

University of Alberta

Forage Responses to Herbicide Weed Control in Grass-Legume Swards

by

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*“There’s nothing like biting off more than you can chew, and
then chewing anyways.”
- Mark Burnett*

“Those who stand for nothing fall for anything” – Peter Marshall

Abstract

Two field studies assessed sward dynamics to legume removal with and without Canada thistle and other broadleaf plants (i.e. forbs). When grown in mixtures with grass, alfalfa had a more consistent negative yield response to legume removal compared to clover swards. Within established pastures, total forage (i.e. legume and grass) had little association with Canada thistle, but was instead associated with perennial forbs such as dandelion. Grass responses (biomass and protein yield) in established swards were unable to compensate for legume removal up to 2 years after spraying. Relative yield ratios were used to identify weed and legume thresholds, and indicated the removal of legume and Canada thistle did not always negatively impact forage production. Instead, select positive yield responses were observed depending on the initial composition, growing conditions and abundance of weed and legume.

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List of Abbreviations

ALF – Alfalfa

CLR – White clover/ alsike clover mixture

CP - Crude protein

CPY – Crude protein yield

E2 – Ellerslie research station study site

LI – Lake Isle

MB – Meadow brome grass

MBALF – Meadow brome grass and alfalfa seeded mixture

MBCLR – Meadow brome grass and white clover/ alsike clover seeded mixture

PCF – Parkland conservation farm

SB – Smooth brome grass

SBALF – Smooth brome grass and alfalfa seeded mixture

SBCLR – Smooth brome grass and white clover/ alsike clover seeded mixture

W240 – Edmonton research station study site

CHAPTER 1.

Why Understand the Impact of Legume Removal in Mixed Forage Swards?

1.1 Background

Invasive weeds are present worldwide and can have a large impact both ecologically and economically in agronomic systems (DiTomaso 2000, Wilson and Kachman 1999). In Canada the annual estimated cost of invasive plants to the agricultural community are estimated at \$2.2 billion, with \$0.6 billion directly attributed to weed control and damage in pastures (CFIA Summary Report 2008). In addition to the direct costs related to forage yield loss and added control measures in range and pasture lands, invasive plants reduce weight gains of animals (Dewhurst *et al.* 2003).

Canada thistle (*Cirsium arvense* L. Scop.) is one of the most prevalent and economically damaging invasive species in Canada and the US (CFIA Summary Report 2008, Wilson and Kachman 1999, Behrens and Elakkad 1981). Most of the US (i.e. 42 states) and 6 of the 10 Canadian provinces have listed Canada thistle (CT) as a noxious weed (USDA National Plant Data Centre 2009). This regulatory status, in turn, requires landowners to control the abundance and spread of CT where present. In Alberta alone, 44 of 47 counties have reported significant infestations of CT in agricultural land (Agriculture and Rural Development 2009). The presence of CT is known to decrease yields in canola, barley and wheat (O'Sullivan *et al.* 1982, O'Sullivan *et al.* 1985), and has also been recognized as a major ecological and economic concern in perennial crops in Australia (Hartley and James 1979), Europe (Haagar *et al.* 1986, Holm *et al.* 1977), the US (Schreiber 1967) and Canada (Goodwin *et al.* 1986, Moyer *et al.* 1991, Grekul and Bork 2004).

Weed control options available for CT include mowing (Beck and Sebastian 2000, Schreiber 1967), tillage (Lukashuk *et al.* 2008), biological control (Pipers and Andres 1995), grazing (De Bruijn and Bork 2006), burning (Emery and Gross 2005, Tranicek *et al.* 2005), and herbicides (Enloe *et al.* 2007; Bork *et al.* 2007). Other methods that have been investigated to suppress CT in pastures include increasing grass competition from neighboring grasses, either through the use of grass over-seeding (Wilson and Kachman 1999) or the use of annual fertilization, with or without direct weed control (Grekul and Bork 2007). However the most commonly used control method in annual cropping systems and pasture areas is through the use of herbicides, either non-selective or selective (Bixler 1991, DiTomaso 2000).

The use of herbicides for control of CT within pastures containing mixed legume-grass swards is often accompanied by the loss of beneficial legumes. Legumes provide many benefits within pastures relative to grass monocultures, including increased overall pasture productivity (Sleugh *et al.* 2000). Legume-grass mixtures are reported to yield more biomass than any individual species in the mixture grown in monoculture, a phenomenon known as ‘over-yielding’ (Gokkus *et al.* 1999, Polser *et al.* 1993). Increased biodiversity of swards may explain over-yielding through the more efficient capture and use of plant resources (i.e. soil, water, light etc.), while simultaneously helping to resist the invasion of weeds (Tracy and Sanderson 2003). The most desired benefit of the inclusion of legumes may be their ability to fix atmospheric nitrogen, and the potential transfer of excess nitrogen to neighboring non-leguminous plants such as grasses (Ta and Faris 1987, Burity *et al.* 1989, Heichel and Henjum 1991, Dubach and Russelle 1994, Walley *et al.* 1996). Despite these benefits, over-yielding has not been

consistently demonstrated in forage swards containing legume (Gabruck 2010, Sleugh *et al.* 2000), and therefore does not guarantee increases in forage yield, suggesting that the factors contributing to this relationship are not yet fully understood. Despite this, the presence of a legume is known to provide other benefits such as improved forage nutritional value (i.e. crude protein), in turn leading to greater individual animal weight gains and associated overall herd production (Bertisson and Murphy 2003).

Legumes such as alfalfa, clover and birdsfoot trefoil are able to fix atmospheric nitrogen through the association of their roots with *Rhizobium* bacteria (Walley *et al.* 1996 Burity *et al.* 1989). Alfalfa, white clover and alsike clover can provide up to 258 (Burity *et al.* 1989), 545 (Elgersma and Hassenk 1997) and 86.2 kg ha⁻¹ (Fairey 1986) respectively, of nitrogen (N) annually in stands two years or older. Nitrogen accumulated by legumes through this process can be subsequently deposited into the soil during nodule and/or root decomposition, at which point these nutrients become available for uptake by neighboring grasses (Brophy *et al.* 1987, Ta and Farris 1987, Burity *et al.* 1989, Ledgard 1991, Heichel and Henjum 1991). Burity *et al.* (1989) reported up to 27 kg ha⁻¹ of N was transferred from alfalfa to smooth brome grass (*Bromus inermis* Leyss.) in two year old swards. The benefit of this relationship is a reduced need for the application of industrial-derived external sources of N that can be easily lost through leaching, volatilization and/or immobilization (Saikia and Jain 2007). Most importantly, the availability of fixed N reduces the cost of production for producers (Haby *et al.* 2006).

The use and overall importance of legumes as forage sources have been widely reported in the literature, and their numerous benefits have made them a highly desirable component of many pastures and hay lands (Haby *et al.* 2006, Sleugh *et al.* 2000, Burity

et al. 1989). Consequently, producers are often hesitant to control undesirable broadleaf weed infestations occurring within mixed forage swards due to the drawbacks associated with legume removal. To date, few studies have evaluated the overall net effect of spraying mixed forage swards, including the compensatory response of grasses within swards following the removal of N rich legumes. The intent of this research was to investigate the overall net forage responses associated with spraying mixed forage swards containing legume. This includes an evaluation of weed and/or legume abundance thresholds that producers may use as decision making tools in order to identify the optimal conditions under which weed control and/or legume removal are justified.

1.2 Research Objectives

This thesis reports on research investigating two complementary field studies. Both studies evaluate overall sward response to legume removal following the application of a broadleaf herbicide commonly used for the control of a noxious weed (i.e. Canada thistle). While the first study was conducted in the absence of Canada thistle within mixed legume-grass swards varying in forage species as well as in initial legume abundance, the second study evaluated forage species composition and yield dynamics within established pastures containing variable amounts of legume, grass and the noxious weed Canada thistle. Both experiments examined forage dynamics following herbicide application. Chapter 2 contains a literature review of information relevant to the research topic.

The specific objectives of the first experiment reported in Chapter 3 were to (1) quantify the overall net changes in forage responses to spraying with respect to forage

yield and nutritional quality, (2) assess the presence and amount of grass compensation in response to varying amounts of legume removal, and (3) determine how the first two objectives may be altered by the presence of different forage species within the initial legume-grass mixtures.

The second experiment reported in Chapter 4 had similar objectives to the first study, but sward dynamics were assessed before and after spraying, and included more complex relationships given the additional inclusion of the common noxious weed, Canada thistle. Specific objectives of this experiment were to (1) evaluate the competitive and facilitative interactions among grasses, legumes and Canada thistle, (2) evaluate the impact of CT and legume removal from the sward on subsequent forage dynamics, and (3) quantify changes in relative forage yield and quality arising from broadleaf removal.

Overall, the intent of this investigation was to assist in determining the overall effect of spraying with respect to aggregate responses in forage yield and quality. Chapter 5 contains a synthesis of the study findings, including the management implications and future research needs arising from these experiments.

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CHAPTER 2. Literature Review

2.1 Introduction

The goal of most livestock producers is to establish and maintain a healthy and vigorous plant community that is both ecologically and economically sustainable. The invasion of noxious weeds such as Canada thistle is often due to stress on ecosystems from climatic conditions and/or poor management decisions and as a result, can reduce the overall productivity of stands, decrease biodiversity, and lead to reductions in livestock profitability (Masters and Sheley 2001, DiTomaso 2000). Once established in a plant community, the eradication of weeds like Canada thistle is unlikely, and instead methods and strategies must be implemented to control the spread or re-invasion of these weeds (DiTomaso 2000). Successful long-term management strategies require the integration of multiple control methods paired with consistent monitoring in the field to reduce the impact of noxious and invasive weeds.

2.2 Canada thistle

Canada thistle (CT) is a widespread aggressive perennial weed that can invade both arable and non-arable land. CT is native to southeastern Europe and the eastern Mediterranean (Moore 1975) and is now found in large areas across Asia, Europe, Africa, Australia, North and South America. Introduction to North America was most likely due to the movement of contaminated seed in the 1600's from Europe. CT is also known as California thistle, creeping thistle or field thistle (Holm *et al.* 1977) and is commonly found in urban yards, roadsides, right-of-ways, forestlands, croplands, perennial pastures

and rangelands (Sheley and Petroff 1999). It is well adapted to a wide range of soil types (Reed and Hughes 1970) and habitats, but is usually found in open areas with moderate moisture conditions in temperate areas (Moore 1975). Four distinct ecotypes or subspecies have been identified including var. *vestilum* (Wimm and Grab.), var. *integrifolium* (Wimm and Grab.), var. *arvense* (Wimm and Grab.) and var. *horridum* (Wimm and Grab.), which are most easily identified by differences in plant morphology (Moore and Frankton 1974).

2.2.1 Biology

CT is an erect perennial dicot with an extensive creeping root system that can grow up to 4 ft (120cm) tall (Moore 1975). Sessile and clasping deeply lobed oblong leaves with spiny toothed margins are attached alternately to grooved hairy stems. Plants as young as 4-5 weeks (2 leaf stage) will begin to develop lateral roots that give rise to adventitious root buds from which new plants form asexually (Friesen 1968).

Canada thistle is an imperfectly dioecious plant that can reproduce both sexually and asexually (Heimann and Cussans 1996). Female plants produce seed heads that can be pink, purple or white and are 1-3 cm in diameter. Each new shoot can produce up to 1-5 female heads per season with each head producing an average of 75 seeds per head (Moore 1975). The seeds or achenes of CT have a fluffy pappus and are dispersed by wind, water, animals or humans. Seeds can survive up to 22 years in the soil if buried to 20 cm depth (Madsen 1962, Sheley and Petroff 1999). However optimal germination depth is 1-1.5 cm. Long seed dormancy and longevity in the soil significantly contributes to the long-term impact and magnitude of CT infestations. Dioecious sexual

reproduction is largely dependent on pollinators. Instead the main form of reproduction is done asexually through adventitious roots (Sheley and Petroff 1999, Hamdoun 1972), which are known to spread up to 12 m annually (Amor and Harris 1975). Roots of CT can penetrate up to 1.8 m deep although more than half are found in the top 40 cm of soil (Nadeau and Vanden Born 1989). When disturbed, the roots of CT have the ability to form new plants from root sections as short as 1 cm long (Hamdoun 1972), making control of the weed difficult. Patches can become worse if disturbed and broken up mechanically, leading to new dense populations.

2.2.2 Economic Impacts

The presence of CT can increase land management costs due to producers having to comply with provincial or state laws and associated weed legislation. CT is known to reduce yields (O'Sullivan *et al.* 1982, O'Sullivan *et al.* 1985), forage availability (Hagggar *et al.* 1986), livestock production (Reece and Wilson 1983), and overall species diversity (Stachlon and Zimblahl 1980) in grasslands. Reductions in annual crop yields of up to 26, 34 and 51% in Alberta were reported in canola, barley and spring wheat, respectively (O'Sullivan *et al.* 1982, McLennan *et al.* 1991). In seed alfalfa, Moyer *et al.* (1991) reported yield losses up to 48%. Grekul and Bork (2004) found in perennial pastures losses of up to 2 kg of forage biomass for each 1 kg of additional CT biomass present. In addition to the potential loss of forage through the displacement of desirable grass and forbs by CT, older and coarser plants are less palatable to cattle and can deter grazing (Scheiber 1967, Hartley and James 1979). More recent studies have reported that CT

may be controlled in pastures using high intensity rotational cattle grazing systems (De Bruijn and Bork 2005).

2.2.3 Control Methods of Canada Thistle

The control of CT can be difficult in pastures due to the complex ecological relationships present within perennial systems, and the effect that key management decisions can have on long-term sward productivity. The success of CT control is highly dependent on root carbohydrate reserves remaining low, as this can lead to plant death (McAllister and Haderlie 1985). The abundance of root carbohydrates fluctuates through the growing season but is lowest during spring and fall (Wilson *et al.* 2006). There are many options available to producers for controlling CT, including mechanical, cultural, biological and chemical, together with increasing crop competition (Donald 1990). Alone each of these options may provide some level of control or suppression of CT, but successful long-term control consists of an integrated management technique that involves two or more management methods (DiTomaso 2000).

Chemical control is one of the most widely used control methods for CT on range and pasture areas. Herbicide applications should ideally be timed to the late rosette, bolting or bud stage in the spring, or when new shoot regrowth and new rosettes appear in the fall. The use of herbicides within mixed pastures often carries the undesirable cost of removing beneficial species such as legumes (i.e. clovers, alfalfa etc.) in addition to the target weed(s). Herbicides such as MCPB, benzone, imazamox or imazethpyr can be applied at lower rates and provide between 27 and 80% control, with limited damage to alfalfa pastures (Meshah and Miller 2005). However, this level of control may not be

acceptable for many producers looking for long-term weed management. Current herbicides recommended for use in range and pasture areas include picloram, clopyralid, dicamba, 2,4-D amine or ester, triclopyr, chlorosulfurin, metsulfuron, and aminopyralid (Dewey *et al.* 2006, Enloe *et al.* 2007).

Previous studies have demonstrated that the best control of CT in range and pasture is through the use of picloram and clopyralid. Picloram + 2,4-D amine (0.28 +1.12 kg ha⁻¹) applied in the fall over two years resulted in the elimination of CT the following year (Beck and Sebastian 2000). Alley and Humberg (1977) found good control of CT with clopyralid (0.42 kg ha⁻¹) applied in the bud stage with up to 90% control one year after treatment. In a recent study by Enloe *et al.* (2007), the best control of CT was with the use of picloram, clopyralid and aminopyralid. Spring and fall applications with aminopyralid (0.11 kg ai ha⁻¹) and picloram (0.42 kg ai ha⁻¹) resulted in 95 and 97% control, respectively, one year after treatment. Due to its reduced mobility in soil relative to picloram, aminopyralid has a reduced risk status that allows it to be used in a wider range of areas within fields, including riparian areas where CT infestations can be particularly problematic (Jachetta *et al.* 2005, Enloe *et al.* 2007).

Mechanical methods such as tillage and mowing are commonly used to control CT in agricultural systems. Tillage in pasture systems is generally used to control small densely infested areas of a field, or when re-establishing a new pasture sward. Repeated tillage within a growing season increases the level of control (Seely 1952, Donald 1990), but is accompanied by the additional risk of distributing root fragments to new locations where new populations may establish (Nadeau and Vanden Born 1989). The use of repeated mowing can also decrease CT populations by depleting root carbohydrate

reserves and reducing seed production within fields (Schrieber 1967, Beck and Sebastian 2000). Hodgson (1968) reported that mowing pastures with alfalfa twice a year reduced CT populations by 86% after one year. Furthermore, Beck and Sebastian (1993) concluded that mowing combined with chemical control could double the effectiveness of CT control compared to mowing alone.

The use of biological agents such as pathogens and insects on CT have been used with limited success and require several treatments (Bourbot *et al.* 2006), but provide an additional option for use in areas where the use of commercial equipment is difficult to undertake. Larvae of the painted lady butterfly (*Cynthia cardui* L.) is one of the few known native insects that will defoliate CT (Moore 1975). However, most of the other insects used to control CT have been introduced from other areas, and carry concerns over their impact on native thistle species and other plants with the same ecosystems. The Canada thistle stem weevil (*Ceutorhynchus litura* L. Scop), Canada thistle bud weevil (*Larinus planus* F.) and the thistle gall fly (*Urophora cardui* L.) are the most common insects that attack the vegetative and reproductive parts of CT, thereby increasing the susceptibility of pathogens to enter and kill plants (Rees 1990, Rees *et al.* 1996). Two pathogen rust fungi *Sclerotinia sclerotiorum* (Lib). de Bary and *Puccinia punctiformis* (F. Strauss) Rhol have demonstrated control of CT. *S. sclerotiorum* is also known to attack economically important crops such as canola, and *P. punctiformis* is rarely found within the prairie provinces of Canada (Harris 1996, Sheley and Petroff 1999) thereby limiting their utility.

Sheep, goats and cattle have also been shown to decrease CT populations within pastures. The use of cattle in high intensity rotational grazing systems reduced CT

growth through increased utilization coupled with the effect of trampling (De Bruijn and Bork 2005). Similarly, both goats and sheep can be effective in controlling CT (Amor and Harris 1975, Popay and Field 1996, Thomson and Power 1993). Booth and Skelton (2009) reported that goats grazing fields infested with CT reduced the total number of shoots by 30% after only two years.

The suppression of CT has also been reported through the use of increased crop competition with perennial grasses and legumes alone or seeded together in mixed pasture swards. Wilson and Kachman (1999) reported that hybrid wheatgrass could control CT up to 85% after three years, which is consistent with other studies (Thrasher *et al.* 1963, Drescheid *et al.* 1961). Seeding alfalfa alone within pastures also reduced CT densities, with increased control up to four years when coupled with mowing treatments (Schrieber 1967, Ominiski 1999). However Spandl *et al.* (1997) reported increased control within fields with alfalfa in mixture with either smooth brome (*Bromus inermis* Leyss) or timothy (*Phleum pratense* L.) than fields with alfalfa grown in monoculture.

2.3 Forage Grasses

2.3.1 Bromegrasses

Smooth bromegrass (*Bromus inermis* Leyss.) is a wide spread perennial grass native to Europe and Asia, and is grown in most temperate regions around the world. Also known as Austrian brome, Hungarian brome and Russian brome, this species was introduced to Canada in 1888 due to its ability to persist in a variety of soil types and survive in periods of drought and extreme temperatures. Smooth brome grows best on well drained deep fertile soils (Otfinowski *et al.* 2007).

Smooth brome is a tall leafy perennial grass with smooth stems containing closed sheaths. Smooth brome is a deep rooted species that has rhizomes in the upper portion of the soil profile. Abundant vegetative reproduction from rhizomes allows smooth brome to form dense sods over time (Otfinowski *et al.* 2007). Unlike smooth brome, meadow brome (*Bromus riparius* Rehm.) is a more grazing tolerant perennial grass. Tightly packed tillers at the base of the plant protect the growing point and give meadow brome its ‘bunchlike’ appearance (Knowles *et al.* 1996). Meadow brome is a long lived perennial grass that is found in cool moist areas (Vogel *et al.* 1996). Nutritional quality (i.e. crude protein) for SB is highest in the spring but decreases as plants mature, while meadow brome has greater digestibility than SB but lower crude protein content. Smooth brome is well adapted to mixtures with high yielding legumes such as alfalfa, and is ideal for hay or pasture production with low frequency of defoliations (Otfinowski *et al.* 2007), however meadow brome-alfalfa mixtures are more suited to increased defoliation harvest regimes (Knowles *et al.* 1996).

2.3.2 Other Perennial Grasses

Timothy (*Phleum pratense* L.) is a cool season perennial grass that is well adapted to temperate environments with high annual moisture (Kunelius *et al.* 2006). It is found in many temperate regions of Europe, Australia, Asia, North and South America. It was first introduced to North America as a cultivated forage species in the 1700’s. Timothy is a perennial bunchgrass that can reach up to 100cm in height. Tiller production is annual from haplocorms (a collection of compact and swollen nodes that act as a carbohydrate reserve), that plays a large role in the plant’s ability to regrow and persist after defoliation

(Emoto and Ikeda 2005). The root system of timothy is reproduced annually by each individual tiller and therefore is quite fibrous, with up to 80% of its root mass being found in the top 5 cm of the soil profile (Garwood 1967). Due to the shallow fibrous root system, timothy is unable to tolerate drought conditions and regrowth after heavy defoliations without adequate moisture. Timothy is commonly grown in monocultures or mixtures with legumes such as alfalfa, red clover or birdsfoot trefoil for hay production (Wedin and Huff 1996).

Kentucky bluegrass (*Poa pratensis* L.) is a low statured, cool season grass found throughout the world that can quickly form sods once established within an area. Cool temperatures, high moisture and fertility encourage persistence and productivity of Kentucky bluegrass within pastures. Reproduction is done either sexually or asexually through the production of creeping rhizomes that can rapidly colonize new areas within pastures (Wedin and Huff 1996). Kentucky bluegrass can become invasive in pastures as a result of poor management and overgrazing (McCartney and Bittman 1994).

Production of leaf area close to the soil allows Kentucky bluegrass to provide valuable soil erosion control in areas that have diminished plant communities due to continuous heavy trampling and grazing. Kentucky bluegrass can be grown in mixture with white (*Trifolium repens* L.), red (*T. pratense* L.) or alsike clover (*T. hybridum* L.) and persists in mixtures with orchardgrass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.) (Wedin and Huff 1996).

2.4 Forage Legumes

2.4.1 Alfalfa

Alfalfa (*Medicago sativa* L.) is one of the most widely grown forage legumes and accounts for approximately 2.5% of the total agricultural acreage worldwide (Sengupta-Gopalan *et al.* 2007). It is found in most temperate regions across Europe, Africa, Asia, Australia, South and North America. Alfalfa, also known as Lucerne, is native to Eastern Europe and Asia. It was first grown in the US as early as 1736, but by the mid 1800's was being grown in Canada (Rumbaugh 1978).

2.4.1.1 Biology

Alfalfa is an erect glabrous perennial legume with alternate trifoliate leaves. The crown of the plants is located at or just below the soil surface and is the main source of regrowth, particularly after defoliation. Plants can grow up to 90 cm tall, depending on management practices and environmental conditions. The majority of alfalfa roots (60-70%) are located in the upper 25 cm of soil (Heidel 1982); taproots of alfalfa can penetrate down to 9 m within the soil profile. Alfalfa is intolerant to flooding. Secondary fibrous roots in the upper 20 cm of soil bear most of the nodules that are important in N fixation (Frame 2005).

2.4.1.2 Production and Nutritional Value

Alfalfa grows best on well-drained soils with a pH of 6.0-6.5 and has greatest yield potential on irrigated soils (Frame 2005). It is drought tolerant due to its deep root system and is more tolerant of saline soils than other legumes. Alfalfa is best suited for

hay or silage production due reduced frequency of defoliation, which allows nutrient reserves (particularly nitrogen) to be maintained within the roots (Barber *et al.* 1996, Frame 2005).

As a forage, alfalfa is widely grown for its high yield and favorable forage quality (i.e. palatability) when grown in monocultures or mixed stands. Alfalfa has a high nutritional value with excellent production potential of up to 20 t ha⁻¹ in moisture rich soils (Frame 2005, Sheaffer *et al.* 1988). Growth stage is the most important determinant of forage quality, with the bud stage having the greatest digestibility and CP content; as alfalfa matures yields continue to increase but forage quality declines (Brink and Marten 1989, Hersterman *et al.* 1993). The inverse relationship between forage yield and quality often results in most alfalfa being cut in the early-mid bloom stage to maximize forage quality while capturing as much yield potential as possible. Alfalfa is most compatible when grown in mixes with smooth brome grass (*Bromus inermis* Leyss.), orchardgrass (*Dactylis glomerata* L.), meadow brome grass (*Bromus riparius* Rehmman), reed canary grass (*Phalaris arundinacea* L.), meadow fescue (*Festuca pratensis* L.) and timothy (*Phleum pratense* L.) (Frame 2005).

