, $p<0.05$. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

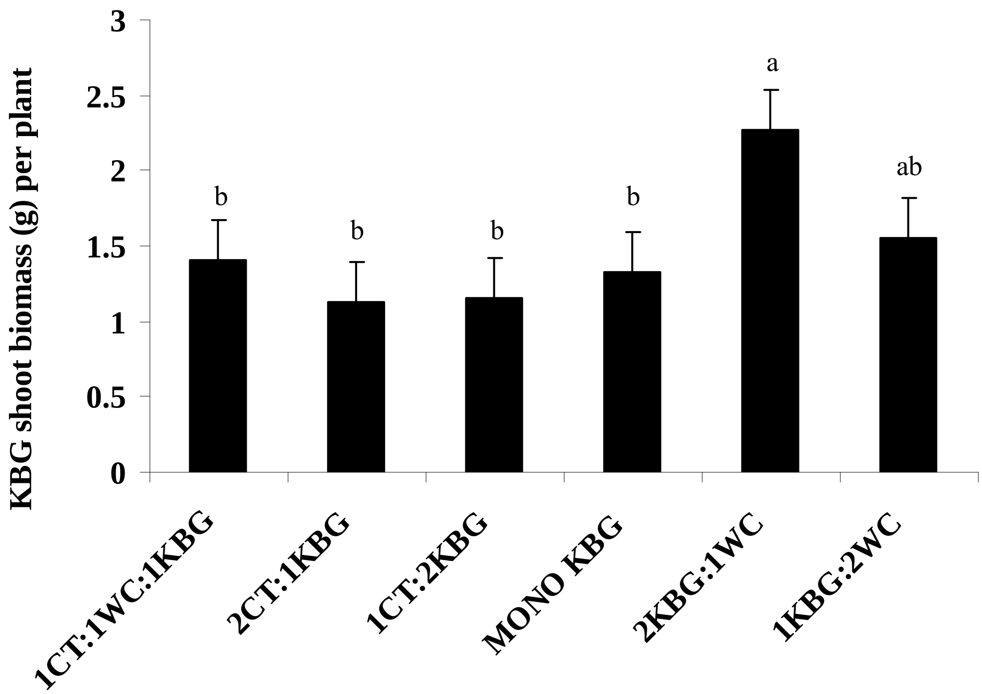

Figure A1.5. Comparison of mean (SE) bluegrass shoot biomass per plant within trial 1. Means with different letters differ *p*<0.05. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

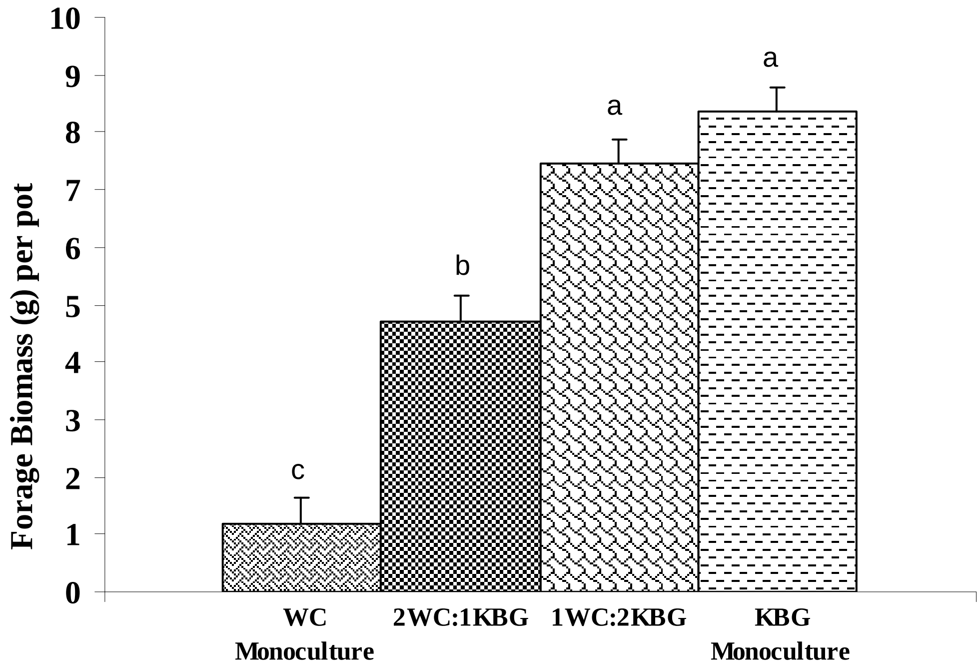

Figure A1.6. Mean (SE) total forage (white clover and bluegrass combined) per pot grown in the absence of Canada thistle within trial 1. Means with different letters differ p<0.05. WC and KBG are white clover and bluegrass, respectively.