2.4.2 White Clover

White clover (*Trifolium repens* L.) is a perennial forage legume grown in moist temperate regions around the world including Australia, Asia, Mediterranean, South and North America (Frame 2005). White clover is an important legume that is tolerant of severe grazing (Burdon 1983) and is able to quickly colonize bare spaces in pastures and

hay land. It grows well on a wide range of soils and environmental conditions but thrives on well drained soils with adequate moisture (Pederson 1995).

2.4.2.1 Biology

White clover plants can reach up to 30cm tall depending on environmental conditions. Root systems of white clover are relatively fibrous with adventitious roots arising from stolon nodes. Clover roots can grow as deep as temperate grasses (Caradus 1990) but remain adept at taking advantage of moisture and soil resources close to the surface due to the creeping nature of the adventitious creeping root system. Stolons are the main vegetative form of reproduction, which allows the plant to spread and colonizes new areas; however, plants commonly reproduce through sexual reproduction as well. Stolon branching and internode length are strongly influenced by the amount and quality of light penetrating to the base of the sward (Frame 2005). As a result, shaded conditions such as those associated with infrequent defoliation can reduce stolon formation and overall productivity of white clover swards (Frame and Harkess 1987, Frame 2005, Sheaffer 1989).

2.4.2.2 Production and Nutritional Value

Production of white clover ranges between 7 and 11 t ha⁻¹ (Frame and Newbould 1984, Fraser and Kunelius 1995) when grown in monoculture. White clover is a weak competitor against perennial grasses for inorganic N (Hogh-Jensen and Schjoerring 1997), but has the ability to produce its own source of N through association with *Rhizobium* spp. White clover is rich in crude protein and minerals and retains higher

digestibility throughout the growing season (Frame 2005). White clover does best when grown in mixtures with non-aggressive grasses, but under intense defoliation can persist in mixtures with more aggressive grasses (Frame 2005). Perennial grasses most commonly grown with white clover include perennial ryegrass (*Lolium perenne* L.) orchardgrass (*Dactylis glomerata* L.), meadow fescue (*Festuca pratensis* Huds.) and tall fescue (*Festuca arundinacea* Schreb) (Frame 2005, Annicchiarico and Piano 1995).

2.4.3 Alsike Clover

Alsike clover (*Trifolium hybridum* L.) is a short lived perennial legume mainly used for pasture and hay production. Alsike clover is native to Europe and is now found in temperate regions of Asia, North and South America. It was first introduced into Canada as a forage legume in 1839 (Fairey 1986). Alsike clover is adapted to a wide range of soils and environments but prefers cool temperate regions with good moisture (Frame *et al.* 1998) and is more tolerant to acidic and alkaline soils than many other clover species as well as moderate flooding, drought and colder temperatures (Frame 2005).

2.4.3.1 Biology

Alsike clover can grow up to 50-60 cm tall with branched slender stems arising from basal crowns (Frame 2005). The fine stems of alsike clover make it susceptible to lodging when grown in monoculture, however when grown in a mixture with a stronger stemmed species it does not affect production. The primary root system of alsike clover is a tap root with many lateral roots originating from the basal crown area. Reproduction

is only sexually with the production of globular inflorescences pale pink to pinkish white. Flowers are self-incompatible and are cross-pollinated by insects, primarily honey bee (Frame 2005).

2.4.3.2 Production and Nutritional Value

Alsike clover can be used for grazing or cut for hay or silage when grown in a mixture. Alsike clover is most productive in the year of establishment with yields reported at 4.08 t ha⁻¹ with subsequent annual yields declining (Frame 2005, Fariey 1986). Nutritional quality is similar to red clover (*Trifolium pretense* L.) but yields are lower, with the exception of saturated soils (Fariey 1986). Alsike clover can be grown with red or white clover, or in swards with non-aggressive grasses such as timothy (*Phleum pratense* L.) (Frame 2005).

2.5 Benefits of Legumes

The inclusion of legumes can have many benefits resulting in the increase of overall forage productivity in pasture swards. Legumes grown with grasses offer several advantages over grasses grown in monoculture (Sleugh *et al.* 2000). Mixtures are associated with increases in forage yield, nutritional quality and improved seasonal distribution of available forage, all of which can enhance animal production (Bertlison and Murphy 2003, Dewhurst *et al.* 2003, Haynes 1980, Fraser and Kunelius 1995). The proportion of legume present in a sward has been positively correlated to the N content of the grass component in mixed swards, including the overall total forage of the pasture (Mallarino and Wedin 1990). In the case of grass-legume mixtures, legumes are thought

to exploit the deeper soil profile relative to grasses, thereby minimizing competition (Buxton and Wedin 1970).

Compatible mixtures of legumes and grasses have been reported to yield either higher amounts of biomass than either component grown in monoculture. This effect, also known as ‘overyielding’, has been reported in the literature by Gokkus *et al.* (1999), Polser *et al.* (1993), Robert and Olson (1942) and Aberg *et al.* (1943), however this relationship has not been consistently demonstrated. The increased diversity with legume-grass mixtures is also recognized for reducing weed encroachment, soil erosion and increasing the length of stand longevity, compared to monocultures of grasses or legumes (Droslom and Smith 1976).

2.5.1 Nitrogen Fixation

Legumes have the unique ability to fix atmospheric N through symbiotic association with *Rhizobium*. Fixed N in turn, has been shown to be transferred to non-leguminous plants within mixed swards (Brophy *et al.* 1987, Ta and Faris 1987, 1988, Burity *et al.* 1989, Heichel and Henjum 1991, Dubach and Russelle 1994). Different *Rhizobium* species are specific to each type of legume (e.g. *Rhizobium meliloti* for alfalfa and *Rhizobium leguminosarum* for white clover) with soil presence and abundance of the microbe dependent on either inoculation of legumes or naturally occurring populations. *Rhizobium* populations are generally highest within fields where the specific host legume species has been previously grown (Frame 2005). Plant deficiencies in the soil such as excess acidity, frequent and heavy defoliation, high soil N, or applications of N fertilizer, can all limit N fixation (Lamb *et al.* 1995, Frame 2005). Other climatic factors that affect

N fixation are soil moisture and temperature (Hardarson and Atkins 2003). Optimal temperatures for nodulation in legumes is between 20-30°C (Gibson 1971). Atmospheric N fixation ranges from 54 to 545 kg N ha⁻¹ yr⁻¹ in white clover (Peoples *et al.* 1995, Elgersma and Hassink 1997), and ranges from 80 to 258 kg N ha⁻¹ yr⁻¹ (Burity *et al.* 1989, Haby *et al.* 2006) in alfalfa.

Nitrogen fixation by legumes is higher in legume-grass mixtures as compared to legumes grown in monocultures as was reported by Brophy *et al.* (1987) in alfalfa-orchardgrass mixtures and by Carlsson and Huss-Danell (2003) for white clover. Conversely, high levels of N fertilizer addition can reduce the amount of N fixed. White clover and alfalfa both demonstrated lower N fixation rates when higher applications of N were applied to swards (Carlsson and Huss-Danell 2003). Higher N availability within soil nutrient pools encourages legumes to obtain N from the soil instead of through atmospheric N assimilation, and suggests plants can reallocate photosynthate depending on needs. Additionally the preference of legumes to utilize available soil N can have a detrimental effect on legume sward longevity due to strong competition with grasses. Grasses with aggressive growth such as smooth brome, meadow brome and Kentucky bluegrass are able to outcompete other plants, including legumes, for N within the soil, thereby decreasing legume abundance within mixed swards.

2.5.2 Nitrogen Transfer

The transfer of N between legumes and non-leguminous plants such as perennial grasses can occur by mycorrhizal fungi, decomposition of leaf litter, roots and nodules, or root exudations of N directly into the soil profile. The pathways for N transfer can be

affected by the age of the plant as well as the legume species itself. Nitrogen can be transferred short term to grass through mycorrhizae or root exudations into the rhizosphere immediately surrounding the roots (Paynel *et al.* 2001). Excretion of N from roots and nodules has been reported by Ta *et al.* (1986) for alfalfa grown in hydroponics, for alfalfa grown in soil by Lory *et al.* (1992) and for white clover by McNeill and Wood (1990). In all studies the excretion of N from roots and nodules in living plants was small relative to the total amount of N fixed into soil and surrounding roots and nodules (Lory *et al.* 1992).

Instead the dominant pathway for the release of N from legumes was through the decomposition of dead roots and nodules (Dubach and Russelle 1994). Dubach and Russelle (1992) found that the rate of decomposition of fine roots was 64% for alfalfa and 34% for birdsfoot trefoil, which occurred up to 12 cm deep in the soil profile during the year of establishment. Dubach and Russelle (1994) also confirmed the findings of Ta and Faris (1987), suggesting that alfalfa transfers fixed N through root decay while birdsfoot trefoil contributes more N through decomposing nodules than roots. The breakdown and transfer of plant biomass can provide an important N source to neighboring grasses over the long-term given the persistence of legumes within swards (Dubach and Russelle 1994, Tomm *et al.* 1994, Russelle *et al.* 1994, Johansen and Jensen 1996). Limited evidence suggests that there may also be a reciprocal transfer of N from grasses to legume within grass-legume pastures (Tomm *et al.* 1994).

Age of plants can also affect the type of N transfer from legumes to grasses. Older plants tend to transfer more N through biomass degradation (Johansen and Jensen 1996, Høgh-Jensen and Schjoerring 2001), while young plants transfer N through

mycorrhizal fungi (Rogers *et al.* 2001) and root exudation (Paynel *et al.* 2001, Paynel and Cliquet 2003). As a forage sward matures the rate of N transfer to grasses can increase (Elgersma *et al.* 2000) provided legumes are retained within the mixture and do not decline due to drought, defoliation or intense competition from neighboring grasses. Nitrogen transferred from clover to associated grasses varies 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 grass and legumes (Brophy *et al.* 1987). In general, the amount of N transferred from legume to grasses ranges from 29 to 454 kg ha⁻¹ (Elgersma *et al.* 2000, Elgersma and Hassink 1997).

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CHAPTER 3.

Production Dynamics of Grass-Legume Swards Following Broadleaf

Herbicide Application

3.1 Introduction

Noxious weed management within mixed forage swards can be complicated by the presence of desirable broad-leaf plants such as legumes. Many weed control options are available to producers including mechanical methods such as tillage (Lukashyk *et al.* 2008) and mowing (Schreiber 1967) or cultural practices such as burning or grazing (De Bruijn and Bork 2006). While all these methods provide some degree of control either alone or in combination (Beck and Sebastian 2000), herbicides have become the most widely used for the majority of noxious weeds in pastures (DiTomaso 2000).

One of greatest challenges with the management of mixed swards is the ability to retain higher amounts (> 40%) of legumes within the stand over a longer period of time. Baylor (1974) noted that the inclusion of legumes usually resulted in increased yield, greater forage quality and improved seasonal distribution of forage (Sleugh *et al.* 2000). The addition and retention of legumes within a sward can increase overall diversity, and complementary growth forms can enhance the capture and use of available resources such as light, soil moisture and soil nutrients (Gross *et al.* 2007, Berendse 1982, Naeem *et al.* 1994). Other benefits include increasing soil nitrogen through atmospheric fixation in association with *Rhizobium* spp., bacteria unique to legumes (Walley *et al.* 1996) that contribute to increased forage nutritional quality (including crude protein yield – CPY) and subsequent animal weight gain (Dewhurst *et al.* 2003). Alfalfa, white clover

(Carlsson and Huss-Danell, 2003), and alsike clover (Fairey, 1986) have been shown to fix up to 350, 545 and 86 kg ha⁻¹, of nitrogen (N), respectively. Moreover the presence of N rich legume plants within a mixed sward may facilitate an increase in overall grass biomass through the subsequent release of N during decomposition (Ta and Faris 1987, Burity *et al.* 1989, Dubach and Russelle 1994).

Use of herbicides for weed control in swards containing legumes assumes that the effect of legume loss or removal necessarily leads to net production declines. However, similar to other plants, legumes are likely to demonstrate at least some degree of competition with neighboring forage grasses for available space and resources (Donald 1990, Gross *et al.* 2007). As a result, legume removal may facilitate an increase in grass abundance via release from competition. Consequently, the desired positive benefit of weed control through the use of herbicides could off-set the loss of legumes within mixed swards. Based on the presence of both facilitative and competitive dynamics within mixed swards before and after legume removal, there are several possible outcomes with respect to the observed compensatory response of grasses within the sward and changes in associated total net forage yield (Fig. 3.1).

The first outcome (O₁) is that total forage sward biomass or CPY may decrease following spraying in swards with greater initial legume removed, suggesting that spraying will have a detrimental effect on overall forage production. In this situation overall grass production gained would not compensate for the loss of legumes initially within the sward. In the second outcome (O₂) total forage sward biomass or CPY may remain equal before and after spraying despite the removal of legumes, indicating changes in grass biomass were able to fully compensate for the removal of legumes. The

third potential outcome (O₃) is that total forage sward biomass or CPY may increase relative to the initial total forage biomass prior to spraying. This could occur due to a large increase in grass biomass post-spraying that more than compensates for legume loss from the initial mixed sward.

Despite the widespread risk of legume loss associated with the use of broadleaf herbicides for perennial weed control, few studies have examined the net effect of spraying mixed swards containing legumes, including the nature and magnitude of compensatory grass responses. The goal of this study was to quantify grass and total forage dynamics within mixed swards following legume removal through the use of broadleaf herbicide application. This information should improve our understanding of the agronomic impact of legume removal within mixed forage swards and the associated opportunity cost of legume loss following spraying.

3.2 Research Objectives

- 1) Quantify overall net changes in total forage biomass and nutritional quality responses within mixed swards for up to three years following herbicide application.
- 2) Assess the presence and magnitude of grass compensation with respect to legume removal within mixed swards over a three year period after herbicide application.
- 3) Determine how observed sward dynamics and compensatory responses may be altered by different grass and legume species presence within grass-legume mixtures.

3.3 Materials and Methods

3.3.1 Study Area

This research was conducted at two sites located approximately 25 km apart near Edmonton, Alberta, Canada, from 2005 through 2008, inclusive. Sites included W240 located at the University of Alberta Edmonton Research Station, and E2 situated at the University of Alberta Ellerslie Research Station. Both sites are in the Aspen Parkland natural sub-region and are on well developed Black Chernozemic soils (Table 3.1).

Average annual temperatures for the region are 4.3°C and total annual precipitation averages 460 mm, with the majority of precipitation falling during the growing season from April through September (Figs. 3.2 & 3.3). Analysis of soil samples was done at each site prior to seeding to determine fertilization requirements. All fertilizer application occurred in 2004 where both sites received 127 kg ha⁻¹ of a complete fertilizer blend (18-20-10-15), with the W240 site (Site 2) having an additional application of 56 kg ha⁻¹ of ammonium phosphate (11-52-0).

3.3.2 Experimental Design and Forage Mixtures

This investigation utilized plots from a pre-existing study examining the long-term forage dynamics within mixed swards. Plots for the forage dynamics study were established in May 2004 at each site in a RCB design with four replicates of each of the four forage mixtures: mixtures represented a 2 x 2 factorial of combinations of grasses and legumes. Each forage mixture was further seeded at 6 different initial proportions of legumes (0, 11, 22, 33, 67 and 100%) with the remainder of the forage mix containing a companion grass, up to a total seeding rate of approx 14 kg ha⁻¹ (Fig. 3.4).

The four forage mixtures examined contained either alfalfa (ALF) (*Medicago sativa* L.) a tap-rooted legume, or a 50:50 mixture of white (WC) (*Trifolium repens* L.) and alsike (AC) (*Trifolium hybridum* L.) clovers, both of which have shallow creeping root systems. Companion grasses seeded with the legumes included either a 40:60 mixture of the rhizomatous grasses Kentucky bluegrass (KBG) (*Poa pratensis* L.) and smooth brome grass (SB) (*Bromus inermis* L.), or the deeper rooted bunchgrass meadow brome grass (MB) (*Bromus riparius* Rhem.). Ultimately, the forage mixes examined were designed to represent a range of common pasture communities in the region, including newly seeded high performance ‘power’ pastures (e.g. ALF-MB mixes) to long-standing pastures commonly dominated by rhizomatous grasses and volunteer legumes (e.g. SB/KBG-clover). Seeding rates for each mixture were calculated by multiplying the recommended seeding rate for the area by the proportion of each legume and grass component in the mix (Alberta Forage Manual, 1988). Finally, each legume proportion (0, 11, 22, 33, 67 and 100%) and corresponding grass component of the mix was totaled and adjusted for pure live seed (PLS) content.

In the fall of 2005, two growing seasons after the initial establishment of the long-term forage plots, half of each original plot (i.e. a 3 x 6 m subplot) was sprayed with a broad-leaf herbicide using a strip plot design (Fig. 3.5). Herbicide application of aminopyralid + 2,4-D ester was applied in early September at a rate of 120 g ae ha⁻¹ + 1440 g ai ha⁻¹ within 100 L ha⁻¹ water using a CO₂ quad sprayer with 8003 nozzles at 32 psi. Additional maintenance spraying (i.e. spot-spraying) with clopyralid was done using a hand sprayer in August 2006 to sprayed sub-plots to remove any surviving or volunteer legumes, and to prevent legume encroachment from neighboring unsprayed sub-plots.

Surviving and/or volunteer legume presence was very low in most sub-plots at both sites (< 3% plot area) in 2006 and thereafter.

3.3.3 Field Sampling

Peak biomass was assessed annually in July from 2006-2008 within both sprayed and unsprayed sub-plots. Within each sub-plot two randomly located 0.25 m² (i.e. 50 x 50 cm) quadrats were harvested to ground level and pooled into an aggregate sample. Each aggregate sample was further sorted into grass, legume and forb (e.g. other dicots), before being dried at 60°C to a constant mass and weighed. At the end of each growing season in September all plots were cut and baled to remove large accumulations of biomass and prevent excess litter accumulation in subsequent years.

3.3.4 Forage Quality Analysis

All grass and legume biomass samples were ground separately with a Wiley Mill through a 2 mm screen and assessed for protein. Crude protein (CP) values were calculated from nitrogen (N) values, the latter of which were determined using a Leco TruSpec C/N Autoanalyzer. Values of N were subsequently converted to CP using equation (1).

$$\%CP = \%N \times 6.25 \quad (1)$$

Crude protein concentrations were also combined with biomass to derive estimates of crude protein yield (CPY) values (see equation 2) for each respective sub-plot. Values of CPY represent the overall forage nutritional content of each forage mix sampled.

$$CPY \text{ (kg/ha)} = [\%CP/100] \times \text{biomass (kg/ha)} \quad (2)$$

3.3.5 Statistical Analysis

Initial legume seeding treatments established as fixed seeding rates in 2004 were no longer distinguishable as discrete (i.e. nominal) treatments in 2006, even within unsprayed sub-plots. As a result, an ANOVA analysis was not appropriate for evaluating post-spraying treatment responses. Instead, an empirical approach was used whereby quantitative forage dynamics were examined using regression modeling for each forage mix. Additionally, each study site was analyzed separately due to apparent differences in site characteristics, including forage establishment. A total of 24 plots were used within a forage mix at each site to examine forage dynamics. Finally, all data at each site were tested separately for normality and homogeneity of variances using Proc Univariate in SAS (SAS Institute Inc. 1988). Due to the absence of discrete categorical treatments, a graphical distribution of normality and homogeneity of variances was used in conjunction with the Kolmogorov-Smirnov test to evaluate data, with no transformations required.

Linear least squares regression was performed in SAS using Proc REG (SAS Institute Inc. 1990) to determine the relationship between forage components (biomass and CPY) of both total forage or grass (dependent variable) and initial legume abundance (independent variable). All biomass estimates were regressed against the initial proportion of legume removed from swards at the time of spraying in 2005 for three consecutive years (2006-2008). Preliminary assessment indicated that using the proportion (%) of legume rather than biomass (kg ha^{-1}) as the independent variable to quantify legume removal consistently resulted in a superior model fit (i.e. greater R^2), and was therefore used in the final regression analysis. Although both linear and polynomial models were initially evaluated, only small changes in model co-efficient of

determination were found (i.e. R^2 increases were $< 5\%$), with marginal to no alteration in observed model significance (p-values). As a result, simple linear models were used in subsequent data analysis, and had the additional benefit of facilitating direct comparison of models from different response variable, location and forage mixes.

Significant variation was observed in the abundance of legumes throughout the study period, even in the absence of spraying (Figs. 3.6 and 3.7). In order to adjust for these naturally occurring temporal changes in the presence and abundance of legumes, all data within sprayed sub-plots were adjusted using data from paired unsprayed sub-plots, which in effect, served as a temporal ‘check’ to provide estimates of forage growth in the absence of spraying. Failing to do so would have altered estimates of legume removal on total forage production in the absence of spraying. Net changes (i.e. sprayed sub-plots-unsprayed sub-plots) in forage attributed to spraying were then determined for each sprayed sub-plot using equation 3, where $Biomass_{SS}$ was the biomass of the sprayed sub-plot within a year, and $Biomass_{US}$ was the biomass of the paired unsprayed sub-plot within the same year.

$$\Delta Biomass \text{ (or } \Delta CPY) = Biomass_{SS} - Biomass_{US} \quad (3)$$

Resulting net change (Δ) values for each year were then regressed against initial legume abundances (i.e. % of total biomass) found within the sward just prior to spraying in 2005.

3.4 Results

Legume removal based relationships for each forage mixture following spraying at both the W240 and E2 sites are shown in Figs. 3.8 through 3.15. Both biomass and CPY responses are shown for grass and total forage components of the swards for three consecutive years (2006-2008) post-spraying. Summary results of all regressions performed for all forage mixtures are provided in Table 3.2. All remaining statistical equations, p-values and model fit parameters for each regression can be found in Appendix A.

3.4.1 MBALF Sward Dynamics

At W240, total forage CPY declined under greater levels of initial legume within MBALF swards for 3 consecutive years following legume removal (Fig. 3.8b, d & f). Reductions in total forage CPY peaked in year 2, declining by 5.3 kg ha^{-1} for each additional 1% of legume removed at the time of spraying. Notably, total forage biomass was negatively associated with initial legume removal in year 2 but not years 1 and 3, suggesting the reductions in total forage CPY during years 1 and 3 may be associated more with decreases in forage quality rather than biomass. The consistent reductions in total forage CPY also occurred despite increases in grass (i.e. meadow brome) CPY of up to 2.9 and 1.7 kg ha^{-1} for each initial 1% of legume removed 1 and 2 years after spraying. Increases in grass CPY paralleled significant increases in grass biomass with greater legume removal (Fig. 3.8b, d, & f). Although grass biomass also exhibited a positive relationship with increasing legume removal 3 years after spraying, this increase did not translate into a positive response in grass CPY (Fig 3.8f).

Sward dynamics in MBALF at E2 following spraying were generally similar to those at W240, but with fewer significant relationships. Total forage CPY once again declined under increasing levels of legume removal in years 1 and 3, although this relationship remained non-significant in year 2 (Fig. 3.9b, d & f). By year 3, total forage CPY continued to decline by 5.5 kg ha⁻¹ for each additional 1% of legume removed 3 years earlier. As might be expected, decreases in total forage CPY were mirrored by a reduction in total forage biomass, but only during year 1 (Fig. 3.9b). However, the lack of a relationship between total forage biomass and legume removed in year 3 (Fig. 3.9e) suggests that the loss of forage quality associated with legume removal was an important determinant of the decline in total forage CPY at that time. Grass responses were particularly limited, with positive responses in each of the biomass and CPY components during the second year after spraying (Fig. 3.9c & d). Notably, this positive grass response coincided with non-significant total forage responses in that year.

Forage responses in plots that initially contained no legume (i.e. 0% legume, 100% grass) at W240 revealed consistent decreases in total forage biomass (year 3) and total forage CPY ranging from 109 kg ha⁻¹ (year 1) to 221 kg ha⁻¹ (year 3). The lone positive grass biomass response evident in non-legume seeding treatments was an increase of 1093 kg ha⁻¹ two years after spraying

3.4.2 SBALF Sward Dynamics

Forage responses within SBALF swards at W240 demonstrated a marked decline in total forage CPY under increasing removal of legume in the first two years (Fig. 3.10b & d). Reductions in total forage CPY were greatest in year 1 at 5 kg ha⁻¹ for every 1% of

ALF removed, with significant reductions of 3.4 kg ha^{-1} still apparent in year 2. In year 3 a non-significant ($p = 0.074$) but negative trend in total forage CPY was also evident. Reductions in total forage CPY reflected total forage biomass decreases in year 1 and 3 (Fig. 3.10a, b, e & f) but were not evident in year 2 (Fig. 3.10c & d). The absence of a negative relationship in total forage biomass in year 2 suggests that decreases in total forage CPY may be more related to changes in forage quality (i.e. N or CP concentrations) rather than biomass. Notably, these observed decreases in total forage CPY during years 1 and 2 occurred despite a significant positive response in grass CPY and biomass for those years (Fig. 3.10a-d).

At E2, forage responses in SBALF were similar to W240 but remained less pronounced in both the grass and total forage components. Total forage CPY exhibited significant declines in the first two years (Fig. 3.11b & d), with the greatest CPY loss in year 1 at 125 kg ha^{-1} for every 1% of ALF removed. No significant relationships were evident in grass CPY for all three years (Fig. 3.11b, d & f) at E2, which may have contributed to the smaller magnitude of responses in total forage CPY to ALF removal compared to the W240 site. Reductions in total forage CPY coincided with total forage biomass reductions in year 1 (Fig. 3.11a & b) but the latter response was absent in year 2 (Fig. 3.11c & d). Similar to the W240 site, this indicates that continued reductions in total forage CPY at E2 during year 2 were associated with decreases in forage quality rather than biomass. However, by year 3 both total forage CPY and biomass did not display any significant responses to alfalfa removal (Fig. 3.11e & f).

Forage responses in SBALF swards free of legume (i.e. SB grass only) at W240 exhibited net losses in both grass and total forage biomass and associated CPY variables,

but only in year 1 (Fig. 3.10a & b). Net reductions in total forage biomass and CPY were 1088 kg ha⁻¹ and 66 kg ha⁻¹, respectively. By year 3, total forage biomass continued to exhibit a net reduction due to spraying of 1225 kg ha⁻¹, as did total forage CPY of 144 kg ha⁻¹ (Fig. 3.10e & f). At E2 responses to spraying within legume free swards exhibited mixed effects depending on the vegetation component and year of sampling. During year 1, total forage biomass declined by 1340 kg ha⁻¹ (Fig. 3.11a). In year 2, a marked increase in grass biomass (2027 kg ha⁻¹) and grass CPY (216 kg ha⁻¹) were evident (Fig 3.11c & d), with no response in total forage biomass or CPY. By year 3, total forage CPY was 170 kg ha⁻¹ lower ($p = 0.058$) in swards initially containing no legume.

3.4.3 MBCLR Sward Dynamics

Total forage CPY within the MBCLR mix at W240 demonstrated a negative relationship with a decrease of 1.9 kg ha⁻¹ of total forage CPY for each 1% of legume removed from the sward in year 2. Notably, this occurred despite a positive response in grass biomass and CPY with increased legume removal (Fig. 3.12c & d). The absence of this relationship in year 1 appeared to be linked to a large increase in grass biomass (Fig. 3.12a) and associated grass CPY (Fig. 3.12b), particularly within swards initially dominated by very high levels of clover (i.e. > 75% clover).

At E2, total forage CPY declined markedly in year 1 by 4.1 kg ha⁻¹ with increased initial legume removal (Fig. 3.13b), despite a pronounced increase in grass CPY of up to 3.9 kg ha⁻¹ for each 1% legume initially present. Both grass and total forage biomass showed no significant relationship in year 1 (Fig. 3.13b), suggesting responses in CPY may be associated with changes in forage quality as well as quantity. By year 2 both

grass biomass and CPY increased in response to legume removal, but failed to lead to an increase in total forage biomass or CPY (Fig. 3.13c & d). No relationship existed between grass and total forage (either biomass or CPY) in regards to legume removal in year 3. Finally, no impacts of spraying were evident in swards initially containing no clover (i.e. MB grass monocultures) at W240 or E2.

3.4.4 SBCLR Sward Dynamics

In SBCLR swards, total forage CPY exhibited no relationship with initial legume removal at W240 in any year of sampling (Fig. 3.14). However, grass biomass and CPY increased sharply in response to increasing legume removal during both year 1 (Fig. 3.14a & b) and year 2 (Fig. 3.14c & d). At E2, limited responses to spraying and legume removal were observed. Total forage CPY declined under increasing legume removal in year 1 by 4 kg ha⁻¹ of CPY for each 1% of clover removed (Fig. 3.15b). This reduction occurred despite a significant increase of 22.8 kg ha⁻¹ in SB biomass for each 1% of clover removed (Fig. 3.15a). By year 2, only grass biomass exhibited a response to legume removal, with grass biomass continuing to rise under increasing legume removal (Fig. 3.15c). Despite the grass increase, no significant relationships were evident between the CPY of grass or total forage in SBCLR swards during years 2 or 3. Similarly, no responses to spraying were evident in legume free (i.e. SB grass monocultures) swards.

3.5 Discussion

3.5.1 Responses to Spraying in Alfalfa Mixtures

Although variable among study sites, forage mixes and timing of response, legume removal consistently resulted in a decrease in total forage CPY in this study. Moreover, decreased total forage CPY occurred despite frequent and relatively widespread increases in the grass component of swards under increasing legume removal. Although grass CPY increases were clearly able to compensate for limited legume removal within the swards examined, this competitive release remained insufficient to fully compensate for the loss of high amounts of legume biomass and associated CPY.

Reductions in total forage CPY and biomass in this study were largely dependent on the type of forage mixture as well as the study site. In some circumstances forage reductions were immediate and consistent over the three year period after spraying. Within alfalfa swards at W240, annual total forage CPY reductions were 3.5-5.5 kg ha⁻¹ in MBALF swards, and 1.8-5.0 kg ha⁻¹ in SBALF swards. Lower or less consistent losses observed at E2 suggest that differences in growing conditions (i.e. precipitation, temperature and soils) may have had a larger impact on initial legume abundance, and thus subsequent responses to legume removal. For example alfalfa was much greater at E2 than W240 within unsprayed swards during all four years (Fig. 3.6 and 3.7). Within swards containing alfalfa, average production removed by spraying (2005) was 2795 kg ha⁻¹ at E2 compared to 1717 kg ha⁻¹ at W240. Average differences of up to 1000 kg ha⁻¹ removal between sites may account for the greater yield loss coefficients observed at E2 relative to W240.

Although both sites were situated on Black Chernozemic soils containing high organic matter with presumably higher soil N, subtle differences in nutrient availability and soil moisture may have accounted for differences in the extent of yield reduction as well as the temporal pattern of yield decline following spraying. Alfalfa is known to vary in performance with soil conditions (Frame 2005, Peterson *et al.* 1992), and lower rainfall at the W240 site (see Fig. 3.2) may also help explain the lower yield loss coefficient at that location. Finally variation in weed pressure may further explain some of the differences in forage responses following spraying between sites. As weed pressure was generally observed to be greater at E2 than W240, the removal of legume at E2 may have resulted in a greater increase in weeds instead of grasses after spraying, which in turn would have limited the ability of grasses to compensate for legume removal. The greater weed population at the E2 site may have also contributed to the inconsistent results observed compared to W240.

Notable differences were observed within pure legume sprayed plots at both sites within swards containing alfalfa, particularly within SBALF mixtures. Observed differences may have been due to the encroachment of adjacent grasses such as MB, SB and KBG into these plots after spraying in 2006 through to 2008. The addition of these volunteer perennial grasses and the presence of weedy species from the soil seed bank may have altered the magnitude of the responses of swards between years and within sites.

Larger and more consistent reductions in total forage CPY were also observed within sprayed swards containing alfalfa compared to clover at either study site. Alfalfa is known for its high production when grown in monoculture or within a forage mix in

western Canada (Popp *et al.* 2000). Although alfalfa (258 kg ha⁻¹ N annually) (Burity *et al.* 1989) fixes up to 250 kg ha⁻¹ N less per year than white clover (545 kg ha⁻¹ N annually) (Elgersma and Hassenk 1997), the overall biomass production potential of alfalfa can be as high as 1800 kg ha⁻¹ per year (Mortenson *et al.* 2005) depending on growing season conditions within inter-seeded fields.

In addition to the high productivity of alfalfa, plots containing this species also demonstrated the ability to maintain a greater proportion of legume over the course of this study within unsprayed plots (Fig. 3.6 & 3.7). Regardless of initial seeding rates (with the exception of non-legume seeded mixtures) and legume abundance, MBALF in this study at both sites tended to reach a stable 'equilibrium' of approximately 30-60% alfalfa (e.g. of total biomass) at the end of the five year study period. Holt and Jefferson (1999) reported that MB in mixture with ALF appeared compatible and led to a good balance of grass and legume that persisted for 7 years. This suggests that the amount of alfalfa present within plots seeded at the lower rates (e.g. 11, 22, and 33% alfalfa) was able to increase and maintain higher abundance despite lower initial seeding rates.

While SBALF demonstrated similar legume 'equilibrium' within swards seeded at different rates after five years, it occurred at a lower level of approximately 25 to 55% legume. These findings suggest SB was a much more aggressive competitor against alfalfa in this study, conditions created in part due to the low frequency of harvest (e.g. single defoliation at peak biomass) (Sleugh *et al.* 2000, Frame 2005). SB is known to be well adapted to infrequent or low intensity defoliation, such as would be encountered under haying or light to moderate grazing (Otfinowski *et al.* 2007). SB is also a tall statured plant capable of shading and outcompeting neighboring plants for resources such

as light, moisture, nutrients and space (Otfinowski *et al.* 2007). In contrast MB is better adapted to heavier grazing (e.g. multiple defoliations) due to its growing points being situated closer to the ground that allow it to recover more quickly (Knowles *et al.* 1993).

3.5.2 Responses to Spraying in Clover Mixtures

In contrast to alfalfa, clover swards appeared to decline in unsprayed plots over time, which also reduced the relative opportunity cost associated with legume removal. Clover swards were difficult to assess given the natural loss of legume from within these swards, and resulted in inconsistent responses at both sites. Clover is known to be sensitive to light availability within the plant canopy, which in turn, may have a direct effect on stolon production and associated forage productivity (Frame 2005, Sheaffer 1989). In this study a single defoliation was carried out later in the growing season. As a result, net clover responses to removal from spraying were minimal and may have been hard to separate from other environmental effects, including high competitive pressure from adjacent grasses, particularly in the SBCLR mixtures. Unsprayed clover plots seeded at high rates were the only ones able to maintain high legume abundance in mixtures with either companion grass due to limited shading by the grass canopy.

The absence and/or disappearance of net yield losses in clover based swards following spraying may have reflected the loss of clover across all plots (including unsprayed plots) during the last two years. Marked declines in clover abundance within unsprayed plots would have limited the ability to detect net yield responses due to spraying. The use of a multiple defoliation treatment may have altered the abundance

and overall productivity of clover based swards, as clover is known to tolerate repeated severe grazing or defoliation (Frame 2005, Sheaffer 1989).

The greater reductions in CPY following spraying in alfalfa rather than clover swards highlight the superior establishment of alfalfa with the companion grasses tested here. Similarly, the reduced alfalfa abundance when grown with SB rather than MB likely led to the high opportunity costs (i.e. forage losses) within MBALF mixtures. These results again reinforce that SB is overall a more competitive companion grass than MB, and that the former may suppress legume establishment and growth in the long term. These findings have implications both for the selection of initial forage mixtures to optimize legume retention (e.g. MBALF > SBALF), as well as the potential for weed control practices to alter forage dynamics (e.g. forage losses and opportunity costs).

Although both MB and SB responded positively to legume removal, SB appeared to respond with greater grass CPY increases following alfalfa removal. For example, during years 1 and 2 following alfalfa removal at the W240 site, grass CPY gains were 2.9 and 1.8 kg ha⁻¹ for each 1% of legume removed from MBALF mixtures, and 3.1 and 3.4 kg ha⁻¹ in SBALF mixtures, respectively. The opposite was true of mixed swards containing clover at W240 one year after spraying, as clover removal led to grass CPY gains of 5.2 and 4.2 kg ha⁻¹ for each 1% legume removed within MBCLR and SBCLR swards, respectively. Differences in the ability of grasses to respond to legume removal may be attributed to the rooting pattern of grasses and their ability to promptly take up soil nutrients (primarily N) released during decomposition of legume roots. In the case of SB and KBG, their extensive creeping root system may be better situated for rapid uptake of mineralized nutrients throughout the soil profile (Wedin and Huff 1996, Etter 1951).

In contrast, being a bunch grass with a large centralized fibrous root system, MB may be very effective at capturing nutrients, but only within a more localized area surrounding each individual plant (Knowles *et al.* 1993).

Net reductions in total forage CPY and biomass to spraying occurred despite a positive response of grasses to legume removal. Short-term increases in grass biomass are indicative of either 1) the release of grass component in the sward through the elimination of neighboring plant competition (Grekul and Bork 2004) such as legumes, and/or 2) the facilitative responses of the grass component due to legume mortality and subsequent release of nutrients (e.g. N) from root decomposition and mineralization (Dubach and Ruselle 1994). Legumes are known to be competitive within mixtures and can directly reduce the growth and overall productivity of neighboring vegetation (Frame 2005, Holt and Jefferson 1999). Therefore the elimination of increasing amounts of legumes due to spraying is likely to lead to an associated increase in grass growth, as evident in this study. Moreover, increases in grass CPY and biomass could be attributed to the release of N from legume root decomposition. In the case of alfalfa and white clover, up to 35% and 79% of N can be released and transferred from these swards due to microbial association (Dubach and Russelle 1994).

3.5.3 Role of Forage Quality in Assessing Cost of Herbicide Application

In this investigation, both forage biomass (kg ha^{-1}) and quality (CPY) data were used to assess forage responses to legume removal. While biomass and CPY responses generally paralleled one another in both the grass and total forage components of swards, the inclusion of forage quality did result in additional relationships between legume

removal and forage dynamics becoming significant. Furthermore, changes in responses through the use of CPY altered the interpretation of legume removal impacts on overall total forage productivity.

In general, CP values at peak biomass ranged from 5 to 11% crude protein with an average of 8% for 2005-2008, while and legume CP values ranged from 12 to 18% with an average of 15%. The most common additive response of including measures of CP into assessments of forage dynamics was to increase the frequency and severity of reductions in total forage opportunity cost with increasing legume removal. Within MBALF and SBALF swards, the inclusion of CP increased the number of significant negative relationships between total forage CPY and legume removal in each of the three years after spraying within both mixtures at W240, and for the first two years at E2. Total costs due to legume removal also increased when measures of CP were included, as represented by the observed increase in the model fit (i.e. R^2), and perhaps more importantly, greater CPY loss coefficients associated with increasing legume removal.

This effect is illustrated at W240 in year 1, when legume removal accounted for only 11.4% of the reduction in total forage biomass and remained non-significant. However when measures of forage nutritional quality were included, legume removal accounted for 46% of the variation in CPY, and led to a significant loss of 3.7 kg ha^{-1} of CPY with each additional 1% of legume removed. Spraying of the SBALF swards at both sites in year 2 led to sharp decreases in total forage CPY despite the absence of a change in overall total forage biomass observed within these swards. These results highlight the importance of including assessments of forage quality in the evaluation of aggregate mixed sward responses to spraying, as doing so is likely to lead to more

accurate quantification and realistic interpretation of the opportunity costs associated with legume removal.

It should also be noted that there were instances where grass biomass release following legume removal became less important following the inclusion of CP measures. Given that grass CPY is a function of both biomass and relative quality, this finding was not entirely unexpected as biomass and quality parameters are often inversely related to one another; with improvements in forage quality often accompanied by lower biomass production (Brink and Marten 1989). For example, within mixed SBCLR swards at E2, the inclusion of CP concentrations in the assessment of grass biomass responses resulted in no relationship between grass CPY and legume removal. This suggests that while grass biomass may have increased in response to legume removal, reductions in grass N (e.g. CP) concentrations were also evident, thereby resulting in little overall change in grass CPY. Similar findings were observed in the MBALF mixture at W240 during year 3 and in MBCLR at W240 during year 2. Overall, the magnification of opportunity costs associated with legume removal using forage quality assessments were more clearly evident in mixtures containing alfalfa as compared to clover mixtures. This observation may be due to the greater retention of alfalfa rather than clover in mixed swards, which in turn, would have enhanced the ability of high quality alfalfa to contribute to observed CPY reductions.

3.5.4 Legume Free Sward Responses to Spraying

Given that the forage dynamics examined in this study were adjusted for unsprayed conditions (e.g. sprayed – unsprayed), these relationships could be used to

assess the impact of spraying on swards that were relatively legume free (i.e. grass dominated) at the time of spraying. Biomass responses in swards with little to no legume at spraying can be expected to respond to 1) the direct impacts of herbicide on the seeded grasses, and 2) the response of non-leguminous neighboring species present, including common weeds.

In this study, grass biomass responses to spraying depended on both the identity of the grass species involved and the study site, and varied further over time. For example, MB biomass typically remained the same or increased in legume free swards of MBALF plots. The favorable response of MB may be associated with the reduction in non-leguminous weedy species following spraying, as species such as dandelion (*Taraxacum officinale* Weber) were relatively common throughout the study plots particularly at the E2 site. However, had weed removal been a factor contributing to MB responses, increases in this grass should also have been evident in low-legume MBCLR plots, which tended to contain even more weeds. As this did not occur, these results suggest that other as of yet unknown factors may have led to the observed MB increase within legume free plots.

MB responses to spraying in plots without legume also varied with study site. At E2, MB biomass and CPY increased the first year after spraying suggesting an immediate response to herbicide application. In contrast, MB biomass at W240 experienced a 2 year lag in response. This lag may be associated with herbicide related damage to the grass species, as significant herbicide injury was observed the first year after spraying at this location (data not shown). Herbicide injury to plants included symptoms typical of a group 4 (i.e. phenoxy) herbicide (2,4-D) such as curling and twisting of stems and heads,

as well as stunting of plants (Hall *et al.* 1999). Silvertop, a common disease in brome grass swards leading to the production of sterile heads within the sward (Bailey *et al.* 2003), was also observed. Silvertop affected a large proportion (>60%) of plant culms in sprayed plots, and thus may have created additional production losses in conjunction with herbicide damage. However, it should also be noted that silvertop was also observed in unsprayed plots, and therefore this condition was unlikely to be tied directly to spraying itself, but instead may have been magnified by the herbicide application. In any case it remains unclear as to why temporal responses in MB monocultures varied between the two study sites, but may be attributed to environmental factors such as the high moisture availability and lower weed competition at E2.

Smooth brome grass responses to spraying also differed markedly between study sites. At W240, both biomass and CPY of this species decreased in legume-free SBALF plots, a trend that was evident both in year 1 after spraying and again in year 3, as well as in year 1 of monitoring within SBCLR plots. At E2 however, prominent biomass and CPY gains were evident in SB with no legume (2027 kg ha⁻¹ and 216 kg ha⁻¹ respectively), but did not occur until the second year after spraying. Additionally, similar to observations of MB at W240, SB at E2 appeared to be detrimentally impacted by spraying.

Unlike grass monoculture responses, which were largely positive, total forage biomass and CPY responses to spraying within legume-free plots were primarily negative and suggestive of small to moderate net losses in productivity. As these changes cannot be attributed to legume removal, and even coincided with select positive responses in

grass biomass, identifying the underlying cause of these reductions remains problematic. One possibility is that spraying may have reduced the vigor of perennial grasses, particularly brome grasses, in turn leading to reductions in forage biomass in subsequent years. It is also important to note that yield reductions within legume free plots in this study tended to increase with the number of years lapsed since treatment, suggesting incremental negative influences on the forage sward. Although plant species composition varied spatially throughout the study, the inclusion of unsprayed ‘temporal checks’ to assess relative yield responses in each sub-plot throughout the study period should have alleviated this concern. Nevertheless, there is the possibility that our methodology (of comparing sprayed and unsprayed subplots) did not adequately account for extreme variability in plant species composition.

3.6 Conclusion

Results of this study highlight that despite some compensatory responses in grass biomass and CPY to legume removal, the overall impact on total forage productivity generally remained negative within mixed swards, and therefore reinforces the substantial opportunity cost associated with practicing weed control in grass-legume swards. Large opportunity costs from spraying were most commonly observed from the removal of alfalfa (e.g. outcome O_1 in Fig. 3.1). Collective sward production dynamics following spraying also varied among grass species, being greater for mixtures containing MB rather than SB.

The inclusion of quality parameters such as CP with biomass (i.e. CPY) resulted in additional significant relationships between forage dynamics and legume removal, and

subsequently altered interpretation of the benefits and losses of spraying mixed swards. Spraying of the swards without legume indicated that broadleaf herbicides created a small to moderate detrimental effect to the sward independent of legume loss. Although the cause of this effect is not fully understood, it may be attributed to the timing of application, rate of herbicide applied, or another unknown cause that should be more fully investigated in future studies.

As most legumes are susceptible to broadleaf herbicides, the information from this study will be useful for assisting decision making when deciding which weed control option to undertake in mixed swards containing legumes. Situations where legume abundance is high and opportunity costs are greater, as in the case of swards with high amounts of alfalfa (particularly those comprised of MBALF) may be more conducive to mechanical methods of weed control (e.g. mowing, grazing or hand-weeding), as these options may have the ability to retain legumes within the sward. In contrast, swards with moderate to low legume abundance, or those more resistant to forage yield declines (i.e. SBALF, or mixes containing CLR) may be better suited for chemical weed control.

3.7 Literature Cited

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Table 3.1 Summary of soil characteristics at each of the W240 and E2 study sites.

Study Site	Soil Type	Location	Texture	Organic Matter	pH	NO₃-N	P	K	SO₄-S
					ppm.....			
W240	Orthic Black Chernozem	53° 31' N, 113° 33' W	Clay-Loam	13%	6.0	33	13	223	8
E2	Orthic Black Chernozem	53° 25' N, 113° 32' W	Loam	11%	6.1	16	26	170	12

Table 3.2 Overview table of all regression relationships for net grass and net total forage for biomass and crude yield protein (CPY) at W240 and E2.

Forage Mixture	Year	W240				E2			
		Grass Biomass	Total Forage Biomass	Grass CPY	Total Forage CPY	Grass Biomass	Total Forage Biomass	Grass CPY	Total Forage CPY
MBALF	Yr 1 (2006)	+	NS	+	-	NS	-	NS	-
	Yr 2 (2007)	+	-	+	-	+	NS	+	NS
	Yr 3 (2008)	+	NS	NS	-	NS	NS	NS	-
SBALF	Yr 1 (2006)	+	-	+	-	NS	-	NS	-
	Yr 2 (2007)	+	NS	+	-	NS	NS	NS	-
	Yr 3 (2008)	+	NS	+	NS	NS	NS	NS	NS
MBCLR	Yr 1 (2006)	+	-	+	NS	NS	NS	+	-
	Yr 2 (2007)	+	NS	NS	-	+	+	+	NS
	Yr 3 (2008)	NS	NS	NS	NS	NS	NS	NS	NS
SBCLR	Yr 1 (2006)	+	NS	+	NS	+	NS	NS	-
	Yr 2 (2007)	+	+	+	NS	+	NS	NS	NS
	Yr 3 (2008)	NS	NS	NS	NS	NS	NS	NS	NS

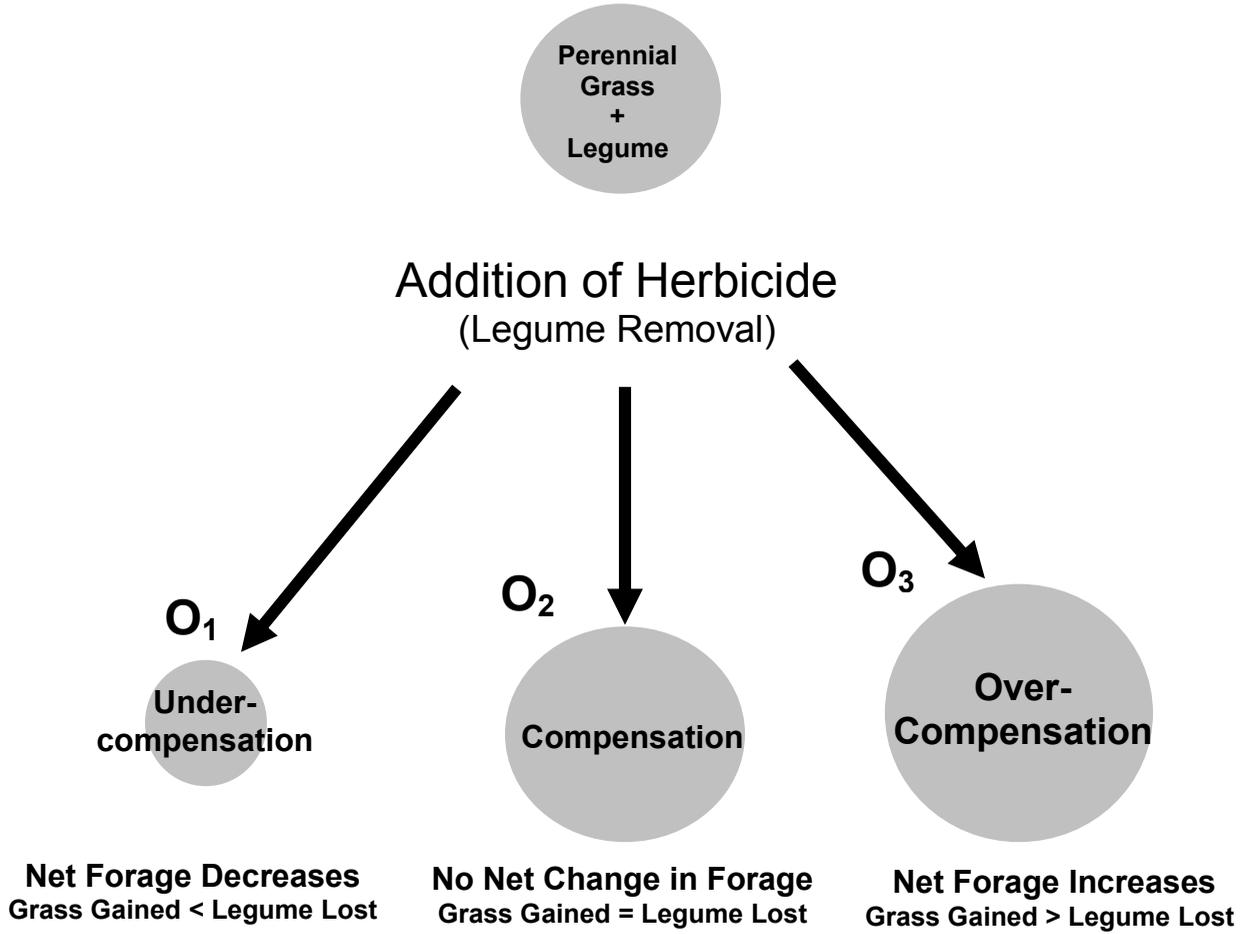


Figure 3.1 Summary of potential outcomes reflecting changes in biomass associated with spraying mixed forage swards containing grasses and legumes.