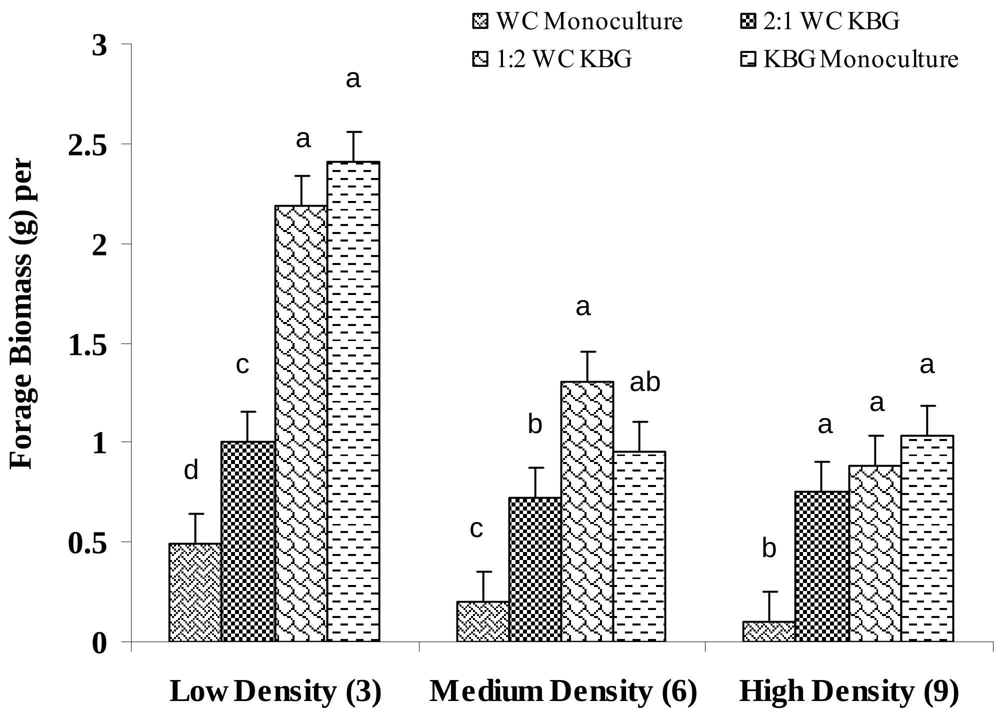

Figure A1.7. Mean (SE) total forage (white clover and bluegrass combined) per plant grown in the absence of Canada thistle at three densities: low (3 plants per pot), medium (6 plants per pot) and high (9 plants per pot) within trial 1. Within a planting density, means with different letters differ, $p<0.05$. CT, WC and KBG are thistle, white clover and bluegrass, respectively.

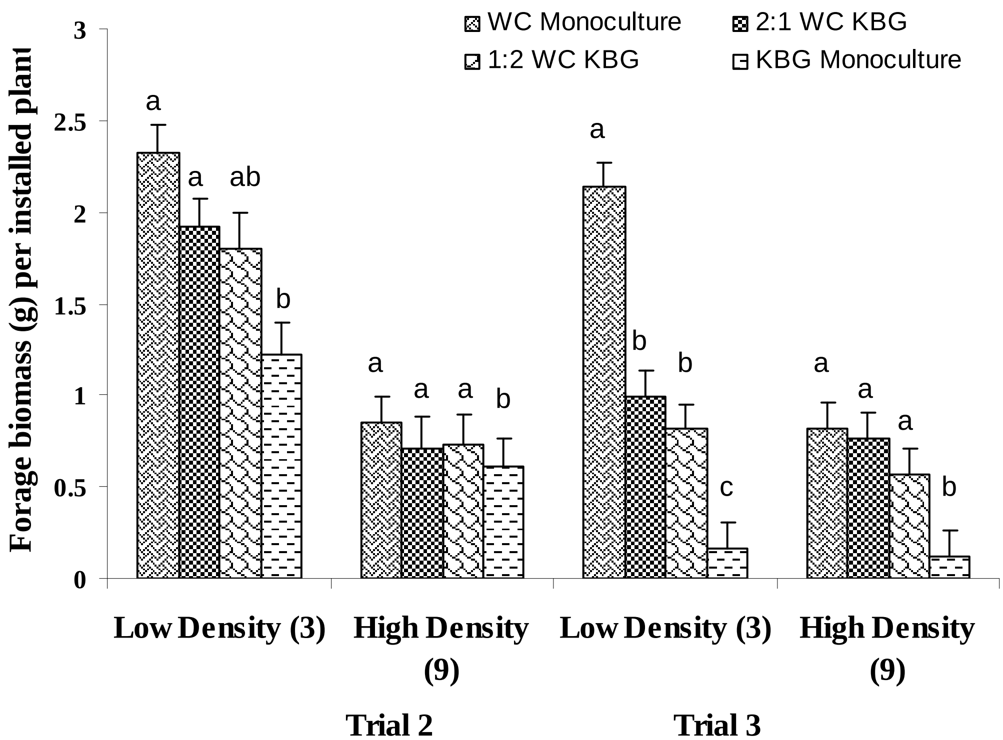

Figure A1.8. Mean (SE) forage biomass (white clover and bluegrass combined) per plant grown in the absence of Canada thistle at low (3 plants per pot) and high (9 plants per pot) density. Within a trial and planting density, means with different letters differ $p<0.05$. WC and KBG are white clover and bluegrass, respectively.