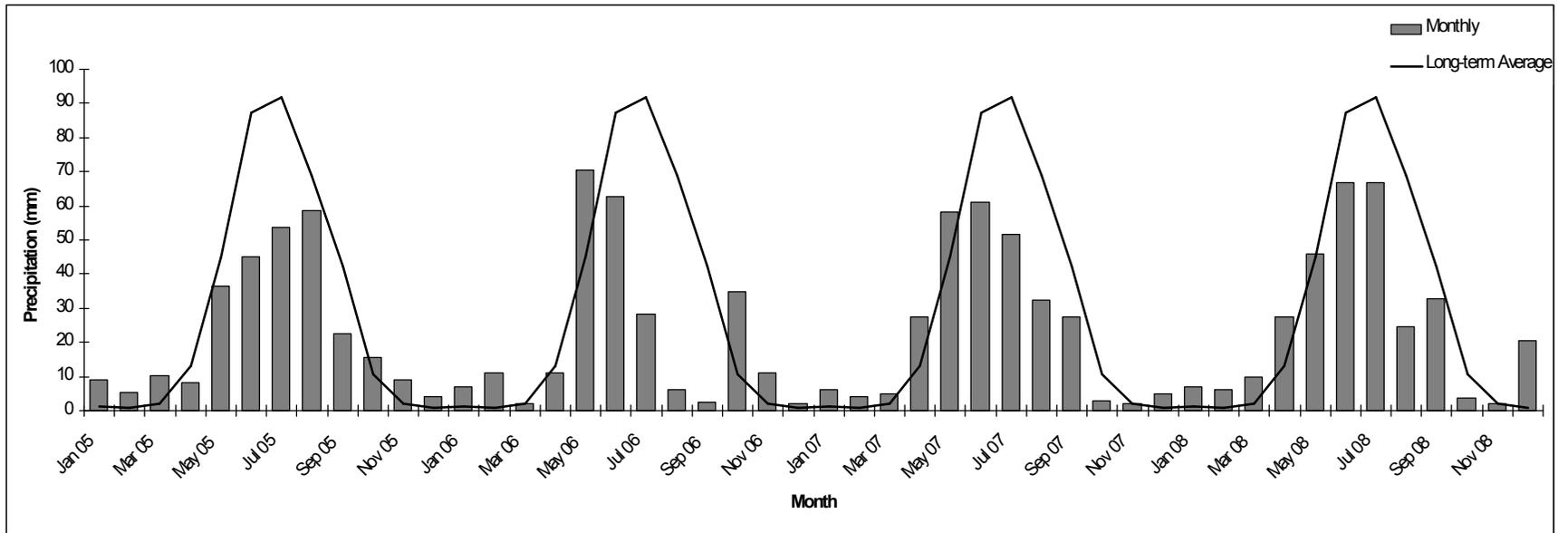


Figure 3.2 Average monthly and long-term annual precipitation (mm) at W240 for 2005-2008.

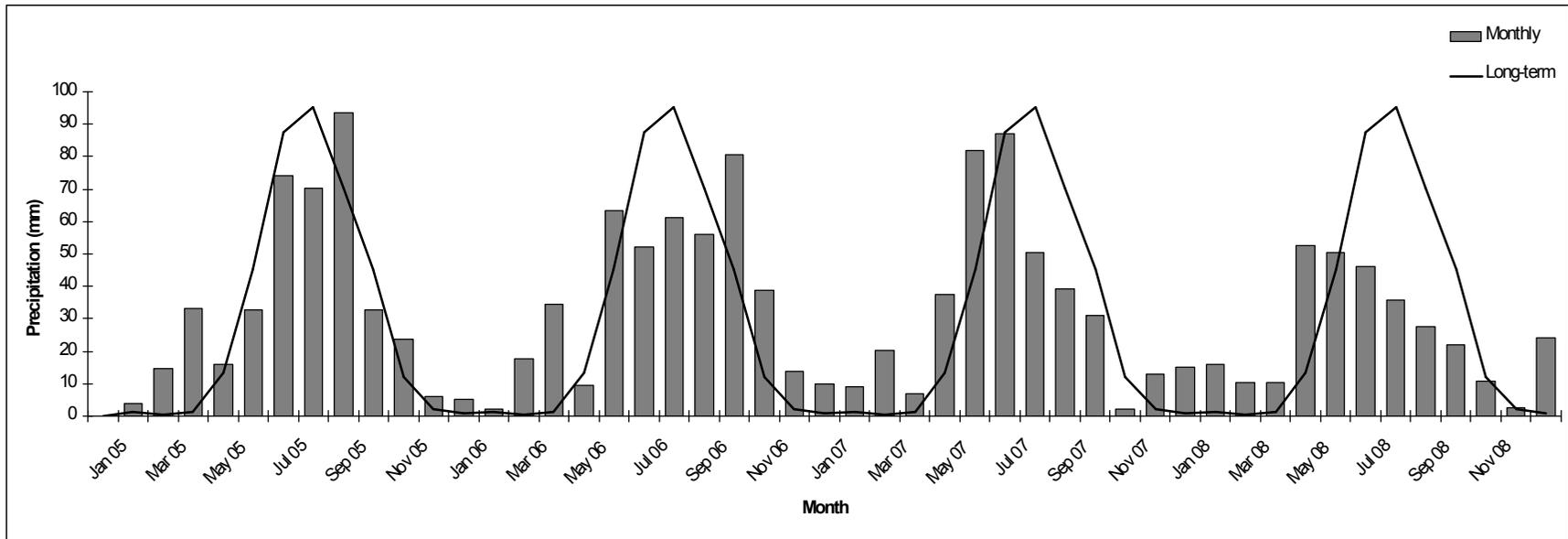


Figure 3.3 Average monthly and long-term annual precipitation (mm) at E2 for 2005-2008.

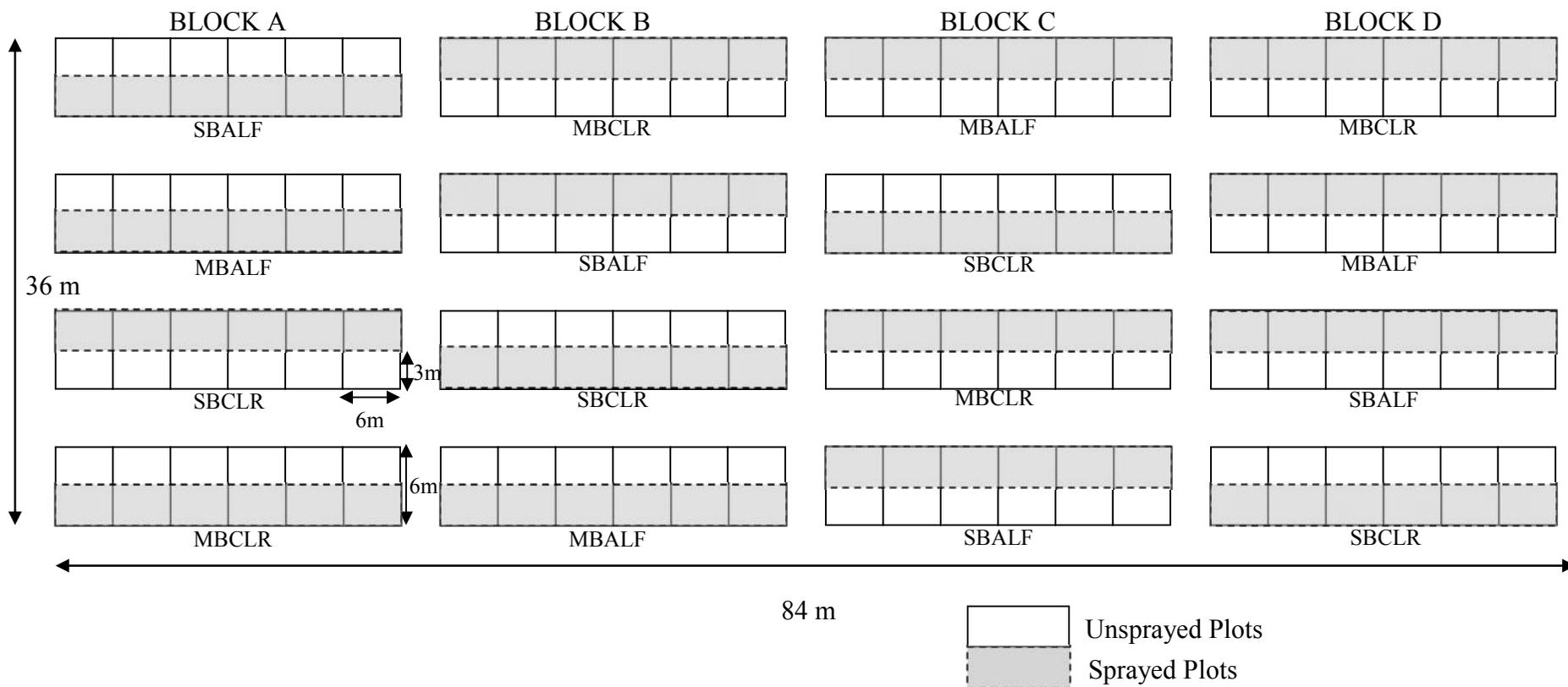


Figure 3.4 Example of a study site trial layout and treatment spraying pattern.

Block A

33 SB 67 ALF	100 SB 0 ALF	78 SB 22 ALF	0 SB 100 ALF	67 SB 33 ALF	89 SB 11 ALF
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SBALF

78 MB 22 ALF	67 MB 33 ALF	100 MB 0 ALF	33 MB 67 ALF	89 MB 11 ALF	0 MB 100 ALF
-----------------	-----------------	-----------------	-----------------	-----------------	-----------------

MBALF

67 SB 33 CLR	0 SB 100 CLR	78 SB 22 CLR	33 SB 67 CLR	100 SB 0 CLR	89 SB 11 CLR
-----------------	-----------------	-----------------	-----------------	-----------------	-----------------

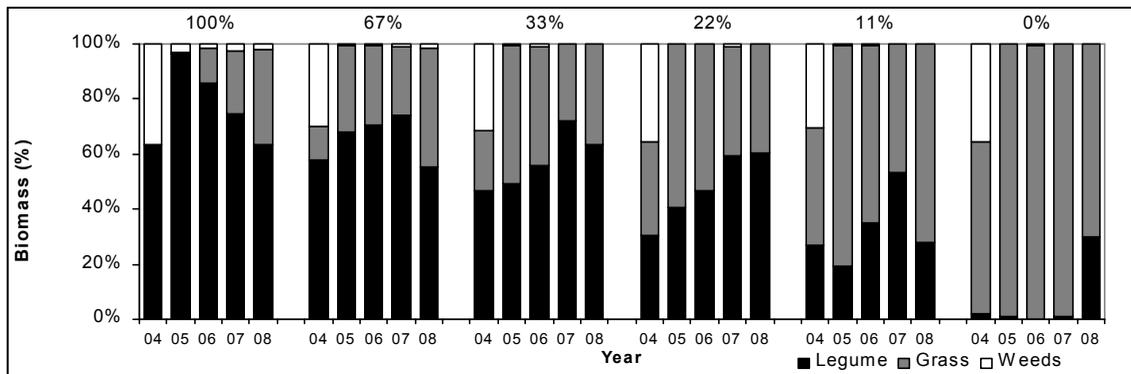
SBCLR

100 MB 0 CLR	89 MB 11 CLR	33 MB 67 CLR	78 MB 22 CLR	0 MB 100 CLR	67MB 33 CLR
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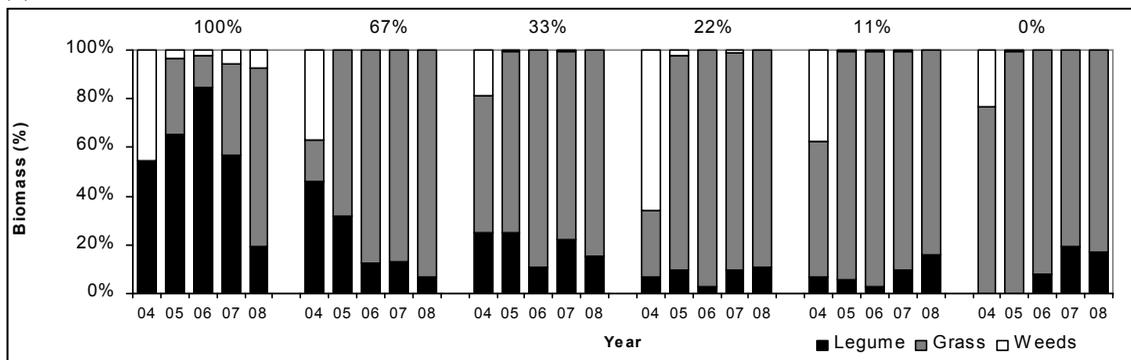
MBCLR

MB = Meadow Brome
 SB = Smooth brome: Kentucky Bluegrass
 ALF = Alfalfa
 CLR = White Clover: Alsike Clover

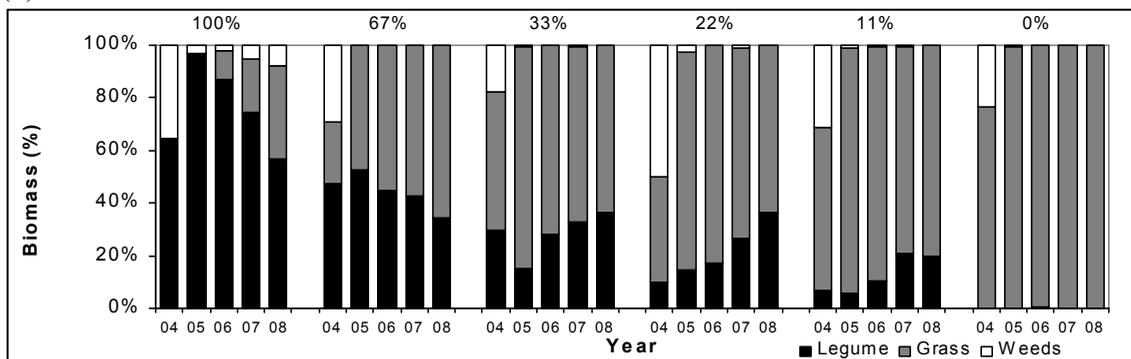
Figure 3.5 Example of the forage seeding mixture map for Block A within a study site.



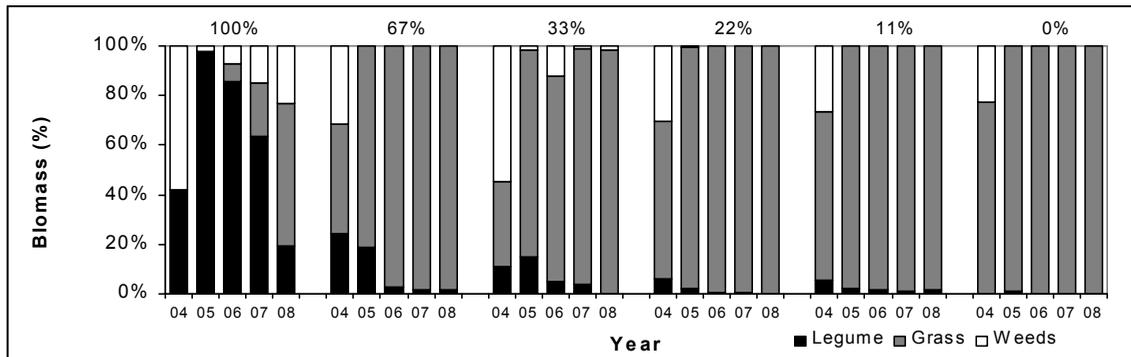
(a) MBALF



(b) MBCLR

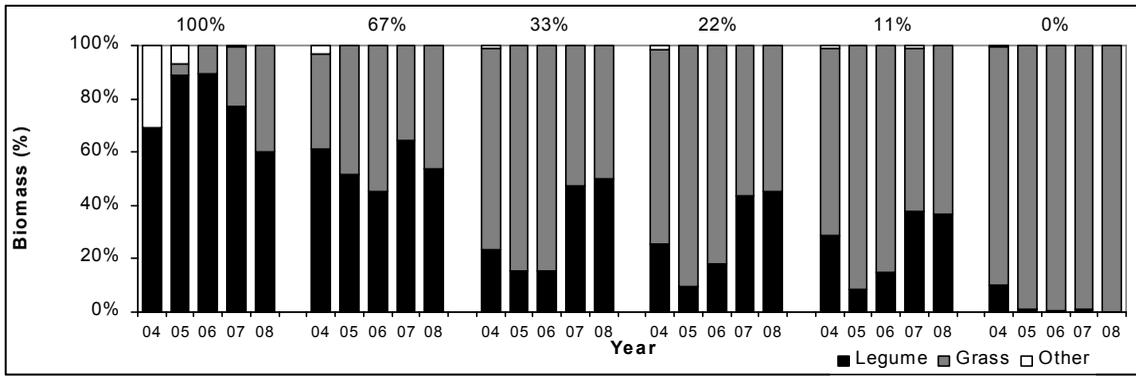


(c) SBALF

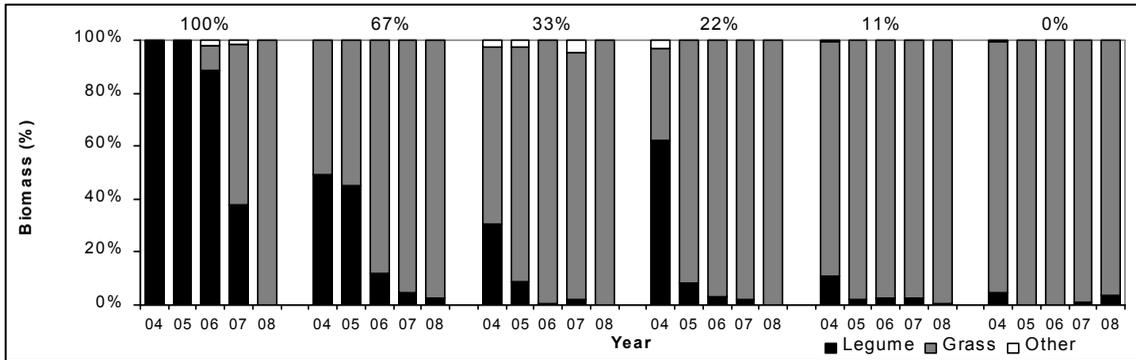


(d) SBCLR

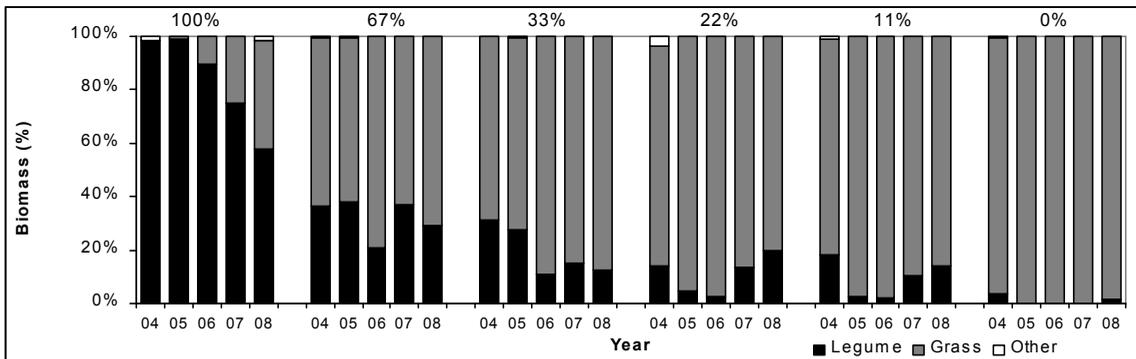
Figure 3.6 Percent biomass forage dynamics for each mixture relative to the initial amount of legume seeded within unsprayed plots at E2 over five years (2004-2008).



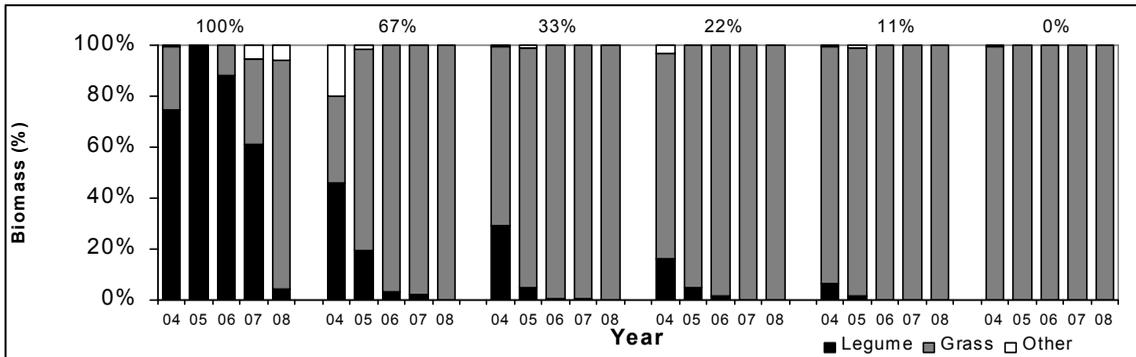
(a) MBALF



(b) MBCLR

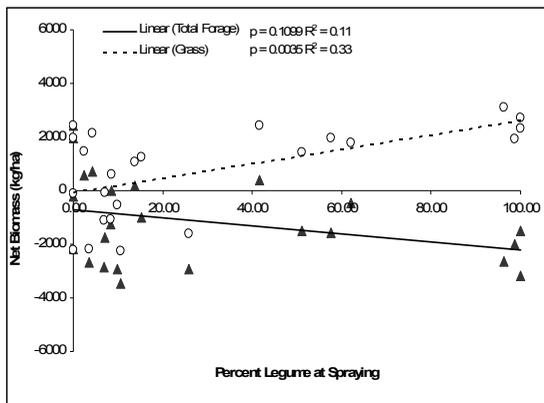


(c) SBALF

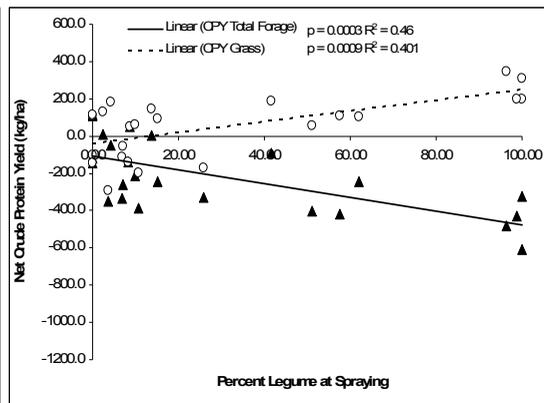


(d) SBCLR

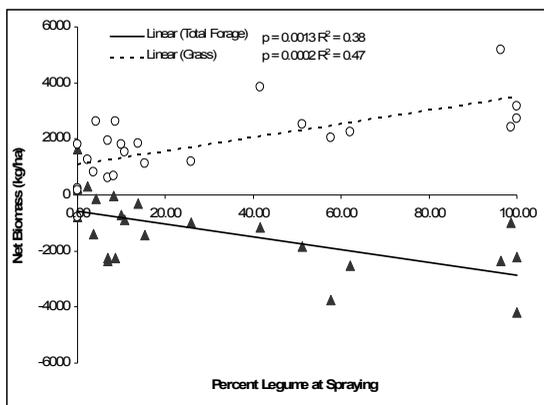
Figure 3.7 Percent biomass forage dynamics for each mixture relative to the initial amount of legume seeded within unsprayed plots at W240 over five years (2004-2008).