APPENDIX A2: Supplementary Results from the Cross-Seeding Study (Chapter 4)

Table A2.1. Summary of ANOVA F-value results from PROC MIXED analysis of total acid detergent soluble yield (ADSY), grass acid detergent fiber (ADF) and legume ADF at Ellerslie 2 over 2 years.

	df	Total ADSY	Grass ADF	Legume ADF
Species Mix (Spp)	3	4.07**	5.82***	50.23***
Legume (Leg)	5	11.53***	2.15	4.36**
Spp*Leg	15	1.44	1.07	0.90
Year	1	755.31***	128.49***	101.43***
Spp*Year	3	0.40	10.68***	4.78**
Leg*Year	5	6.22***	1.24	2.68*
Spp*Leg*Year	15	1.66	0.90	1.41

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table A2.2. Summary of ANOVA F-value results from PROC MIXED analysis of total acid detergent soluble yield (ADSY), grass acid detergent fiber (ADF) and legume ADF at W240 over 2 years.

	df	Total ADSY	Grass ADF	Legume ADF
Species Mix (Spp)	3	0.48	2.94*	39.21***
Legume (Leg)	5	26.26***	1.20	8.75***
Spp*Leg	15	2.04*	0.56	1.04
Year	1	352.51***	125.93***	16.79***
Spp*Year	3	1.12	2.19	4.69**
Leg*Year	5	4.57***	2.12	5.90***
Spp*Leg*Year	15	0.76	0.94	0.24

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table A2.3. Summary of ANOVA F-value results from PROC MIXED analysis of forage biomass, grass biomass, legume biomass, total crude protein yield (CPY), grass CPY, legume CPY, total acid detergent soluble yield (ADSY), grass acid detergent fiber (ADF) and legume ADF at Ellerslie 1 for 3 years.

	df	Forage Biomass	Grass Biomass	Legume Biomass	Total CPY	Grass CPY	Legume CPY	Total ADSY	Grass ADF	Legume ADF
Species Mix (Spp)	3	3.80**	8.05***	25.80***	4.38**	10.05***	17.32***	1.62	23.21***	65.60***
Legume (Leg)	5	5.60***	24.97***	36.74***	8.85***	18.77***	40.63***	5.27***	0.65	5.55***
Spp*Leg	15	1.15	0.4170	0.7595	1.93*	1.62	0.87	1.12	0.90	1.01
Year	2	539.56***	319.13***	106.77***	173.46***	114.99***	74.20***	370.56***	320.04***	217.97***
Spp*Year	6	5.94***	5.12***	14.98***	8.86***	1.59	14.47***	5.15***	6.52***	10.60***
Leg*Year	10	4.16***	9.79***	2.56**	0.24	5.11***	1.67	3.31***	0.46	2.52**
Spp*Leg*Year	30	0.88	1.07	0.58	1.13	0.93	0.69	0.68	0.55	1.50

*, **, *** Indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

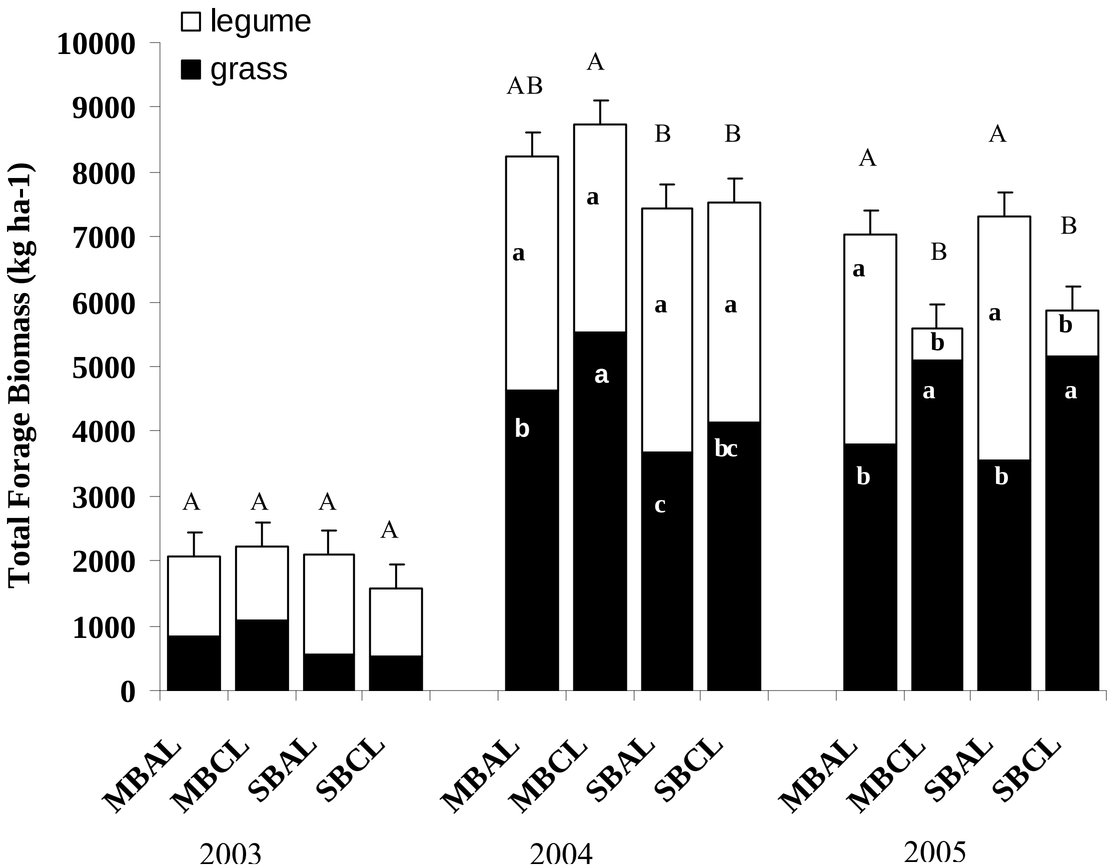

Figure A2.1. Comparison of total forage biomass among species mixtures and years at site E1. Within a year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

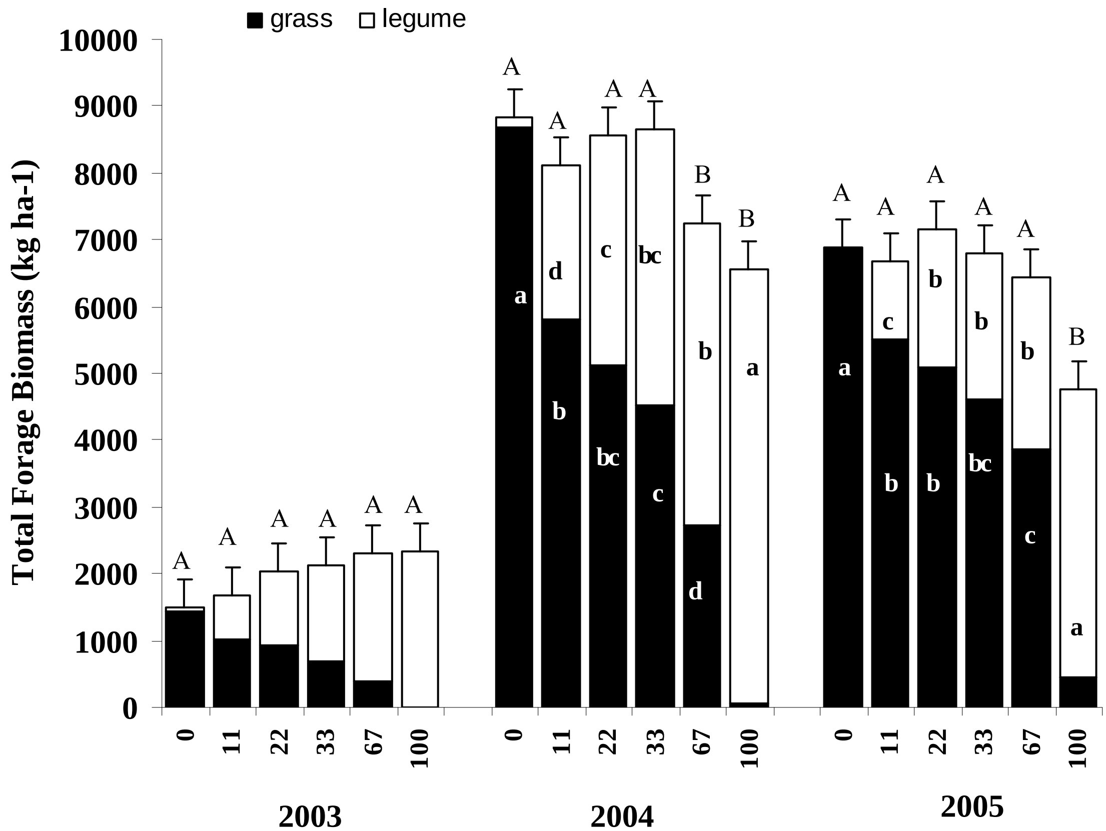

Figure A2.2. Comparison of total forage biomass among legume proportions and years at site E1. Within a year and vegetation component, means with different letters differ, *p*<0.05. Upper case letters compare grand means.

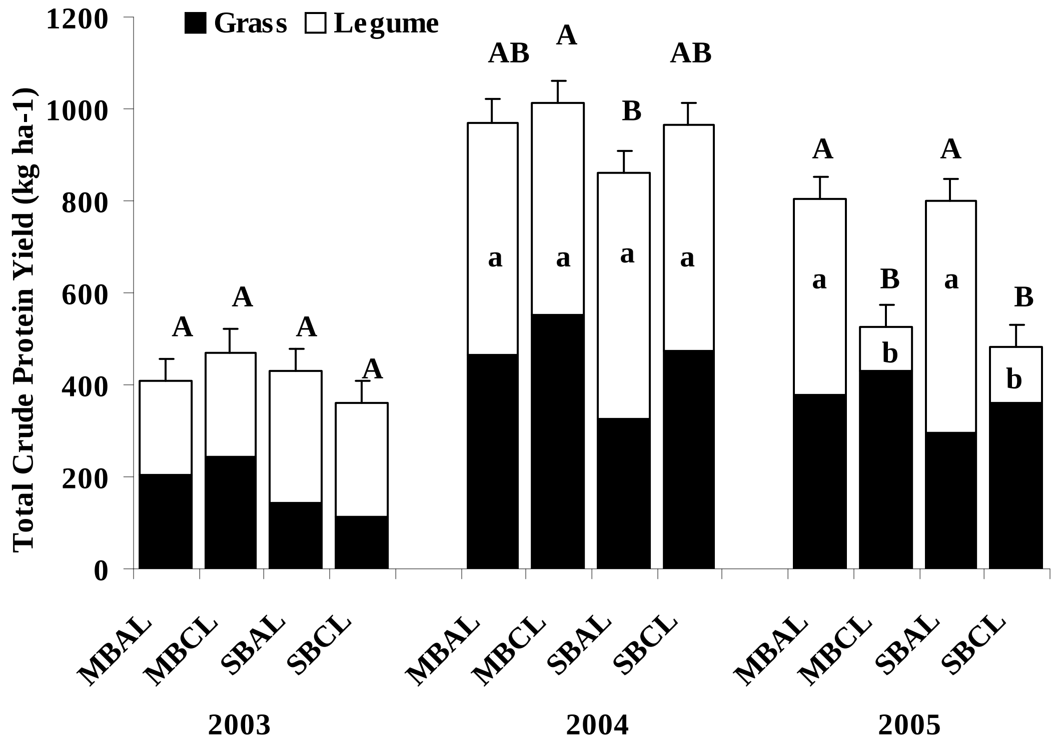

Figure A2.3. Comparison of total crude protein yield (CPY) among species mixtures and years at site E1. Within a year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