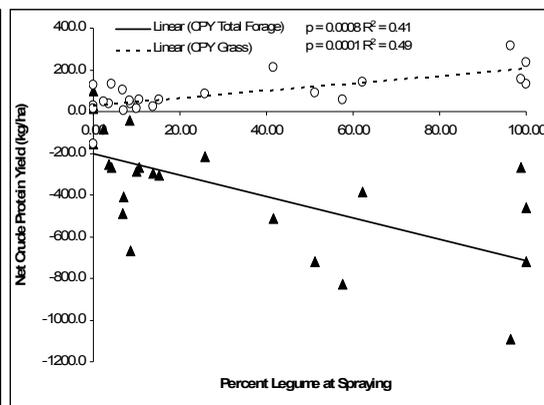
(a) Biomass – yr 1 post-spraying (2006)



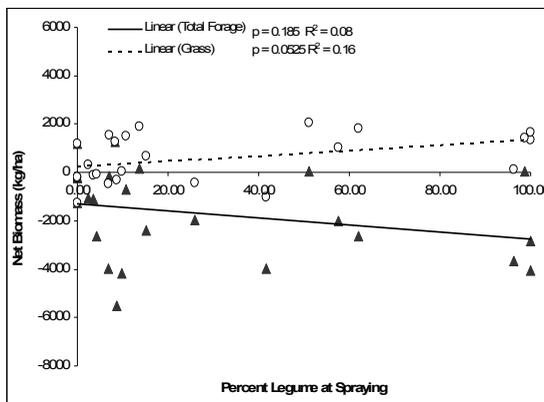
(b) CPY – yr 1 post-spraying (2006)



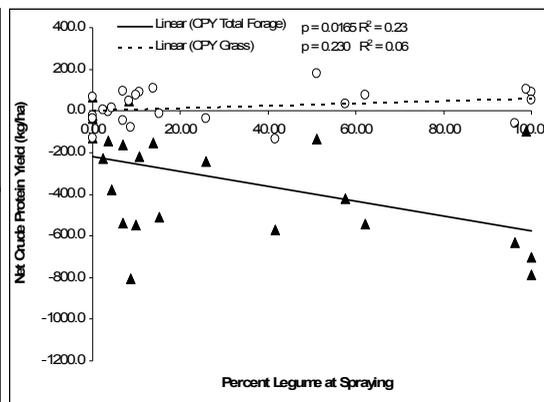
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 2 post-spraying (2007)

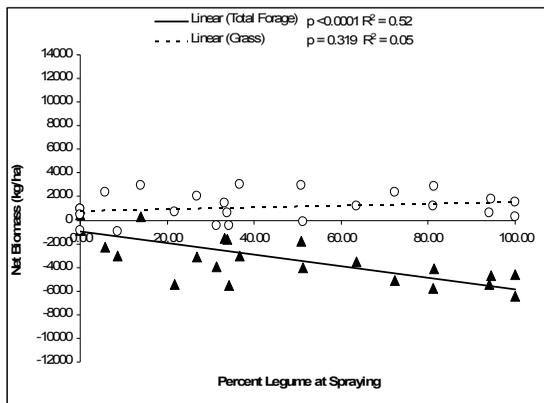


(e) Biomass - yr 3 post-spraying (2008)

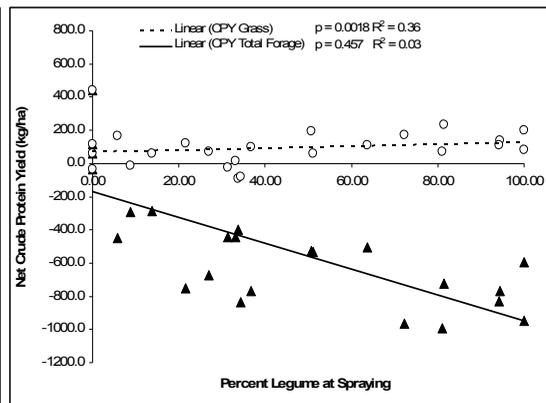


(f) CPY – yr 3 post-spraying (2008)

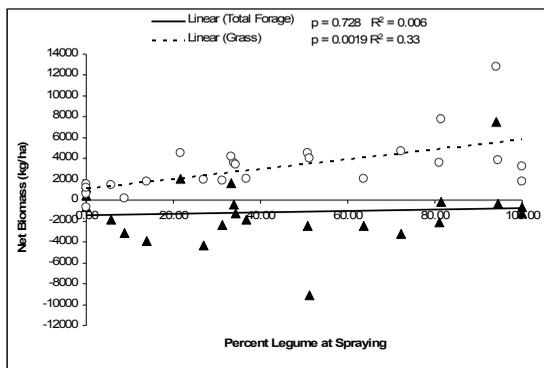
Figure 3.8 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in MBALF at W240 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.



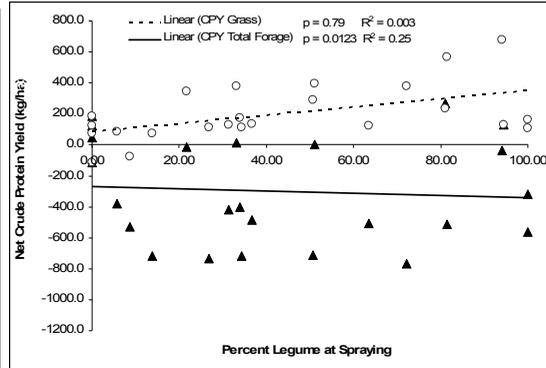
(a) Biomass – yr 1 post-spraying (2006)



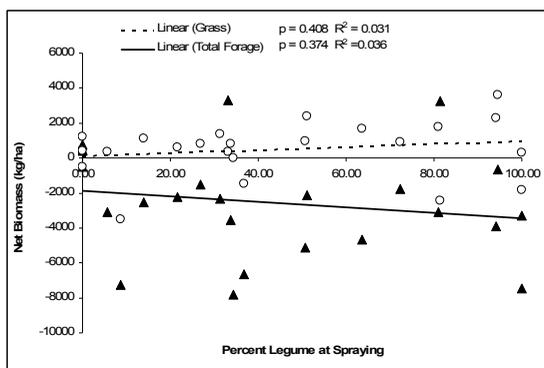
(b) CPY – yr 1 post-spraying (2006)



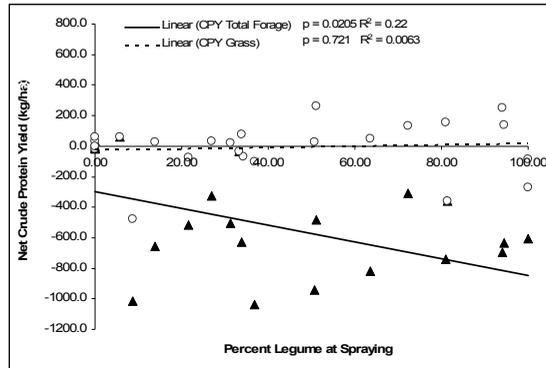
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 2 post-spraying (2007)

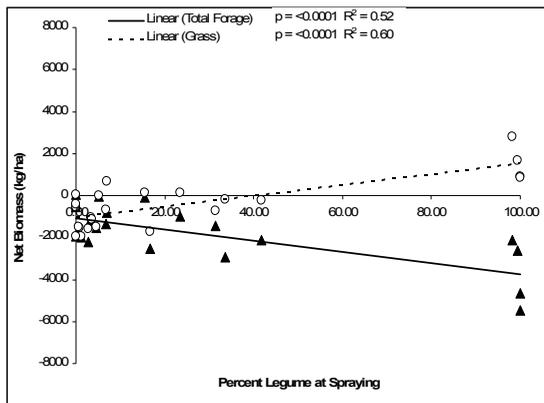


(e) Biomass – yr 3 post-spraying (2008)

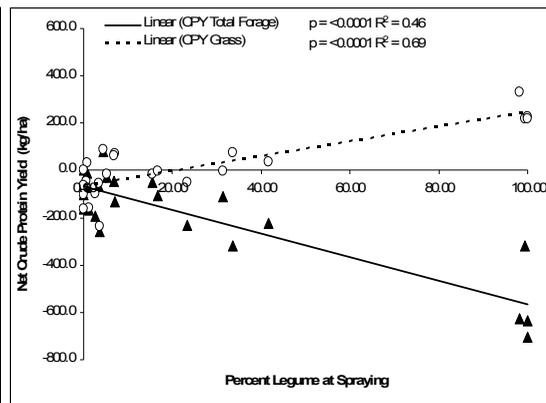


(f) CPY – yr 3 post-spraying (2008)

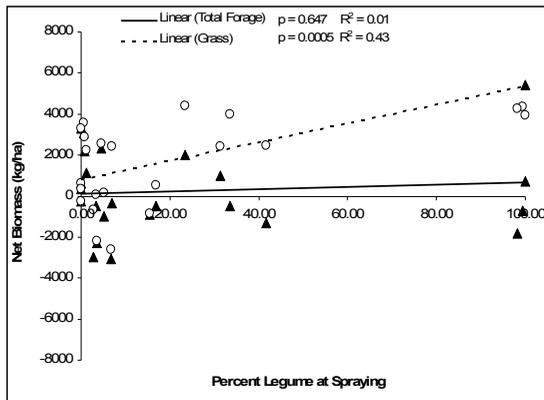
Figure 3.9 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in MBALF at E2 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.



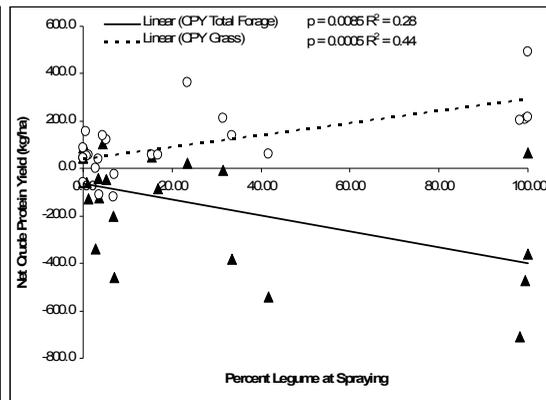
(a) Biomass – yr 1 post-spraying (2006)



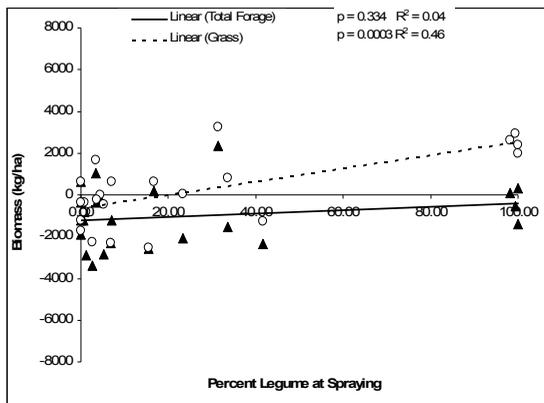
(b) CPY – yr 1 post-spraying (2006)



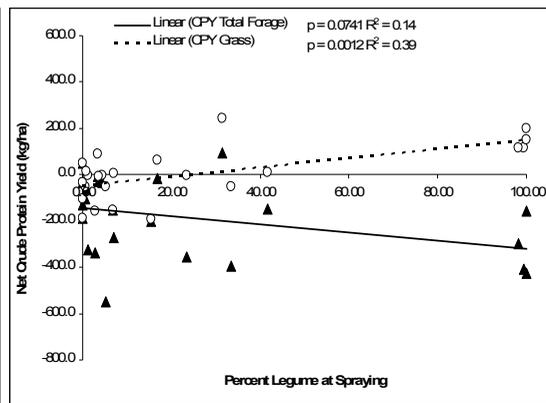
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 3 post-spraying (2007)

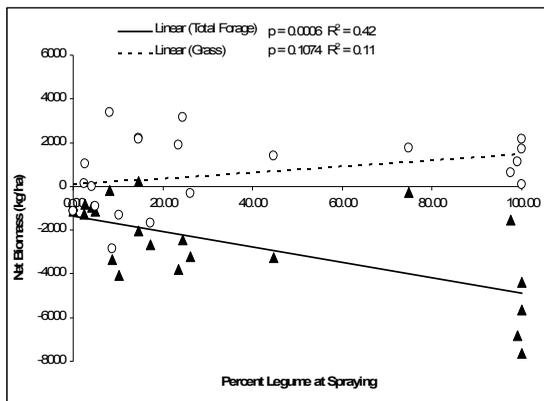


(e) Biomass – yr 3 post-spraying (2008)

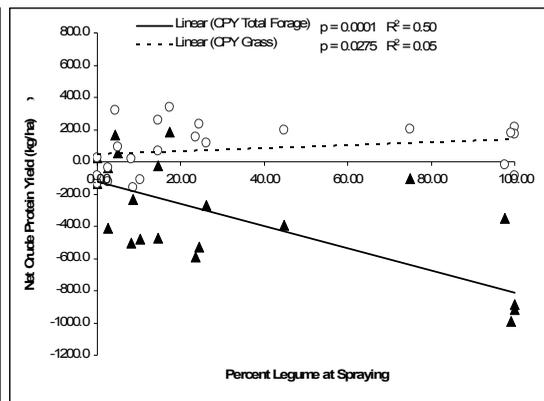


(f) CPY – yr 3 post-spraying (2008)

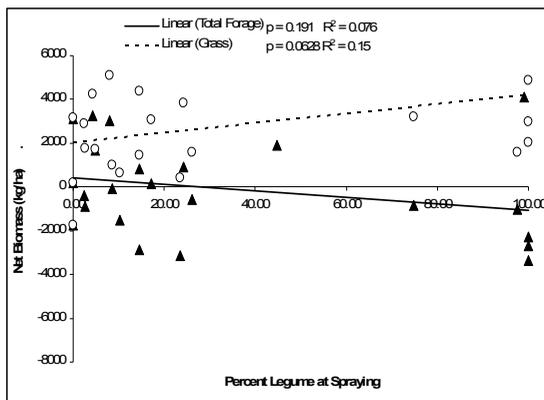
Figure 3.10 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in SBALF at W240 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.



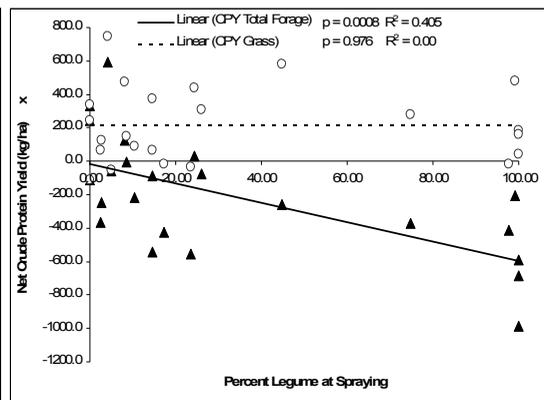
(a) Biomass – yr 1 post-spraying (2006)



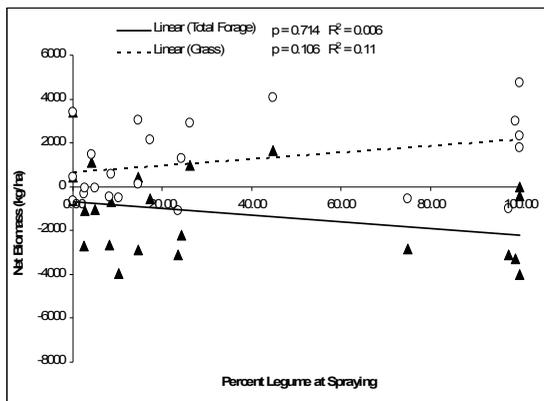
(b) CPY – yr 1 post-spraying (2006)



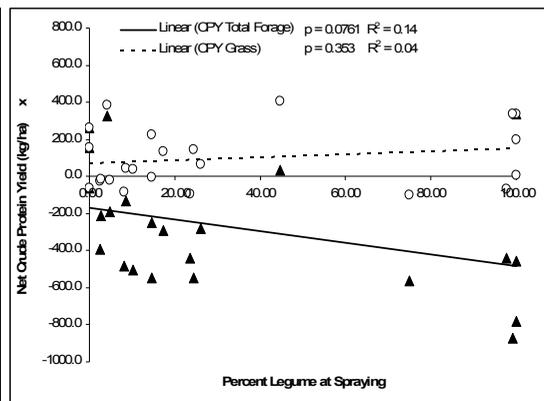
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 2 post-spraying (2007)

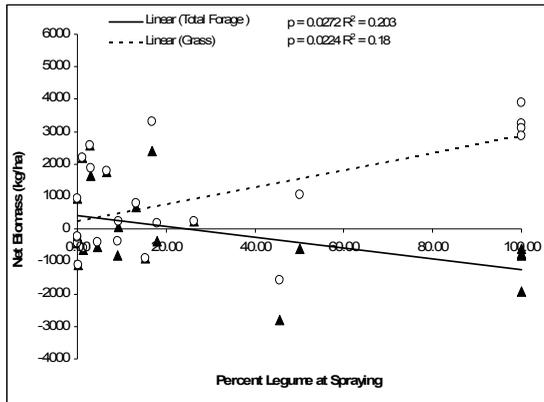


(d) Biomass – yr 3 post-spraying (2008)

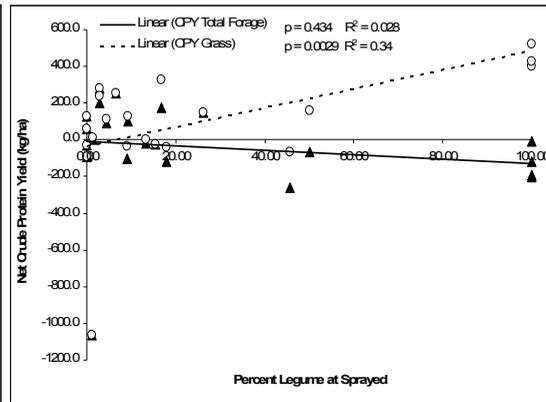


(e) CPY – yr 3 post-spraying (2008)

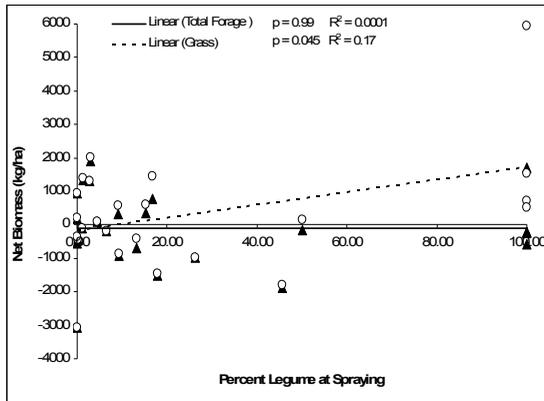
Figure 3.11 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in SBALF at E2 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.



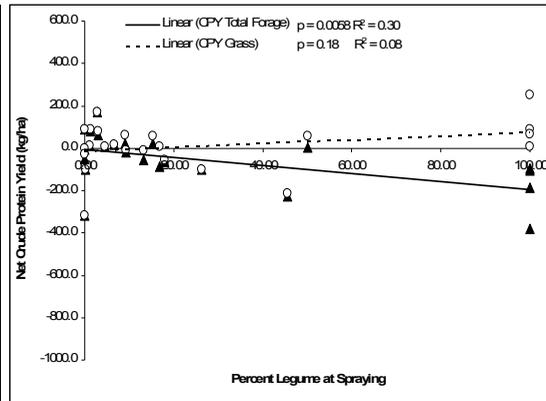
(a) Biomass – yr 1 post-spraying (2006)



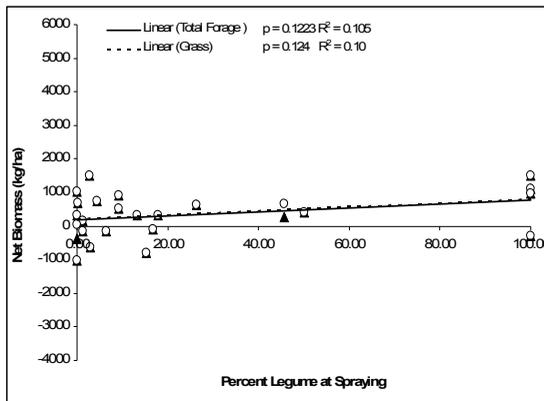
(b) CPY – yr 2 post-spraying (2006)



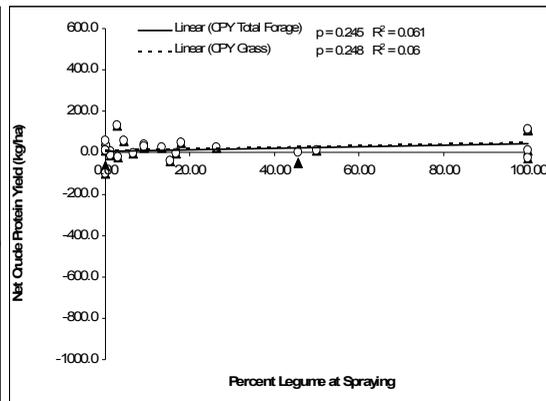
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 2 post-spraying (2007)

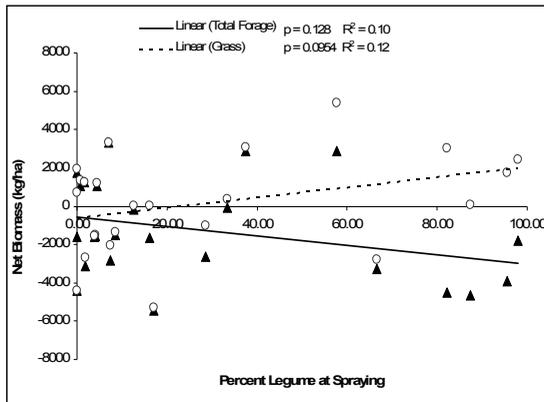


(e) Biomass – yr 3 post-spraying (2008)

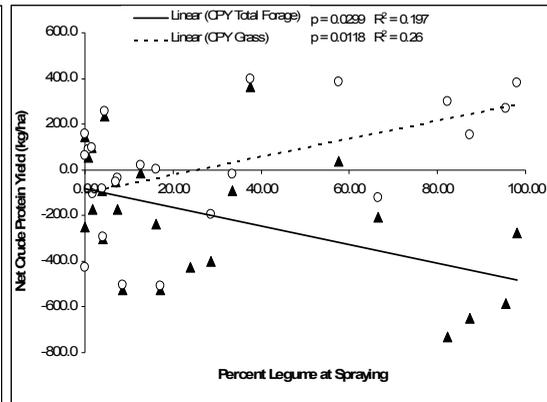


(f) CPY – yr 3 post-spraying (2008)

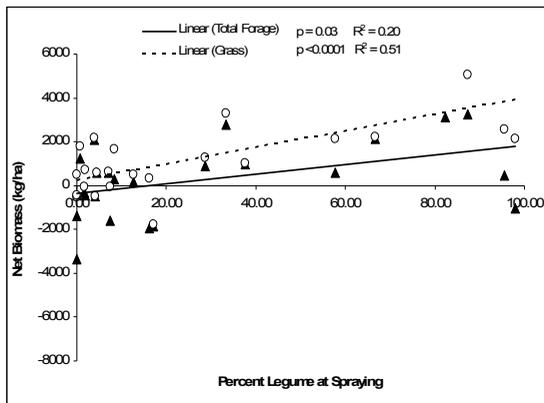
Figure 3.12 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in MBCLR at W240 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.