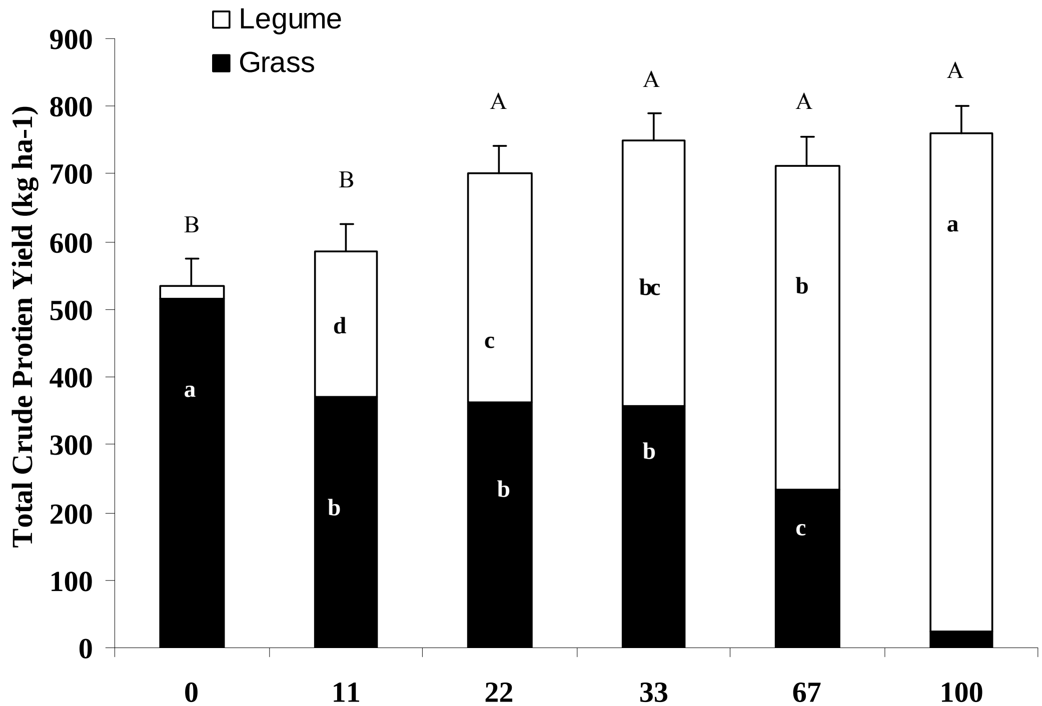

Figure A2.4. Comparison of total crude protein yield (CPY) among plots with varying legume proportions averaged across three years at the E1 site. Within a vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means.

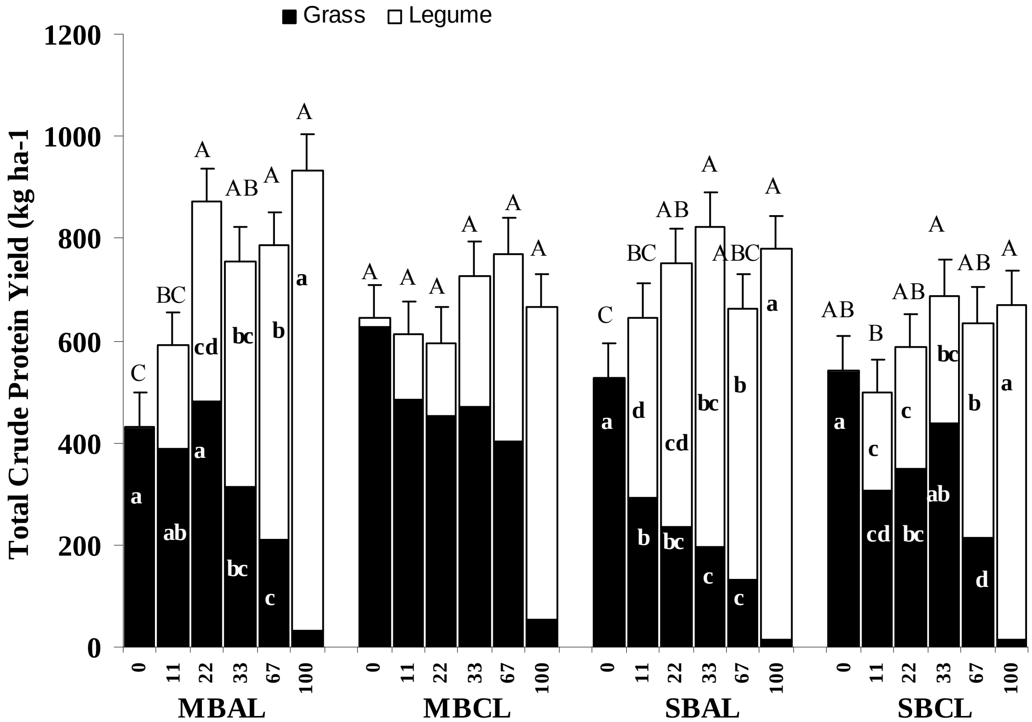

Figure A2.5. Comparison of total crude protein yield (CPY) among legume proportions and species mixtures averaged across three years at site E1. Within a species mixture and vegetation component, means with different letters differ, *p*<0.05. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