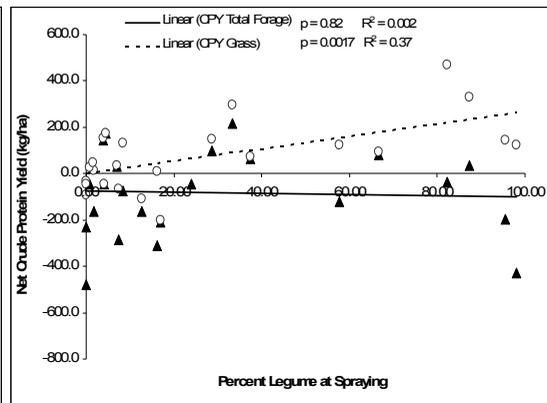
(a) Biomass – yr 1 post-spraying (2006)



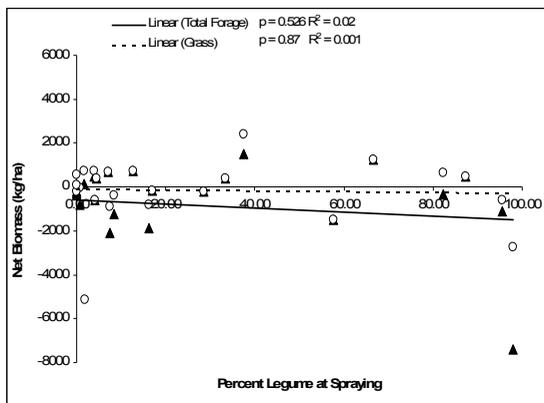
(b) CPY – yr 1 post-spraying (2006)



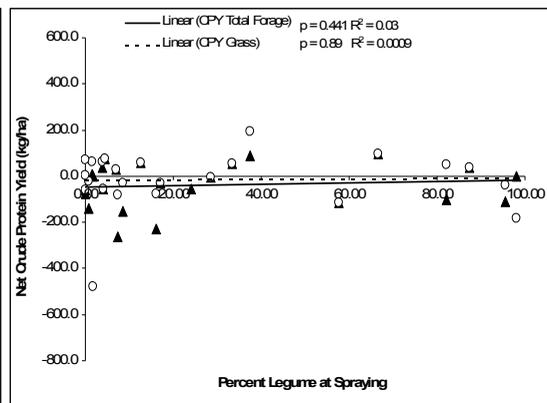
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 2 post-spraying (2007)

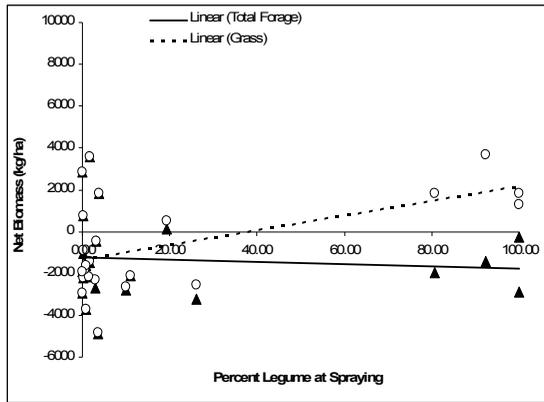


(e) Biomass – yr 3 post-spraying (2008)

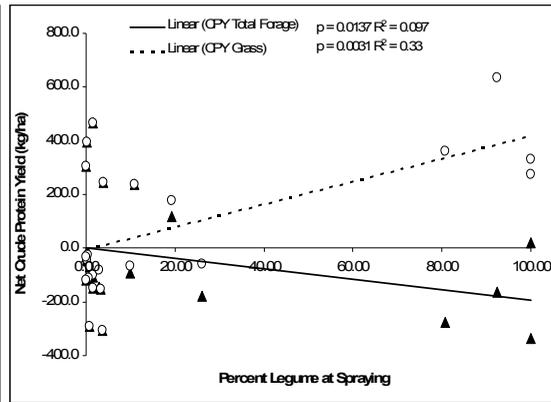


(f) CPY – yr 3 post-spraying (2008)

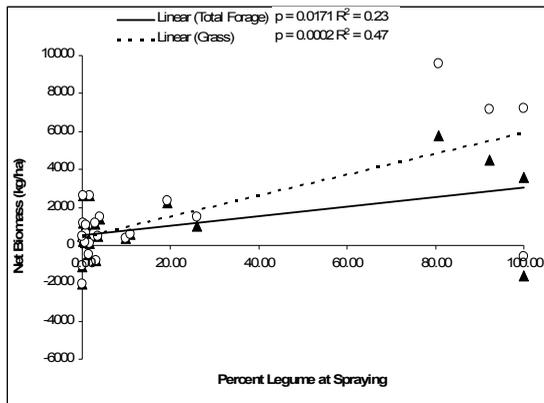
Figure 3.13 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in MBCLR at E2 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.



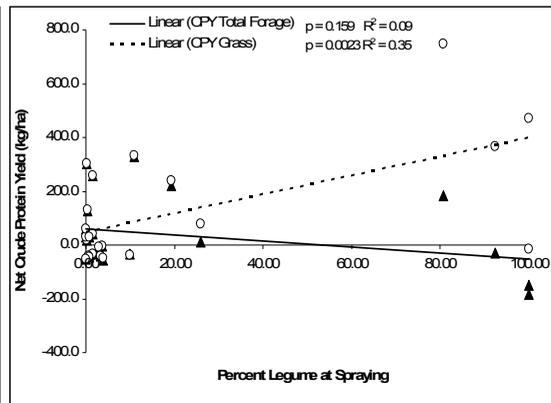
(a) Biomass – yr 1 post-spraying (2006)



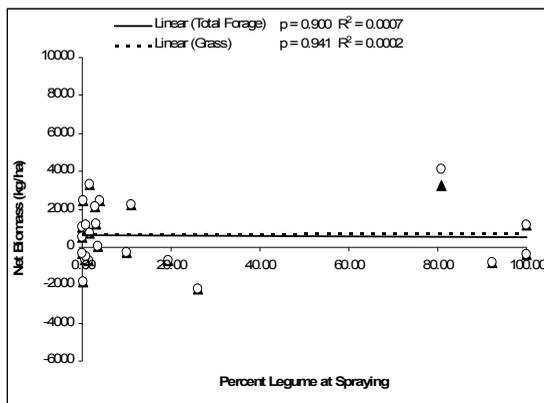
(b) CPY – yr 1 post-spraying (2006)



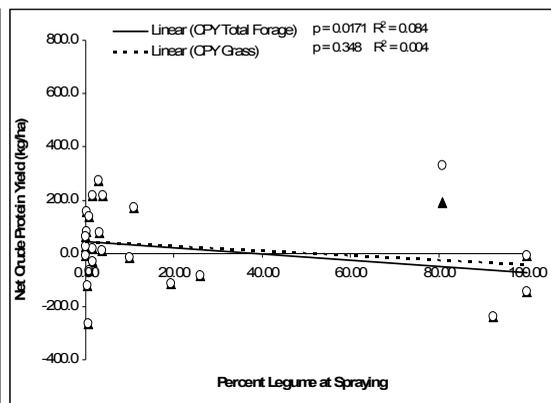
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 2 post-spraying (2007)

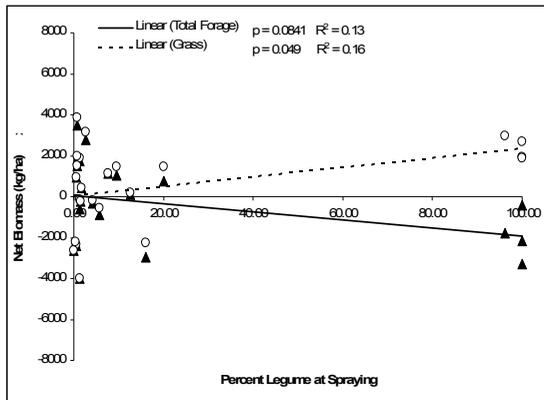


(e) Biomass – yr 3 post-spraying (2008)

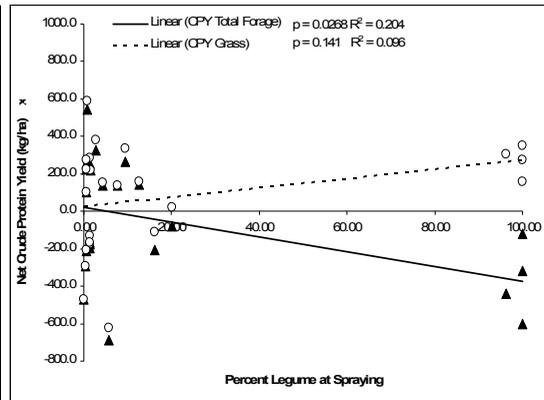


(f) CPY – yr 3 post-spraying (2008)

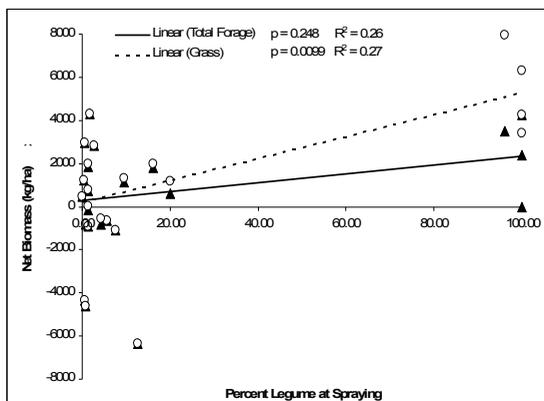
Figure 3.14 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in SBCLR at W240 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.



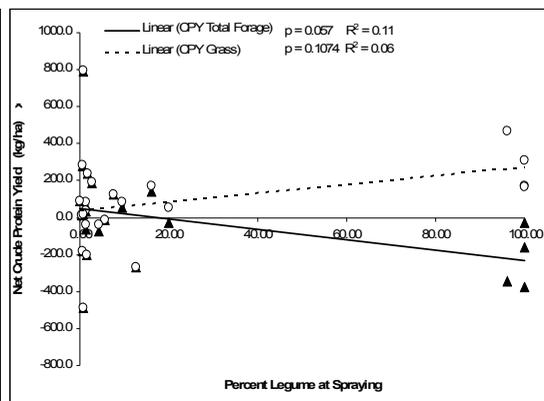
(a) Biomass – yr 1 post-spraying (2006)



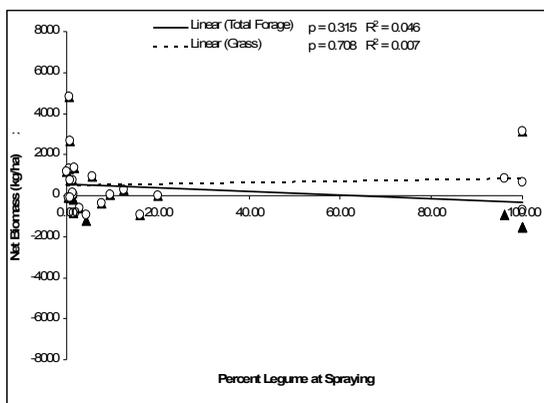
(b) CPY – yr 1 post-spraying (2006)



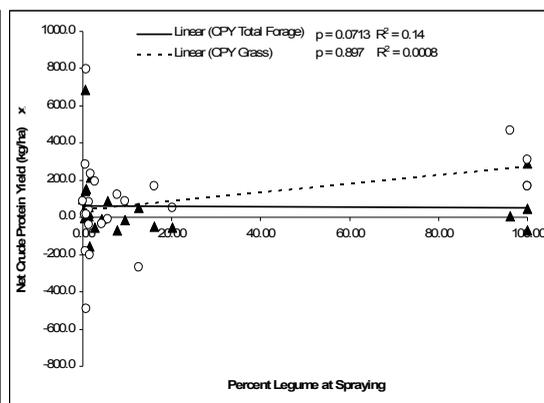
(c) Biomass – yr 2 post-spraying (2007)



(d) CPY – yr 2 post-spraying (2007)



(e) Biomass – yr 3 post-spraying (2008)



(f) CPY – yr 3 post-spraying (2008)

Figure 3.15 Net biomass (kg ha^{-1}) and CPY (kg ha^{-1}) for total forage and grass responses relative to the percent legume at spraying (2005) in SBCLR at E2 for 1, 2 and 3 years post-spraying (2006-2008) $n = 24$.

CHAPTER 4

Mixed Pasture Sward Dynamics Before and After Broadleaf Herbicide Application

4.1 Background

Established pastures are dynamic in composition and prone to the invasion of noxious weeds such as Canada thistle (*Cirsium arvense* L.). Distribution of Canada thistle is found worldwide, including temperate regions of Canada and the northern United States (Holm *et al.* 1997, Wilson and Kachman 1999). The presence of Canada thistle within swards is known to decrease productivity in agricultural crops such as wheat (O’Sullivan *et al.* 1982), barley (Hodgon 1968) and canola (O’Sullivan 1985).

Due to the highly competitive nature of Canada thistle the abundance of this weed is typically significant enough to justify weed control. Options available for weed control include mowing (Beck and Sebastian 2000; Schreiber 1967), tillage (Lukashyk *et al.* 2008), burning (Emery and Gross 2005; Tranicek *et al.* 2005), biological control (Pipers and Andres 1995), grazing (De Bruijn and Bork 2006) and herbicides (Enloe *et al.* 2007, Bork *et al.* 2007). Although the greatest weed suppression often results from an integrated approach of weed management (Masters and Sheley, 2001), broadleaf herbicides remain a popular and effective method for weed control (DiTomaso 2000).

Invasion of noxious weeds into pastures results in complex sward dynamics within pasture communities (Tracy and Sanderson 2004, Sanderson *et al.* 2007 Grekul and Bork 2004), and includes negative impacts on neighboring forage plants. For example, weeds compete for available resources (i.e. water, nutrients, sunlight, etc.) and space (Donald 1990, Booker *et al.* 2007). Within pastures, Canada thistle can reduce

forage availability (Reece and Wilson 1983, Enloe *et al.* 2007), accessibility (Haggar *et al.* 1986) and species diversity (Stachion and Zimdahl 1980). Grekul and Bork (2004) reported a reduction in pasture forage yield of up to 2 kg ha⁻¹ for each 1 kg of Canada thistle present. However, the full extent of ecological and economic impacts of the presence of noxious weeds within pasture environments remain largely unknown (Lym and Duncan 2005).

Many studies have examined the production benefits of grass-legume mixtures (Sleugh *et al.* 2000, Frame and Harkess 1987, Holt and Jefferson 1999), but few have ventured to understand the complex 3-way relationships that exist when a perennial noxious weed such as Canada thistle becomes a significant component of a mixed forage sward. Legumes are known for their ability to directly increase overall forage yields (Malhi *et al.* 2002, Popp *et al.* 2000). However, legumes may also lead to facilitation (i.e. improved growth) of neighboring plants (Nyfeler *et al.* 2009). The presence of legume species such as alfalfa or clover within mixed swards can increase the abundance of available soil nitrogen for neighboring grasses through nitrogen fixation by *Rhizobium* bacteria (Walley *et al.* 1996).

While the facilitation of grass growth in pastures from fixed N may be beneficial, increases in soil N and associated N availability for neighboring noxious weeds could also increase their abundance, and thus be detrimental to overall forage yields, including attempts to achieve weed control. In the case of Canada thistle, previous studies have demonstrated that this species responds positively to nutrient addition (Grekul and Bork 2007). Consequently, where pasture communities consist of complex mixtures that include perennial grasses, weeds and legumes, these swards are likely to experience

complex interactions among species, with both simultaneous competition for resources, and positive interactions through changes in nitrogen cycling. In these situations, the combined effects of competition and facilitation will determine the net response in pasture productivity to simultaneous legume and weed presence.

The use of broadleaf herbicides in mixed pastures containing weeds is likely to lead to a trade-off between the desirable control/suppression of a weed, and the associated undesirable loss of beneficial legumes. Although generally assumed that legume removal will result in a net loss of forage yield and quality within mixed pasture swards, this remains untested. Moreover, as legumes may provide some competition against grasses for soil resources (Hill 1990), grass compensation for legume loss could partly or fully offset the opportunity cost associated with legume removal, a response that may be further augmented by the beneficial effects of weed removal. Quantifying the conditions under which grasses may compensate for legume removal, particularly under variable weed presence, is important for producers. This information is needed to more fully quantify the benefits and opportunity costs associated with herbicide weed control, as well as identify the legume and weed abundance thresholds necessary for producers to make improved pasture management decisions.

4.2 Research Objectives

The goal of this study was to determine the nature of the relationship between grass, legume and noxious weed (i.e. Canada thistle) abundance in perennial pastures, both prior to spraying and after spraying with non-selective broadleaf herbicide. Specific objectives include:

- (1) Determine the competitive and facilitative relationships between grasses, legume, CT and forbs prior to spraying and identify yield losses associated with each component,
- (2) Evaluate effects of varying levels of Canada thistle removal and legume loss following herbicide application on total forage production in mixed pasture swards, including the identification of forage loss thresholds,
- (3) Assess the role of forage quality in altering forage dynamics, including thresholds separating net forage gains and losses, in response to legume and Canada thistle removal from within mixed swards.

4.3 Materials and Methods

4.3.1 Study Site

Two established pastures were selected as the study sites for this investigation from 2004 to 2007 inclusive. Both were located in the Aspen Parkland natural sub-region of central Alberta. Site selection was based on a minimum abundance of Canada thistle and legume, as well as relatively uniform ecosite conditions (i.e. internal slope, aspect, drainage, etc.). Each site initially contained a minimum of 30% legume cover, with abundant Canada thistle as well. Legume and thistle were not uniformly distributed

across each study sites; complete uniformity was not desired as inherent variability in legume and thistle abundance across each site was used to facilitate the assessment of inter-specific relationships among vegetation components within swards.

The first site (53° 39' N: 114° 43'W) was located at Lake Isle (LI) approximately 70 km NW of Edmonton, and was situated on a lake bed adjacent to a marsh on a Gleyed Black Chernozemic soil. This site was a well established pasture with a relatively diverse plant composition dominated by clover (primarily *Trifolium repens* L.), timothy (*Phleum pratense* L.) and smooth brome (*Bromus inermis* Leyss), with other (non-leguminous) forbs comprising up to 15% of total forage biomass.

The second site (53° 39'N: 112° 20' W) was located at the Parkland Conservation Farm (PCF) near Mundare, Alberta, approximately 90 km east of Edmonton, on a well-drained upland Orthic Black Chernozemic soil. Dominant plant species included meadow brome (*Bromus riparius* Rehm.), smooth brome and alfalfa (*Medicago sativa* L.), which had been seeded in 1999 five years prior to the start of the study. Smaller amounts of total forage biomass (up to 10%) were associated with various forb (non-leguminous) species at PCF.

Average annual precipitation for the LI and PCF region was 430 and 378 mm, respectively, with average annual temperatures for the Edmonton region at 4.3°C, with a typical frost free period of 120 days (Fig. 4.1 and 4.2). Average biomass production values for grasses, legumes and Canada thistle (CT) for all years are provided in Tables 4.1 and 4.2.

4.3.2 Experimental Design and Field Sampling

The experimental design and sampling of pasture trials consisted of two major components: (1) pre-spraying forage dynamics during 2004 and 2005, and (2) post-spraying sward responses to herbicide application during 2006 and 2007. The initial experimental design established for the pre-spraying forage dynamics component consisted of establishing 100 permanent 1 m² plots containing varying amounts of legume and Canada thistle at each site. Plots were systematically established along a series of linear transects with a minimum 1 m buffer around each plot. Plots were permanently marked in the first year of the study to facilitate easier relocations and repeated measurement throughout each additional year of the study. Each plot included a 0.25 m² (i.e. 50 x 50 cm) nested quadrat situated within the middle of a larger 1 m² permanent plot within which all biomass measurements were taken.

The second component of the study examining post-spraying responses to herbicide application was conducted by spraying 80 randomly selected plots from the original 100 at each site with a broadleaf herbicide. In the fall of 2005, aminopyralid and 2,4-D were applied at a rate of 120 g ae ha⁻¹ + 1440 g ai ha⁻¹ with a CO₂ quad sprayer using 8003 nozzles at 32 psi. The remaining 20 unsprayed plots at each site remained as unsprayed 'check' plots to account for natural temporal (i.e. inter-annual) variation in the abundance of various vegetation components over the balance of the study period. Maintenance spraying with the same equipment was used in August 2006 to apply aminopyralid at a rate of 120 g ae ha⁻¹ to remove any surviving or volunteer legumes, as well as emergent Canada thistle within sprayed plots.

Biomass data were collected annually from each of the 0.25 m² permanent quadrats at peak biomass (mid July to early August) and sorted to perennial grasses, legumes, CT and other forbs/weeds. All harvested biomass was sorted into each vegetation component (i.e. grass, legume, forb and CT), bagged, oven-dried at 60°C to constant mass, and weighed. Both study sites were fenced and protected from April to August of each year to prevent any grazing prior to sampling. Light fall grazing with cattle occurred on both sites, which helped prevent the excessive accumulation of litter and allowed both sites to remain consistent with typical land use practices in the region.

4.3.3. Forage Quality Analysis

After weighing all biomass samples were ground separately through a 2 mm screen in a Wiley mill grinder and sealed until further analysis for crude protein (CP) concentration. CP values for all samples were derived from nitrogen (N) values obtained using a LECO TruSpec Autoanalyzer. Nitrogen values were converted into CP using equation 1, where N is equal to the nitrogen value obtained from each sample.

$$\%CP = \%N \times 6.25 \quad (1)$$

CP concentrations were further converted into estimates of crude protein yield (CPY) by combining CP and respective biomass values from each plot using equation 2.

$$CPY \text{ (kg/ha)} = [\%CP/100] \times \text{Biomass (kg/ha)} \quad (2)$$

4.3.4 Statistical Analysis

All data pre- and post-spraying at each site were tested separately for normality and homogeneity of variances using Proc Univariate in SAS (SAS Institute Inc. 1988).

Due to the absence of discrete categorical treatments, a graphical distribution of normality and homogeneity of variances was used in conjunction with the Kolmogorov-Smirnov test to evaluate the data. Pre-spraying 2005 data were square root transformed for all vegetative components to obtain normality; in addition post-spraying independent variables of each vegetative component were square root transformed. Post-spraying dependent RYR variables (defined below) for 2006 and 2007 met the assumptions of normality and therefore remained non-transformed.

Data for each study site were analyzed separately due to the large differences in site characteristics, species composition and age of the swards. In this investigation, a regression approach was used to analyze the data to exploit the high variability in biomass components among plots within these pastures, with separate analyses conducted for pre-spraying and post-spraying data.

Data prior to spraying (2005) were analyzed with a stepwise multiple regression using Proc Stepwise in SAS (SAS Institute Inc. 1988) to determine the individual and additive relationship of individual vegetation components (i.e. grass, legume, CT and forb) on one another among the 100 sample plots, as well as on overall total forage biomass. 'Forage' was defined as the combined biomass of grass and legume components. All vegetation biomass data were converted to kg ha^{-1} for analysis. Regressions were evaluated using model significance ($p < 0.05$), goodness of fit (R^2), and regression coefficients (B) for each independent variable to evaluate their importance within each site. Positive coefficients were considered indicative of facilitation (i.e. positive association), while negative coefficients were considered indicative of competition (i.e. yield reduction).

As spraying in the fall of 2005 resulted in removal of the legume and CT from sprayed plots within each site, post-spraying sward dynamics (kg ha^{-1}) were assessed using a modified regression approach. Post-spraying responses were confined to net forage (grass + legume) responses, and were further modified using forage values from unsprayed plots to account for natural variation in the abundance of legumes, even in the absence of spraying. Distinct inter-annual variation was observed in the biomass data, including that of legumes (see Table 4.1 and 4.2) in unsprayed plots throughout the study period. As this variation could have led to important changes in the inferred ‘opportunity cost’ of spraying (i.e. natural declines in legume should not be attributed to herbicide application), these adjustments were necessary to more accurately quantify the impact of legume removal on actual forage sward dynamics.

Forage responses following spraying were therefore adjusted for each sprayed plot by dividing biomass from each sprayed plot by the calculated mean forage production for the same year within unsprayed plots ($n=20$ per site), which still contained legumes. This modification produced a forage relative yield ratio (RYR) that allowed changes in forage productivity following spraying to be determined despite natural changes in the abundance of legume. RYR production values greater than 1 were equal to net increases in total forage relative to unsprayed plots, while RYR less than one indicated a decrease in total forage production. RYR responses within sprayed plots were calculated for biomass and CPY separately for each of 2006 and 2007, 1 and 2 years after spraying, respectively, using equation 3:

$$\text{Relative Yield Ratio (RYR)} = \text{Total Forage}_S / \text{Average Total Forage}_{US} \quad (3)$$

where Total Forage_S is the production of each individual sprayed plot (n=80) within a year (i.e. 2006 or 2007), and Average Total Forage_{US} is the total production of all the unsprayed (n=20) plots averaged within the same year (i.e. 2006 or 2007). The biomass (kg ha⁻¹) of each vegetative component present within each plot was used as the independent variable for all analysis of forage RYR data, and required square root transformation prior to analysis.

Regression analyses included two complementary approaches. First stepwise regressions were performed in SAS using Proc Stepwise (SAS Institute Inc. 1988) for each site-year of data on the RYR of biomass and CPY to determine those independent variables most closely associated with post-spraying yield responses. This analysis was further supported through the use of simple linear regressions performed in SAS using Proc Reg (SAS Institute Inc. 1988) of the primary vegetation components of legume, CT, legume + CT, or legume + CT + forb (independent variables) against RYR biomass and CPY responses (dependent variables). This additive approach enabled direct comparison of the models associated with each component, and therefore assisted in separation of the role of each factor in accounting for observed yield responses. Additionally, one other model was examined that included non-CT forbs (i.e. largely weeds such as dandelion) in combination with legumes and CT removal.

Finally, all significant regression models ($p < 0.05$) were used to solve for the specific threshold abundance of legume, CT, or combined vegetation components, separating increases in net forage (i.e. $RYR > 1$) from decreases in net forage (i.e. $RYR < 1$) following spraying. This was done by solving for X (i.e. component biomass) values

at the point where RYR was equal to 1, and back-transforming (thresholds = X^2) to obtain the original independent variables. RYR thresholds were solved to where $X = 1$ as this was the point where production within the sprayed plots equaled the production of unsprayed plots.

4.4 Results

4.4.1 Pre-Spraying Forage Relationships

Total forage biomass (grass + legume) was negatively associated with forb biomass at both PCF and LI (Table 4.3). In contrast, CT biomass at each site had no relationship with total forage. Among individual biomass components, grass was negatively associated with both legume and forb biomass (Table 4.3). Based on the regression coefficients, forb biomass demonstrated the greatest impact on grass at both LI ($B = -0.352$) and PCF ($B = -0.798$), regardless of pasture composition (See Appendix C. Table C.1).

At the clover dominated LI site, legume biomass was related only to grass ($p < 0.0001$): increases in grass biomass were associated with sharp decreases in clover ($B = -0.567$). A similar result was observed at PCF, but included grass ($B = -0.461$) and forb biomass ($B = -1.111$) with alfalfa (Table 4.3). CT had no clear relationship with legume biomass at either study site ($p > 0.08$).

Biomass of CT was not associated with any of the other vegetation components of the sward at LI (Table 4.3). In contrast, CT biomass was positively related to forb biomass at PCF ($B = 0.165$). Also at PCF, legume ($B = -0.056$) and grass ($B = -0.063$)

biomass had a weak but significant association with forb biomass (Table 4.3). At LI, only grass biomass was negatively related to forbs ($B = -0.165$).

4.4.2. Net Forage Responses Post-Spraying

The legume based mixed pastures examined here demonstrated distinct responses in both forage biomass and CPY to the combined removal of legume, CT and forbs, for two consecutive years after spraying. However, strong differences were observed in the magnitude and directionality of those relationships, depending on the study locations (LI vs. PCF), sampling year (first vs. second year post-spraying), and even growth forms (CT vs. legume), with subsequent implications for the detection of thresholds separating net forage yield gains from losses.

4.4.2.1 Clover Dominated Sward (LI)

Relative yield ratios in the clover based swards at LI revealed few relationships between forage RYR and the independent variables examined. During 2006, the RYR of both forage biomass (Table 4.4) and CPY (Table 4.5) remained non-significant ($p > 0.21$) for most variables, with the lone exception of forbs during 2006 on forage biomass, where a weak association was evident. Stepwise regressions supported the limited effects found during 2006 (Table 4.3), with only forb biomass exhibiting any relationship with forage RYR in that year. However the impact of forb on RYR remained relatively weak ($R^2 = 0.05$; $B = -0.008$).

During 2007, two years after spraying, forage RYR at LI demonstrated more relationships with the abundance of initial broadleaf components of the sward. Stepwise

regression revealed that both legume and other forb components were associated with variations in forage RYR (Table 4.3), with both components negatively associated with changes in RYR. Moreover, the inclusion of forage quality measures in RYR (i.e. through CPY estimates) generally led to stronger relationships (greater R^2) between broadleaf sward components removed and observed forage dynamics. Although changes in legume typically accounted for more variation in forage compared to other forbs, B coefficients for each component suggested that RYR declined more in response to increasing initial forb removal rather than legume (Table 4.4 and 4.5).

Given the limited relationship among sward components at LI, thresholds identifying net increases in RYR could only be quantified relative to the amount of legume removed (i.e. significant models in Tables 4.6 and 4.7) in 2007, the second growing season after spraying. Significant thresholds of legume abundance during 2007 at LI were identified as 2311 kg ha⁻¹ and 3728 kg ha⁻¹ for the RYR of biomass and CPY, respectively (Table 4.8), with levels below this likely to lead to net increases in RYR. The marked increase in the RYR threshold of CPY relative to biomass suggests a greater opportunity cost when measures of forage quality are included in assessing sward dynamics following spraying.

4.4.2.2. Alfalfa Dominated Sward (PCF)

Changes in RYR within the alfalfa based sward at PCF were much more developed than in the clover based sward at LI, with immediate changes in RYR evident in 2006, as well as in longer-term responses in 2007. At this location, the initial abundance of legume consistently appeared as the leading vegetation component

associated with changes in RYR during both years, a finding consistent regardless of whether measures of forage quality were included in the assessment of RYR (Table 4.4 and 4.5). Notably, regression coefficients (B) for legume removal were distinctly negative for both estimates of RYR in biomass (B = -0.0144 and -0.0312) and CPY (B = -0.0102 and -0.0312) for 2006 and 2007, respectively, and were also greater in 2007 than the previous year. In addition to legume, CT demonstrated a relationship, albeit weaker, with both forage RYR responses (biomass and CPY). However, unlike legume, RYR values were positively associated with increasing initial CT removal (Table 4.4 and 4.5), and were associated with high B coefficients (max B = 0.2505 and 0.2234 for biomass and CPY respectively), suggesting CT removal led to substantial forage yield increases. Finally, the inclusion of forage quality into RYR assessments for this component (i.e. CT) generally reduced model R^2 values at PCF, though these relationships remained significant (Table 4.5).

Linear regression of vegetation components at PCF, alone and in combination with one another, against RYR revealed many significant relationships (Table 4.6 and 4.7). Similar to the stepwise regression results, model goodness-of-fit values were greatest for legume, but were also marginally improved with the inclusion of CT and forb biomass. Pronounced variation in model fit was also apparent among years of assessment, and was not based on whether quality was included in the assessment of RYR. In general, the leading models for all vegetation components occurred with biomass RYR during 2006 ($R^2 > 0.16$), with subsequent declines one year later ($R^2 < 0.11$) (Table 4.6). Similarly, explanatory models for RYR of CPY declined from 2006 ($R^2 < 0.13$) to 2007 ($R^2 < 0.10$).

Final linear regressions between vegetation components and RYR values of both biomass and CPY revealed distinct thresholds separating net positive from negative yield responses following broadleaf plant removal via spraying (Table 4.8). While thresholds were relatively low for legume removal the first year following spraying (i.e. biomass: 370 kg ha⁻¹; CPY: 864 kg ha⁻¹), these increased sharply during the second year (i.e. biomass and CPY thresholds were 6658 and 4218 kg ha⁻¹, respectively). In contrast, thresholds for CT removal were very low, ranging from 10 to 107 kg ha⁻¹ (Table 4.8).

Thresholds also generally increased when broadleaf components were combined during analysis against RYR, but only in 2006. For example, the legume threshold increased from 370 kg ha⁻¹ to 567 kg ha⁻¹ with the inclusion of CT, and increased again to 884 kg ha⁻¹ with the inclusion of CT and other forb biomass. A similar trend was evident for CPY in 2006 (Table 4.8). During 2007 however, thresholds were generally maximized with the use of legume biomass only when predicting RYR responses: for example, the inclusion of CT and forb biomass reduced the threshold separating positive from negative RYR values from 6658 kg ha⁻¹ to 5294 kg ha⁻¹ (with CT), which further declined to 4849 kg ha⁻¹ (with CT and forb).

4.5 Discussion

4.5.1 Relationships within Swards Prior to Spraying

Total forage (i.e. grass + legume) present prior to spraying was negatively associated with the abundance of other forbs, but not CT, at both study locations. However, sward dynamics within each of the study sites also appeared to be influenced by species composition, site characteristics, stand age, and management practices, all of

which are known to influence the abundance of weedy species that can enter a pasture (Masters and Sheley 2001, DiTomaso 2000). Despite similar abundances of other forbs at the two locations ($x = 233\text{-}340 \text{ kg ha}^{-1}$ per plot), total forage yield losses at LI were 0.486 kg ha^{-1} for every 1 kg ha^{-1} of forb biomass, which was less than the $-0.759 \text{ kg ha}^{-1}$ observed at PCF.

The study site at LI was situated on a floodplain, leading to abundant moisture availability due to the shallow water table, coupled with periodic nutrient influx during flooding. Abundant resources combined with the presence of a low growing legume and tall statured grasses may have decreased competition between vegetation components at this location. Holmgren *et al.* (1997) reported that moisture and light had a varied effect on competition that depended on microsite conditions. At the more mesic LI site in the current study, positive increases in light availability likely outweighed slight increases in moisture. Moreover, limited defoliation (i.e. once at peak growth) would have resulted in increased grass growth and reductions in light for clover in the understory. In contrast, moisture limitations likely had a greater impact than light availability at the more xeric PCF site. Forage losses may have been greater at the PCF site due to the location of this study site on an upland where plant resources such as water and nutrients were likely to be much more limited, thereby creating a more competitive environment between legumes, grasses and forbs (Holmgren *et al.* 1997, Walker and Chaplin 1987, Callaway and Walker 1997). Additionally, rainfall was markedly lower at PCF than LI during 2005 (Figs. 4.1 and 4.2).

The absence of a strong negative impact from CT at both study sites was unexpected given that previous studies in the region have demonstrated significant

declines in forage availability due to CT (Grekul and Bork, 2004). This observation was particularly surprising at LI given that CT was more than five times as abundant as other forbs sampled (Table 4.2). The apparent impact of forbs rather than CT suggests that other broadleaf plants may be playing a larger role in suppressing total forage production. Dominant broadleaf plants other than CT and legume at the LI and PCF sites included dandelion, common plantain and perennial sow thistle (see Appendix C for full species list). Dandelion was the most common forb and found in all plots at both sites with average abundances of 14% and 33% at PCF and LI, respectively. The widespread distribution of this species and its ability to rapidly adapt and spread throughout a wide range of environments explains the abundant distribution of dandelion throughout both study sites, as well as the observed competitive impact on total forage production. Froese and Van Acker (2003) reported yield losses of between 39% and 64% in canola with 50% ground cover of dandelion.

The limited impact of CT at both sites suggests that either this plant species was not sufficiently abundant to have a detectable association with other vegetation component in 2005, or more likely, that CT may be occupying niches not filled by other key forage components (i.e. grasses and legumes). At LI, despite representing as much as 1584 kg ha⁻¹ of biomass, CT exhibited no association with any other vegetation component, suggesting CT was a relatively common 'passenger' within that community, potentially occupying ecological niches unexploited by other plants (MacDougall and Turkington 2005). In contrast, while CT was decoupled from legume abundance at PCF, CT was positively associated with other forbs at this location. While it is possible that CT and other forbs were facilitating one another, there is a simpler alternative

explanation for the observed covariance between these vegetation components. The latter may simply reflect auto-correlation between these components, as both CT and weeds such as dandelion are both adapted to a wide range of environments. In turn, this allows species such as CT to compete well with other plants for resources (Donald 1990, Stewart-Wade *et al.* 2002), and are therefore both likely to benefit from the decline of neighboring plants and the increased availability of light, water and nutrients.

Outside of CT, several additional relationships were apparent among individual vegetation components at each study site. Consistent with the negative impact of other forbs on total forage, grass biomass tended to decline at both sites with increasing forbs. However, legume biomass was also negatively associated with grasses, suggesting there was direct competition between the primary forage components (legume and grass) within each study site. At LI, reductions in clover were likely exacerbated by the favorable growing conditions for neighboring grasses. Dominant grasses at the moisture rich LI site included smooth brome and timothy, both of which are more aggressive species in a mesic environment (Otfinowski *et al.* 2007, Kunelius *et al.* 2006), which in turn may have contributed to the marked yield loss coefficient (-0.567) in clover. With its low creeping growth habit (Frame 2005), clover is known to be susceptible to reductions in light availability (Frame and Harkess 1987, Sheaffer 1989), and favorable growing conditions would have maximized grass growth (i.e. plant height) and therefore the associated decline in clover at this location. Moreover, as study sites were fenced to prevent grazing prior to September of each year, grasses would have been able to reach their full growth potential by late summer, further adding to the decline in clover. Grazing has been shown to favor the retention of short-statured, light dependent species

such as white clover (Burdon 1983, Evans et al. 1998), and thus, repeated defoliation throughout the growing season could have led to a different outcome.

Similar to LI, the legume component (i.e. alfalfa) at PCF also appeared to be susceptible to competition, albeit at a reduced intensity from grasses based on observed yield loss coefficients ($B = -0.461$). Being a tall-statured plant with a well developed taproot, alfalfa is known to be a good competitor (Sleugh *et al.* 2000, Mortenson *et al.* 2005, Holt and Jefferson 1999). These conclusions are supported here by the relatively lower susceptibility of alfalfa at PCF, as compared to competition from smooth brome, the predominant grass at this location. However, alfalfa was also negatively associated with neighboring forb abundance ($B = -1.11$), which together with the impact of grasses, resulted in a greater net negative impact on alfalfa at the PCF location than clover at LI. Overall, these results reinforce the variable presence of competitive processes among vegetation components across environments (i.e. drier upland > sub-irrigated meadows), and differences in forage species (i.e. clover > alfalfa). In this case, despite the superior competitiveness of alfalfa against neighboring grasses, greater net competition appeared to result at PCF due to the combined impact of grasses and other forbs, coupled with drier conditions.

4.5.2. Forage Responses to Herbicide Application

The removal of legumes typically had the greatest influence on post-spraying total forage production responses at both study sites, although contributions from forb and CT remained important at the LI and PCF locations, respectively. Overall pasture production dynamics following spraying appeared to be a complex response dependent on initial site

characteristics and pasture composition (including legume species). Finally, the inclusion of forage quality into the assessment of RYR resulted in changes to the interpretation of net forage dynamics to spraying; significant responses in CPY tended to closely parallel those of biomass RYR from both sites, with marginally stronger estimates for CPY in some situations, and weaker estimates in others.

4.5.2.1 Forage Responses in the Clover Dominated Sward

At the clover dominated LI site, total forage RYR in 2006 failed to demonstrate any relationship to the removal of broadleaf vegetation components, which is contrary to the yield loss expected with the loss of clover at this location. The absence of any yield loss may be due to the limited biomass present of vegetation components other than grass ($< 638 \text{ kg ha}^{-1}$), or due to the prompt ability of grasses to respond to the removal of neighboring vegetation, largely clover. Clover is known to have abundant fine roots in the shallow soil layer (Caradus 1990) that contribute to the cycling of N and C pools below-ground (Rasmussen *et al.* 2007). Finer roots have been reported by Dubach and Russelle (1994) and Fornara *et al.* (2009) to have more rapid senescence and decomposition than coarse roots (Rasmussen *et al.* 2007). Rapid breakdown of the clover roots, together with removal of competition from clover, could account for why average grass biomass increased from 4070 kg ha^{-1} prior to spraying in 2005 to 7735 kg ha^{-1} in 2006. Moreover the favorable moisture status of soil at the clover dominated LI site would have helped facilitate grass growth, as well as lead to more rapid decomposition of clover (and other dicot) roots due to increased microbial activity. Microbial activity

within the soil is a direct function of soil moisture, temperature and nutrients (Fornara *et al.* 2009).

In 2007, forage RYR of biomass and CPY both declined with increasing legume removal, and to a lesser extent, forb removal, although yield loss coefficients remained relatively small, and thresholds separating yield gain from loss were quite high (2311 and 3728 kg ha⁻¹ for biomass and CPY, respectively). Moreover, the inclusion of forage quality (through CPY) led to greater yield losses than for biomass, suggesting the effects of clover removal at LI were becoming evident on forage production two years after spraying (see Appendix D). These results are important, as they suggest that although the short-term impact of clover removal may be negligible within sites such as this, the long-term impacts of clover removal may lead to increasing forage losses, assuming 1) legumes do not recolonize the site from the seed bank or seeding, and 2) resident grass species remain unable to fully compensate for initial legume removal. By virtue of their ability to exploit the soil rhizosphere more effectively, mixed forage swards generally have been shown to be superior in forage production (Fustec *et al.* 2010). At the LI study site, the predominant grass, smooth brome, is known to lead to production declines in stagnant, long-lived swards (Lardner *et al.* 2001), with large inputs of N necessary to increase production (Otfinowski *et al.* 2007).

Post-spraying forage dynamics exhibited no response to the removal of CT at the LI site, reinforcing the earlier notion that this weed is filling an ecological niche that other plants are not competing directly for. These results suggest CT abundance within sites such as LI originate exclusively from other factors such as moisture regime (i.e. sub-irrigation and enhanced fertility) or management (i.e. overgrazing), flooding, drought,

etc., the combination of which may favor the establishment and persistence of CT over other species.

4.5.2.2 Forage Responses in the Alfalfa Dominant Sward

Forage RYR declined with increasing legume removal both 1 and 2 years after spraying, indicating the loss of legumes resulted in a substantial opportunity cost in forage availability shortly after spraying. This is not surprising given the high initial presence of alfalfa at this location ($x = 1412 \text{ kg ha}^{-1}$), the removal of which would represent an immediate loss of forage, and subsequently lead to the low legume threshold separating net yield gains ($< 370 \text{ kg ha}^{-1}$ initial legume) from net yield losses ($> 370 \text{ kg ha}^{-1}$). Notably, the decline of forage from legume removal was at least partly offset by a positive relationship between forage RYR and CT removal at the PCF location.

Moreover, yield gain coefficients from CT removal remained greater ($B \geq 0.062$) in 2006 than those from legume loss in the same year ($B \sim -0.014$), suggesting grasses within the sprayed sward may have been responding more strongly to CT removal than legume loss.

Yield gains following CT removal suggest that despite the limited abundance of CT at this location, and in contrast to the findings of the pre-spraying relationships, CT appeared to have a negative impact on forage production, and its removal led to prompt yield improvements. However, model RYR responses tended to show little change in observed threshold values, as the inclusion of CT with legume abundance into the model increased the threshold separating positive from negative yield changes to 566 kg ha^{-1} , which theoretically represents little more than the additive effect of legume and CT respectively (i.e. $370 + 168 = 538 \sim 566$).

Forage RYR decreases with increasing legume removal remained present during 2007, the second growing season after spraying, and even increased in yield loss coefficients ($B \sim -0.031$ for both biomass and CPY). The larger yield loss in 2007 may have arisen from limited legume survival the year before, or conversely, suggests that some compensation occurred within the grass component of the sward to legume removal. Despite the importance of legume removal during 2007, it is notable that the modeled threshold for legume separating yield gains from losses rose sharply from the year before (to 6658 kg ha^{-1}). This shift was unexpected, but appears to indicate that the net effect of legume removal may have been a large increase in complementary grass biomass following legume removal.

Grass biomass can be expected to increase at the PCF site due to both 1) the direct removal of alfalfa as a strong competitor (Holt and Jefferson 1999), and 2) the subsequent release of N from the decomposing root systems of alfalfa (Dubach and Russelle 1994, Haby *et al.* 2006), which is known to release as much as 258 kg ha^{-1} of N into the soil (Burity *et al.* 1989). As alfalfa has a relatively deep penetrating root system with a large taproot (Fustec *et al.* 2010, Frame 2005), and the PCF site was situated on an upland with more limited moisture, these conditions would favor slower decomposition of dead roots (Fustec *et al.* 2010), and therefore help explain the two year delay to the observed response. Regardless of the cause, this delay highlights the need for pasture managers to understand that the complete implications of spraying pasture swards may extend well beyond one year, in part due to the long-term dynamics associated with root turnover (i.e. mineralization) and nutrient uptake.

Also of note is that the inclusion of forage quality into RYR models changed forage responses and RYR thresholds substantially. During 2006, the inclusion of quality shifted thresholds from 370 kg ha⁻¹ to 864 kg ha⁻¹, suggesting a greater tolerance for legume removal at the time of spraying. In 2007 however, the inclusion of quality resulted in the opposite shift, with the new threshold of 4218 kg ha⁻¹ (see Appendix D). This shift would be expected, however, as grasses should not be as high in N concentration as legumes themselves, thereby reducing the tolerance to legume loss. Nevertheless, as both the thresholds for RYR biomass and CPY remained at very high levels, these results suggest that the removal of alfalfa within sites such as PCF may result in long-term benefits despite substantive short-term costs, which are also likely to vary further depending on the type and abundance of various weed populations.

4.6 Conclusion

Results of this study indicated that pre-spraying sward dynamics and responses to herbicide weed control were strongly influenced by species composition, site characteristics and management practices. Total forage production before spraying was negatively associated with forb biomass, largely dandelion rather than CT. The lack of a strong relationship between forage production and CT suggests the latter was more of a 'passenger' species within the pastures examined here occupying empty ecological niches rather than competing directly with other vegetation.

Responses to the use of herbicides within the two swards resulted in both negative and positive relationships with respect to forage production. The greatest influence on forage was associated with the removal of legumes from swards at both sites. Legume

removal had the largest negative impact in alfalfa based swards, with the grass component unable to fully compensate for legume removal in either of the two years after spraying. Thresholds calculated for clover and alfalfa suggest that losses were greater for forage quality from 2006 to 2007 but the opposite for biomass.

Observed relationships prior to spraying will help establish the role of each vegetational component with respect to contributing to total forage production. An improved understanding of sward responses to weed control through the use of herbicides will allow for more informed decisions about how different vegetation components, particularly how different legume species affect forage yield and quality. Thresholds calculated in this research help clarify the costs and potential benefits associated with spraying of mixed pastures including legume removal and associated compensatory responses of the grass component.

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Table 4.1 Average annual biomass in unsprayed plots during pre-spraying (2005[†]), one (2006[‡]) and two (2007[‡]) years post spraying at the PCF study site.

Component & Year	Mean	Std Dev.	Max	Min
Perennial Grass				
2005 [†]	4260	1849	7012	0
2006 [‡]	8477	2732	13824	1272
2007 [‡]	5839	2631	8788	676
Legume				
2005 [†]	1412	1691	4808	0
2006 [‡]	956	738	2172	0
2007 [‡]	35	86	276	0
Canada Thistle				
2005 [†]	168	239	932	0
2006 [‡]	149	363	1004	0
2007 [‡]	0	0	10	0
Forbs ^Ψ				
2005 [†]	223	140	580	0
2006 [‡]	844	444	1332	0
2007 [‡]	39	61	204	0
Total Biomass				
2005 [†]	6034	2463	11400	2196
2006 [‡]	10436	2398	14400	4776
2007 [‡]	5913	2570	9428	1100

[†] Parameters are calculated for all plots prior to spraying (n=100)

[‡] Parameters are calculated for remaining unsprayed plots (n=20)

^Ψ Component excludes legume and CT

Table 4.2 Average annual biomass in unsprayed plots during pre-spraying (2005[†]), one (2006[‡]) and two (2007[‡]) years post spraying at the LI study site.

Component & Year	Mean	Std Dev.	Min	Max
Perennial Grass				
2005 [†]	4070	1605	1492	7964
2006 [‡]	7735	2126	3044	12528
2007 [‡]	5138	3326	1332	8332
Legume				
2005 [†]	683	584	4	1732
2006 [‡]	329	581	0	1720
2007 [‡]	32	127	0	556
Canada Thistle				
2005 [†]	1584	1752	12	3348
2006 [‡]	1549	1146	0	4380
2007 [‡]	560	315	0	1244
Forbs ^ψ				
2005 [†]	240	175	4	632
2006 [‡]	1179	552	0	1736
2007 [‡]	85	115	0	484
Total Biomass				
2005 [†]	6577	2344	3868	12888
2006 [‡]	10794	2239	6752	14992
2007 [‡]	5816	3063	2716	8520

[†] Parameters are calculated for all plots prior to spraying (n=100)

[‡] Parameters are calculated for remaining unsprayed plots (n=20)

^ψ Component excludes legume and CT

Table 4.3 Empirical relationship between the biomass (kg ha^{-1}) of various vegetation components assessed prior to spraying (2005) at LI & PCF study sites.