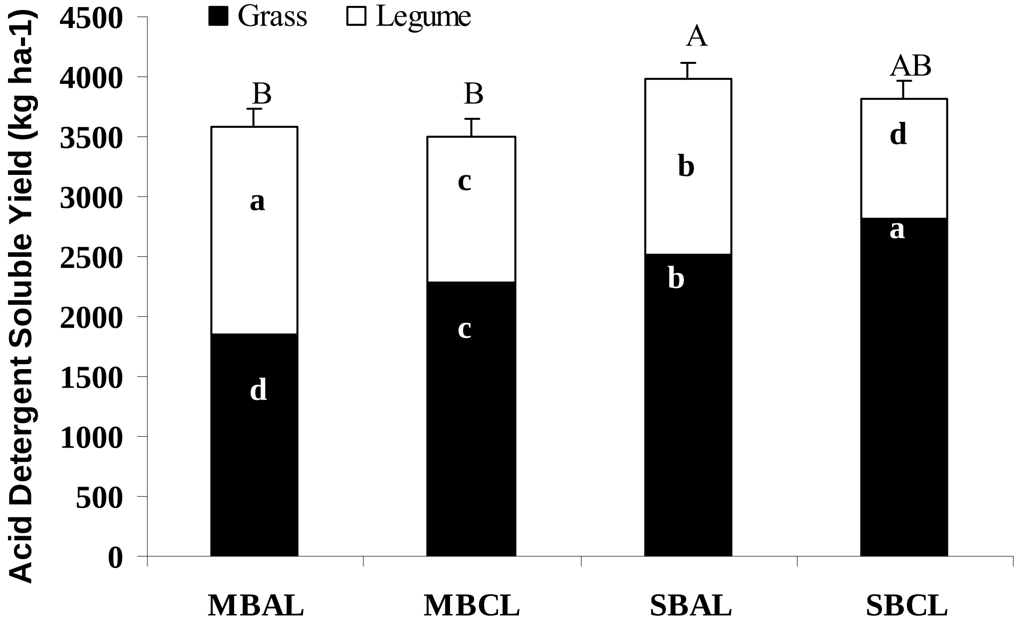

Figure A2.6. Comparison of total acid detergent soluble yield (ADSY) among species mixtures averaged across two years at the E2 site. Within a vegetation component, means with different letters, differ p<0.05. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

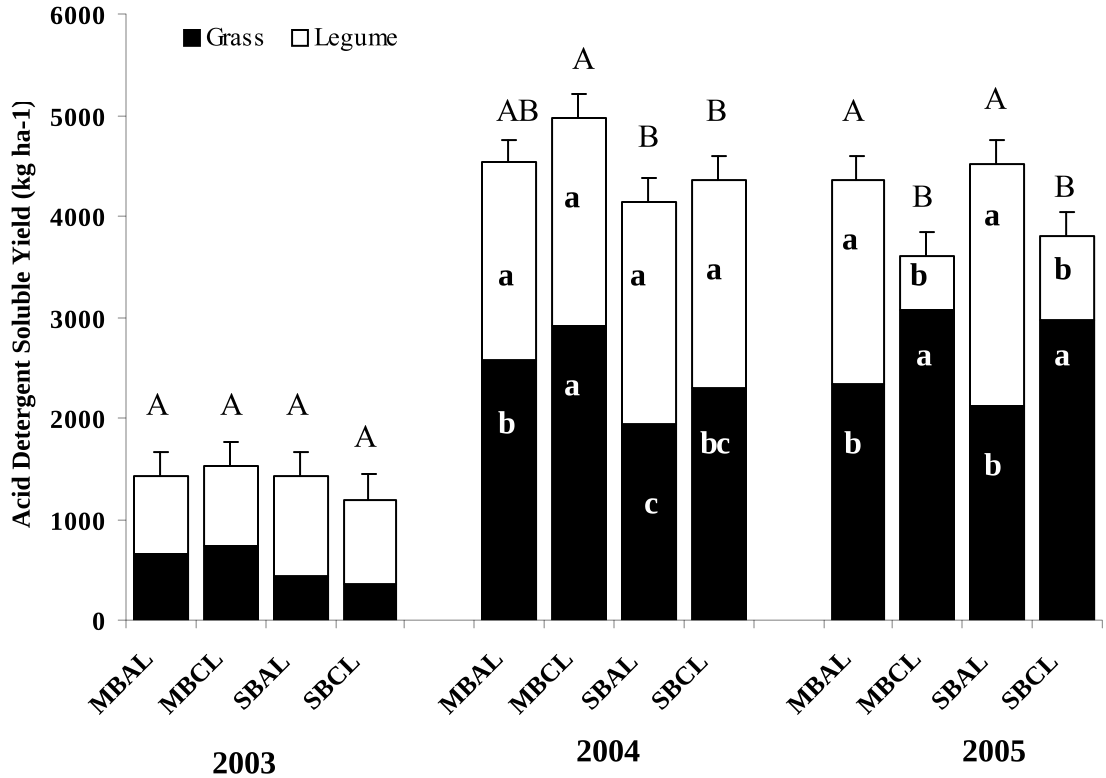

Figure A2.7. Comparison of total acid detergent soluble yield (ADSY) among species mixtures and years at site E1. Within a year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.

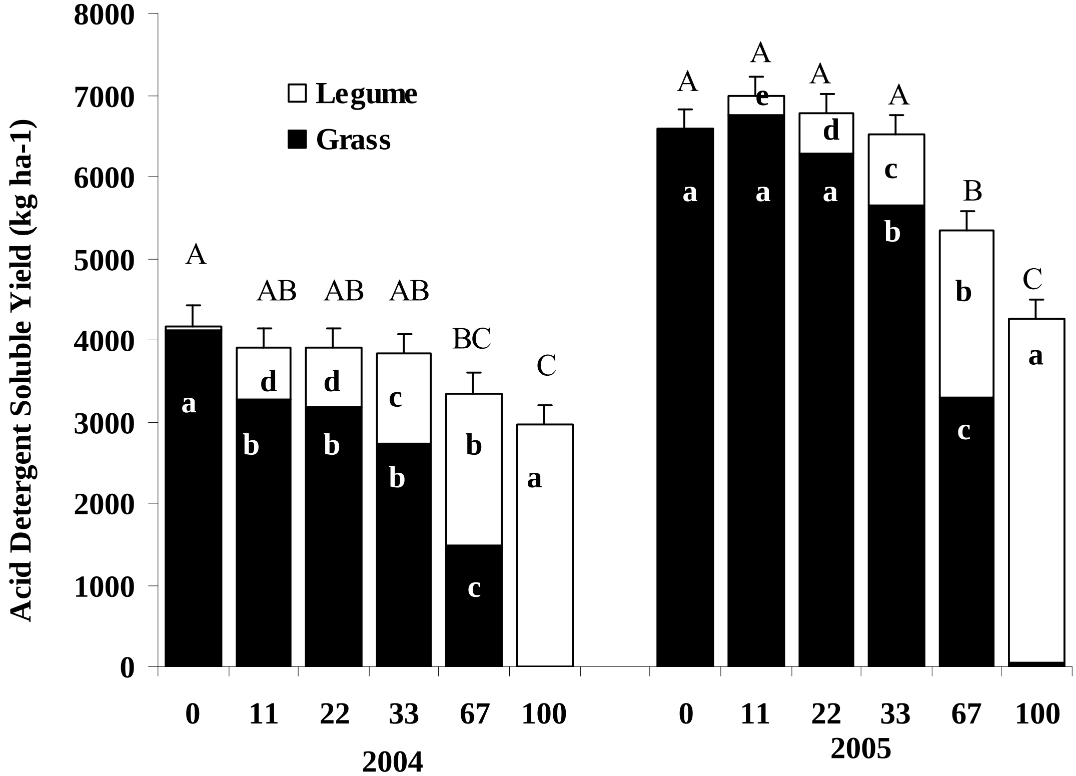

Figure A2.8. Comparison of total acid detergent soluble yield (ADSY) among legume proportions and years at the W240 site. Within a year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means.

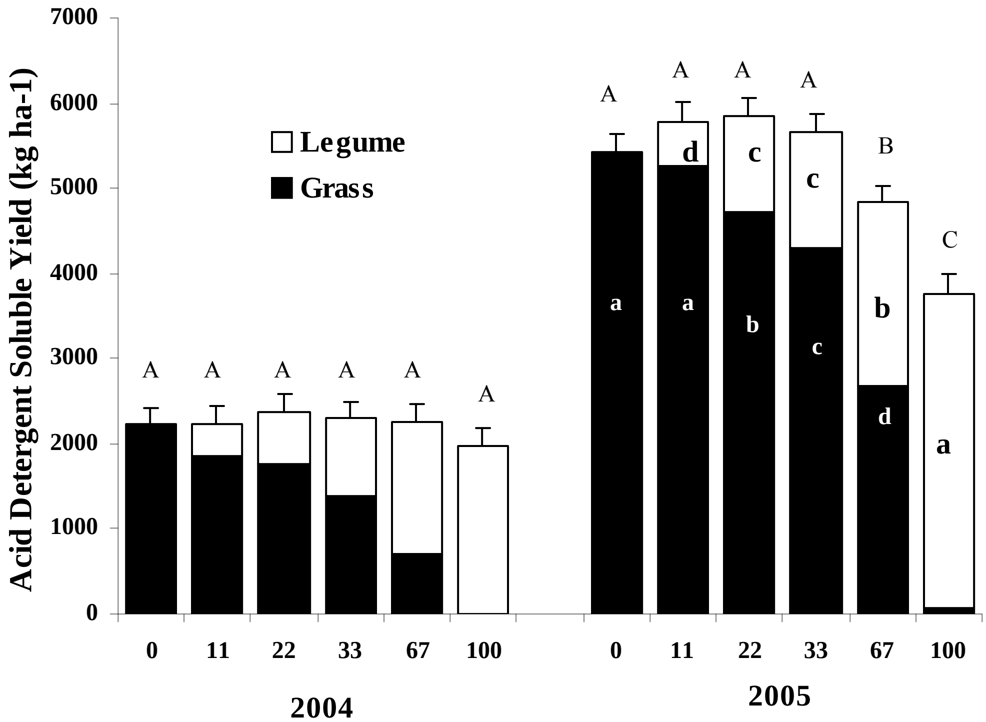

Figure A2.9. Comparison of total acid detergent soluble yield (ADSY) among legume proportions and years at the E2 site. Within a year and vegetation component, means with different letters, differ $p<0.05$. Upper case letters compare grand means.

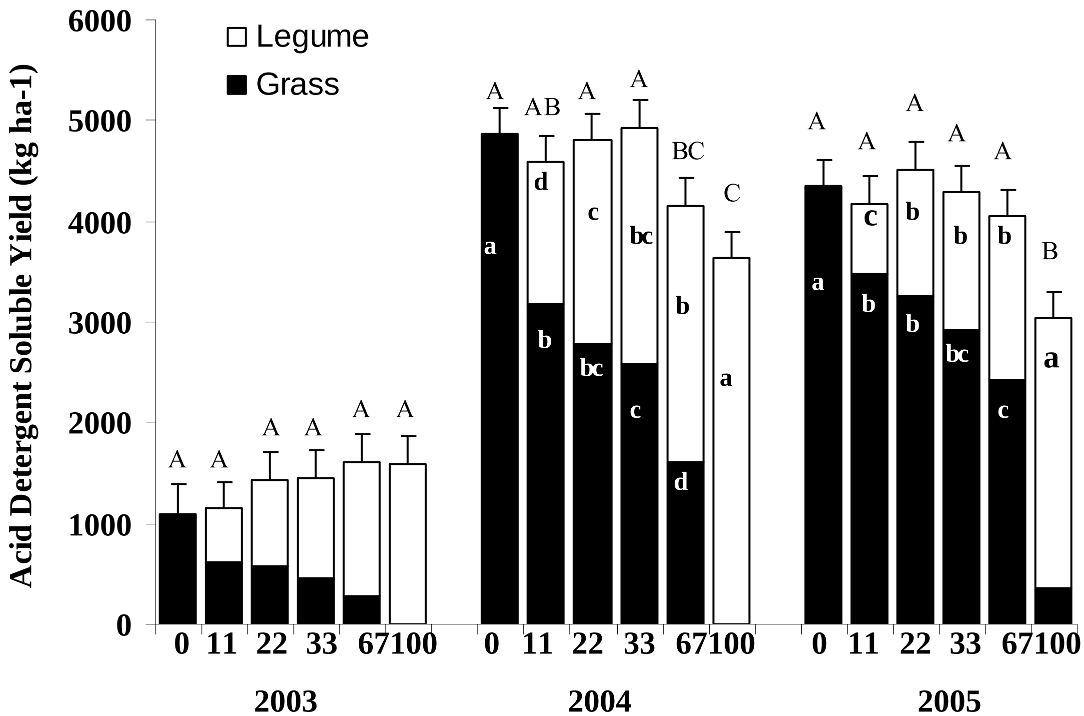

Figure A2.10. Comparison of acid detergent soluble yield (ADSY) among legume proportions and years at the E1 site. Within a year and vegetation component, means with different letters differ, $p<0.05$. Upper case letters compare grand means.

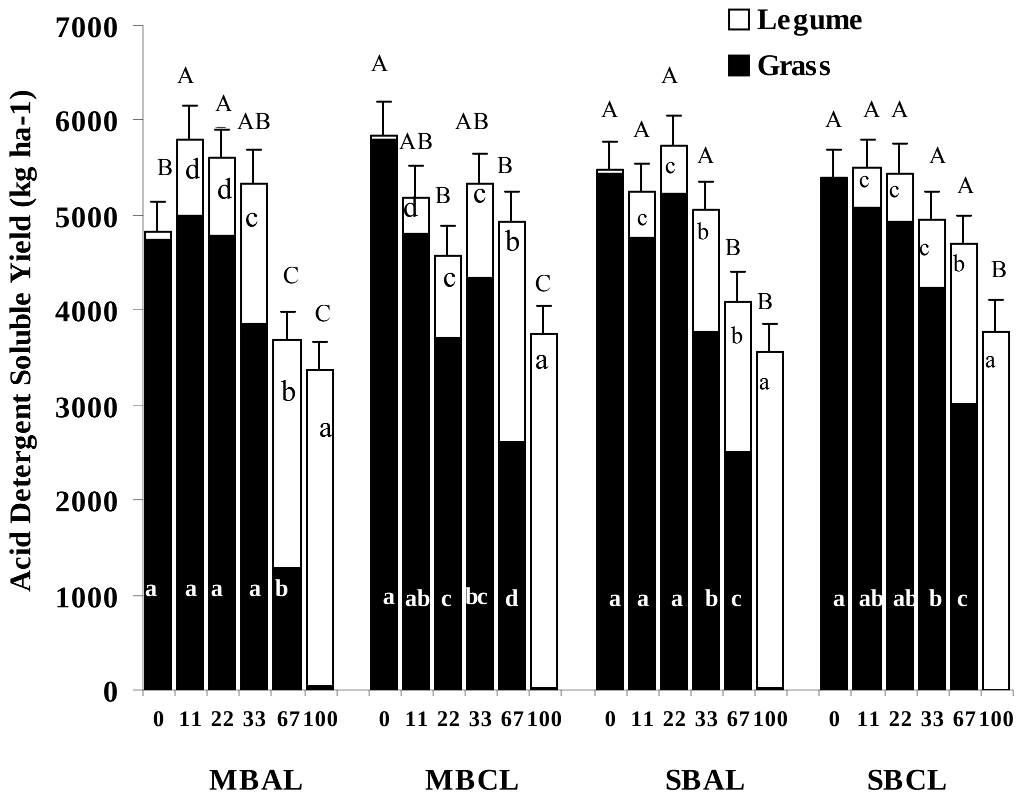

Figure A2.11. Comparison of acid detergent soluble yield (ADSY) among species mixtures and legume proportion averaged across two years at the W240 site. Within a species mixture and vegetation component, means with different letters differ, *p*<0.05. Upper case letters compare grand means. MBAL = meadow brome-alfalfa; MBCL = meadow brome-clover; SBAL = smooth brome-alfalfa; SBCL = smooth brome-clover.