Site	Dependent Component	Adjusted R^2	Independent Component	Partial R^2	Model R^2	B^a	Prob > $ F ^b$
LI	Total Forage ^c	0.0944	Forb	0.094	0.094	-0.486	0.0019
			CT	--	--	--	> 0.5
			Intercept	--	--	77	< 0.0001
LI	Grass	0.257	Legume	0.19	0.189	-0.352	< 0.0001
			Forb Biomass	0.068	0.257	-0.487	0.0038
			CT Biomass	--	--	--	> 0.50
			Intercept	--	--	79	< 0.0001
LI	Legume	0.20	Grass Biomass	0.19	0.189	-0.567	< 0.0001
			Forb Biomass	0.011	0.2	-0.249	0.25
			CT Biomass	--	--	--	> 0.50
			Intercept	--	--	61	< 0.0001
LI	CT	0.029	Forb Biomass	0.021	0.021	-0.44	0.16
			Legume Biomass	0.008	0.029	-0.12	0.37
			Grass Biomass	--	--	--	> 0.50
			Intercept	--	--	44	< 0.0001
LI	Forb ^d	0.098	Grass Biomass	0.071	0.071	-0.165	0.005
			CT Biomass	0.013	0.084	-0.04	0.23
			Legume Biomass	0.014	0.098	-0.057	0.21
			Intercept	--	--	28	< 0.0001
PCF	Total Forage ^c	0.113	Forb Biomass	0.091	0.091	-0.759	0.014
			CT Biomass	0.023	0.114	-0.337	0.12
			Intercept	--	--	93	< 0.0001
PCF	Grass	0.17	Legume Biomass	0.089	0.089	-0.297	0.0002
			Forb Biomass	0.064	0.15	-0.798	0.027
			CT Biomass	0.015	0.17	-0.33	0.19
			Intercept	--	--	91	< 0.0001
PCF	Legume	0.20	Grass Biomass	0.089	0.089	-0.461	0.0002
			Forb Biomass	0.085	0.174	-1.111	0.013
			CT Biomass	0.026	0.20	-0.542	0.0817
			Intercept	--	--	85	< 0.0001
PCF	CT	0.131	Forb Biomass	0.098	0.098	0.348	0.017
			Legume Biomass	0.017	0.115	-0.058	0.082
			Grass Biomass	0.016	0.131	-0.054	0.19
			Intercept	--	--	9	0.035
PCF	Forb ^d	0.172	CT Biomass	0.098	0.098	0.165	0.017
			Legume Biomass	0.031	0.129	-0.056	0.013
			Grass Biomass	0.044	0.172	-0.063	0.027
			Intercept	--	--	18	< 0.0001

^a Regression coefficient.

^b Probability of the *F*-test for each variable within the model to determine the best overall fit of the regression model.

^c Total forage includes grass + legume.

^d Forb biomass included all broadleaf weeds excluding CT.

Table 4.4 Stepwise multiple regressions for biomass relative yield ratios (RYR) relationships one (2006) and two (2007) years after spraying relative to the vegetation components removed at spraying (2005) at each of the LI and PCF study sites.

Site	Dependent	Adjusted R ²	Independent	Partial R ²	Model R ²	B ^a	Prob > F ^b
LI	RYR 2006	0.066	Forb	0.049	0.049	-0.008	0.0481
			Legume	0.018	0.066	-0.0021	0.23
			CT	--	--	--	>0.5
			Intercept	--	--	1.19	<0.0001
LI	RYR 2007	0.20	Legume	0.11	0.106	-0.009	0.0027
			Forb	0.096	0.20	-0.021	0.003
			CT	--	--	--	>0.5
			Intercept	--	--	1.75	<0.0001
PCF	RYR 2006	0.31	Legume	0.25	0.25	-0.014	<0.0001
			CT	0.063	0.31	0.084	0.0097
			Forb	--	--	--	>0.5
			Intercept	--	--	0.9701	<0.0001
PCF	RYR 2007	0.24	Legume	0.057	0.057	-0.031	<0.0001
			CT	0.18	0.24	0.25	<0.0001
			Forb	--	--	--	>0.5
			Intercept	--	--	0.98	<0.0001

^a Regression coefficient.

^b Probability of the *F*-test for each variable within the model to determine the best overall fit of the regression model.

Table 4.5 Stepwise multiple regressions for crude protein yield (CPY) relative yield ratios (RYR) relationships one (2006) and two (2007) years after spraying relative to the vegetation components removed at spraying (2005) at each of the LI and PCF study sites.

Site	Dependent	Adjusted R ²	Independent	Partial R ²	Model R ²	B ^a	Prob > F ^b
LI	RYR 2006	0.031	Forb	0.0312	0.0312	0.0076	0.1144
			Legume	--	--	--	>0.5
			CT	--	--	--	>0.5
			Intercept	--	--	1.31	<0.0001
LI	RYR 2007	0.14	Legume	0.07	0.07	-0.0092	0.0122
			Forb	0.0488	0.1187	-0.019	0.0255
			CT	0.0251	0.1438	-0.0042	0.14
			Intercept	--	--	1.97	<0.0001
PCF	RYR 2006	0.138	Legume	0.016	0.106	-0.0102	0.0132
			CT	0.0317	0.1377	0.0617	0.097
			Forb	--	--	--	>0.5
			Intercept	--	--	1.011	<0.0001
PCF	RYR 2007	0.169	Legume	0.0873	0.0873	-0.031	0.0008
			CT	0.0821	0.1694	0.22	0.0072
			Forb	--	--	--	>0.5
			Intercept	--	--	1.14	<0.0001

^a Regression coefficient.

^b Probability of the *F*-test for each variable within the model to determine the best overall fit of the regression model.

Table 4.6 Results of the linear regression between biomass relative yield ratio and various combinations of legume, CT and forb (kg ha⁻¹) one (2006) and two (2007) years after spraying, at each of the LI and PCF study sites.

Site	Independent Component(s)	R _{YR} 2006			R _{YR} 2007		
		P-values	R ²	Equations	P-values	R ²	Equations
LI	Legume	0.21	0.02	$y = -0.002x + 1.08$	0.0031	0.11	$y = -0.009x + 1.46$
	CT	0.63	0.003	$y = -0.0007x + 1.01$	0.83	0.0005	$y = 0.0005x + 1.25$
	Legume + CT	0.86	0.0004	$y = -0.0003x + 1.04$	0.49	0.006	$y = -0.002x + 1.35$
	Legume + CT + Forb	0.66	0.002	$y = -0.0007x + 1.07$	0.29	0.014	$y = -0.003x + 1.41$
PCF	Legume	<0.0001	0.25	$y = -0.006x + 1.11$	0.033	0.057	$y = -0.005x + 1.38$
	CT	0.0003	0.16	$y = -0.041x + 1.13$	0.389	0.01	$y = 0.017x + 1.31$
	Legume + CT	<0.0001	0.24	$y = -0.006x + 1.15$	0.0097	0.08	$y = -0.006x + 1.46$
	Legume + CT + Forb	<0.0001	0.29	$y = -0.007x + 1.22$	0.003	0.11	$y = -0.008x + 1.55$

Table 4.7 Results of the linear regression relationships between relative yield ratio of crude protein yields (CPY) and various combinations of legume, CT and forb (kg ha⁻¹) during one (2006) and two (2007) two years spraying at each of the LI and PCF study sites.

Site	Independent Component(s)	RYR ₂₀₀₆			RYR ₂₀₀₇		
		P-values	R ²	Equations	P-values	R ²	Equations
LI	Legume	0.75	0.001	$y = -0.0007x + 1.22$	0.017	0.07	$y = -0.0089x + 1.54$
	CT	0.75	0.001	$y = -0.0005x + 1.22$	0.38	0.01	$y = -0.0026x + 1.45$
	Legume + CT	0.73	0.002	$y = -0.0006x + 1.23$	0.15	0.03	$y = -0.0005x + 1.57$
	Legume + CT + Forb	0.56	0.004	$y = -0.0012x + 1.26$	0.083	0.04	$y = -0.0061x + 1.65$
PCF	Legume	0.0032	0.11	$y = -0.0038x + 1.11$	0.0078	0.09	$y = -0.0077x + 1.50$
	CT	0.022	0.07	$y = -0.027x + 1.12$	0.084	0.04	$y = -0.046x + 1.48$
	Legume + CT	0.0034	0.11	$y = -0.0041x + 1.14$	0.0044	0.10	$y = -0.0092x + 1.58$
	Legume + CT + Forb	0.0008	0.13	$y = -0.0053x + 1.20$	0.0017	0.12	$y = -0.011x + 1.7$

Table 4.8 Maximum thresholds for the amount of legume, legume + CT, or legume + CT + forb biomass (kg ha^{-1}) removed pre-spraying, which subsequently led to an increase in forage relative yield ratio (i.e. $\text{RYR} > 1$) of either biomass or crude protein yield (CPY), during 2006 or 2007 at each of the PCF and LI study sites.

Site	Sward Component	Biomass (kg ha^{-1})		CPY (kg ha^{-1})	
		2006	2007	2006	2007
PCF	Legume	370	6658	864	4218
	CT	10	--	21	--
	Legume + CT	566	5466	1136	3955
	Legume + CT + Forb	884	4849	1427	3877
LI	Legume	--	2311	--	3728
	CT	--	--	--	--
	Legume + CT	--	--	--	--
	Legume + CT + Forb	--	--	--	--

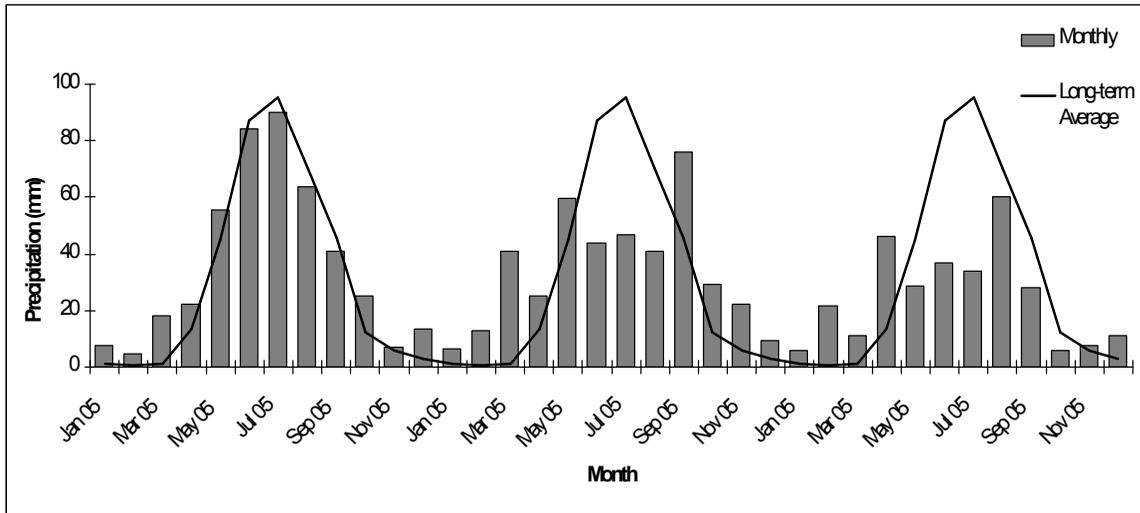


Figure 4.1 Average monthly and long-term annual precipitation (mm) at PCF for 2005-2007.

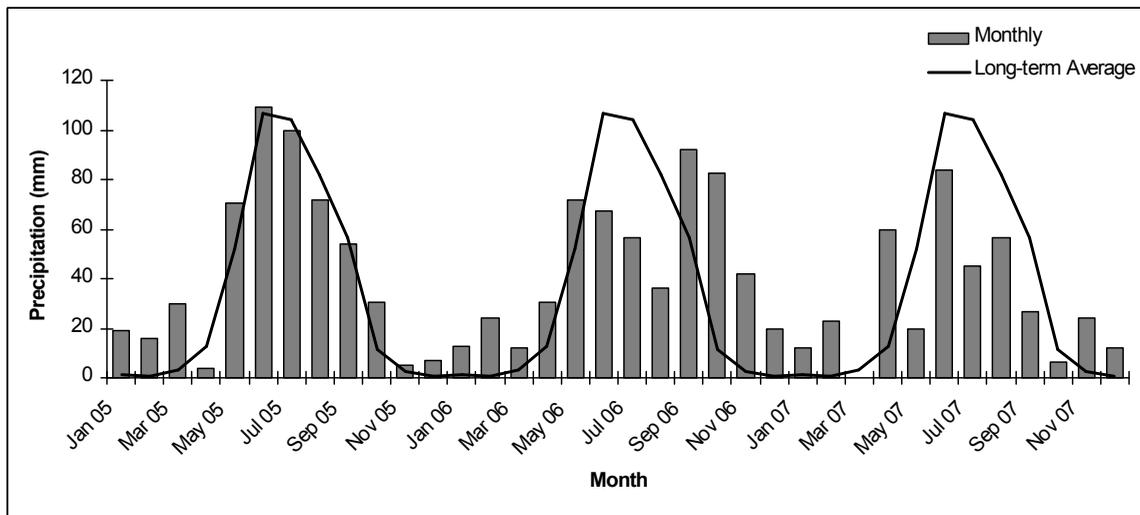
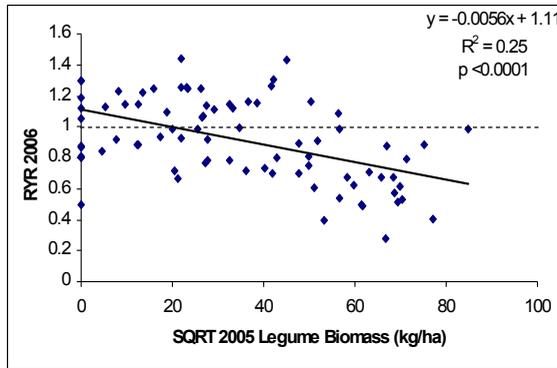
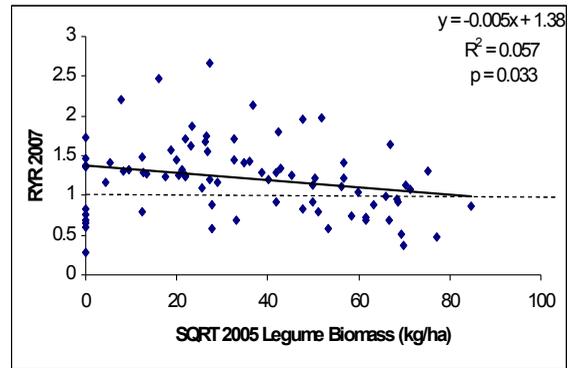


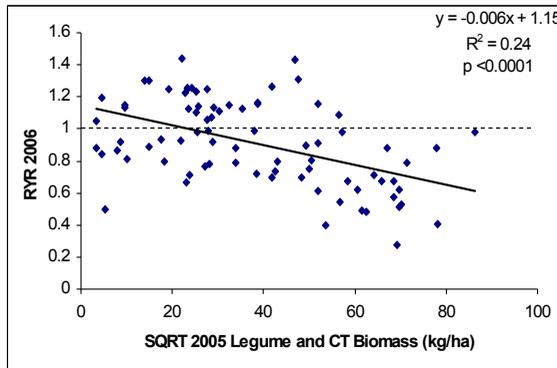
Figure 4.2 Average monthly and long-term annual precipitation (mm) at LI for 2005-2007.



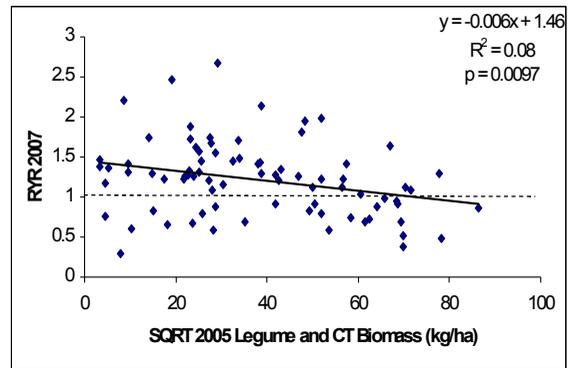
(a) Legume removed



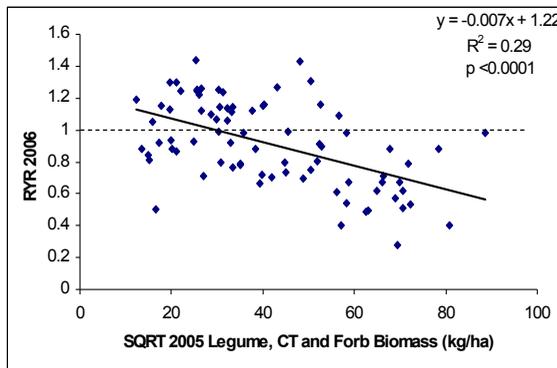
(b) Legume removed



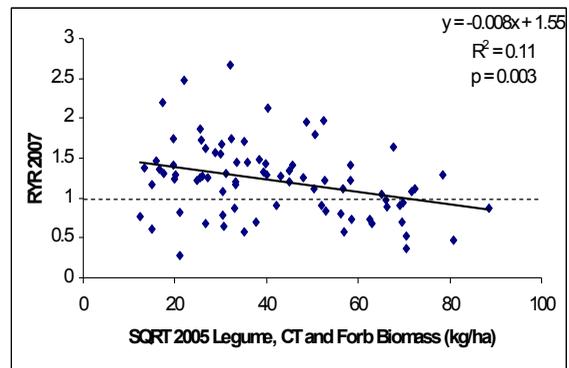
(c) Legume + CT removed



(d) Legume + CT removed

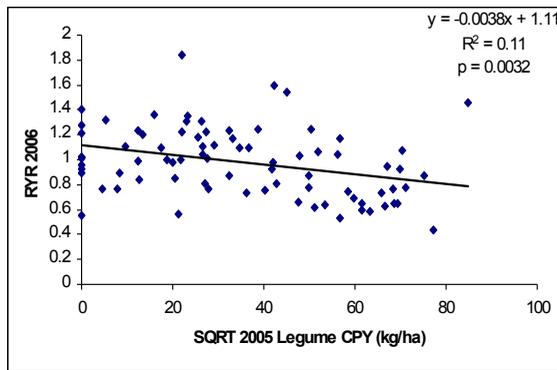


(e) Legume + CT + Forb removed

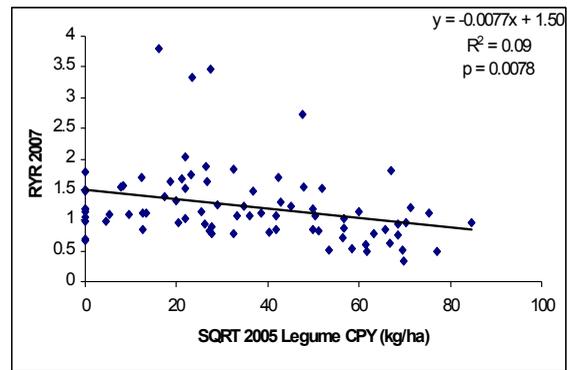


(f) Legume + CT + Forb removed

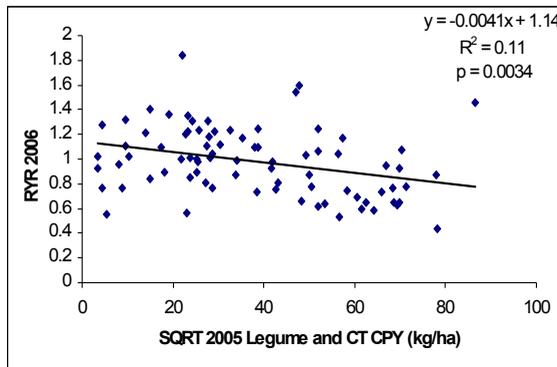
Figure 4.3 Empirical relationship between forage biomass (kg ha^{-1}) relative yield ratio (RYR) in 2006 and 2007 and biomass (kg ha^{-1} square root transformed) of the pre-spraying (2005) sward vegetation components legume, legume + CT, or legume + CT + forb, at the PCF study site.



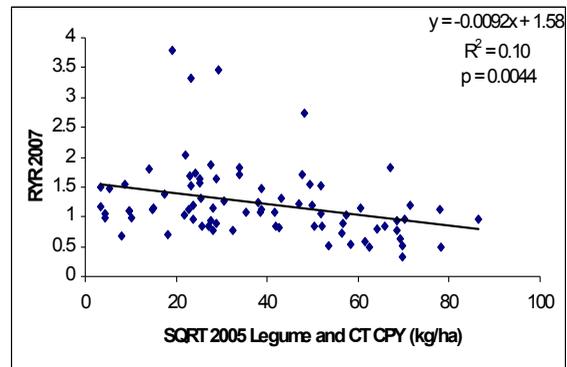
(a) Legume removed



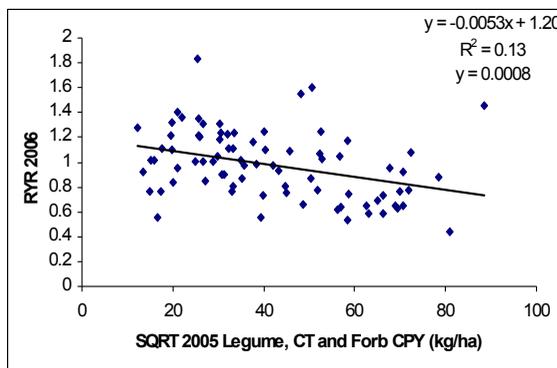
(b) Legume removed



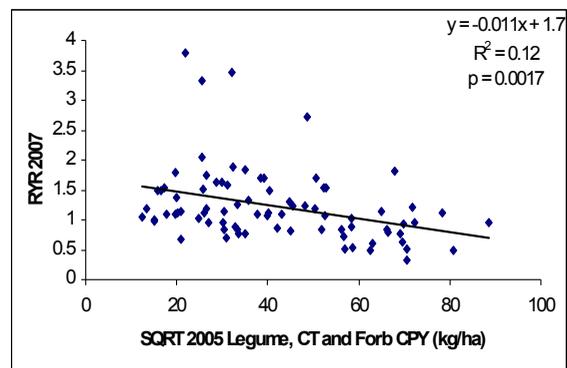
(c) Legume + CT removed



(d) Legume + CT removed

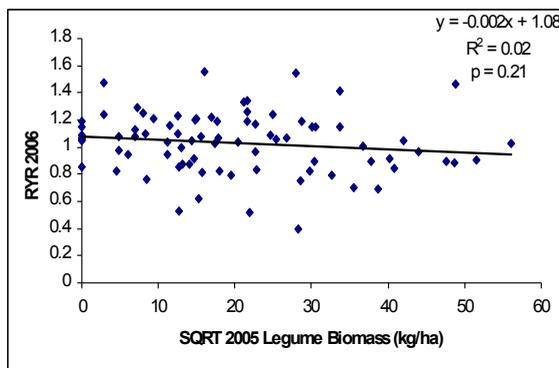


(e) Legume + CT + Forb removed

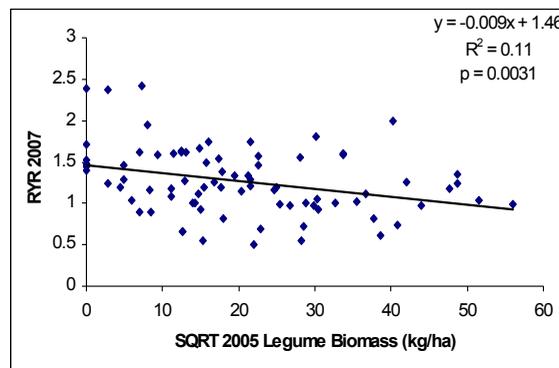


(f) Legume + CT + Forb removed

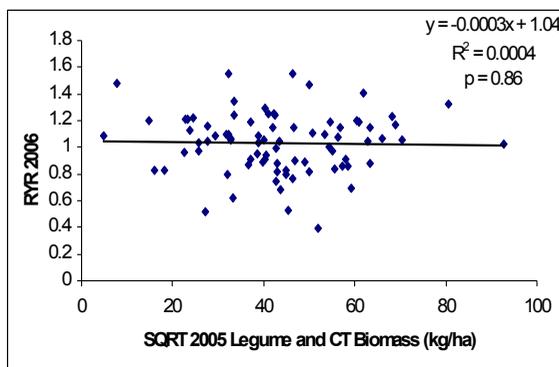
Figure 4.4 Empirical relationship between forage crude protein yield (CPY)(kg ha⁻¹ square root transformed) relative yield ratio (RYR) in 2006 and 2007 of the pre-spraying (2005) sward vegetation components legume, legume + CT, or legume + CT + forb at the PCF study site.



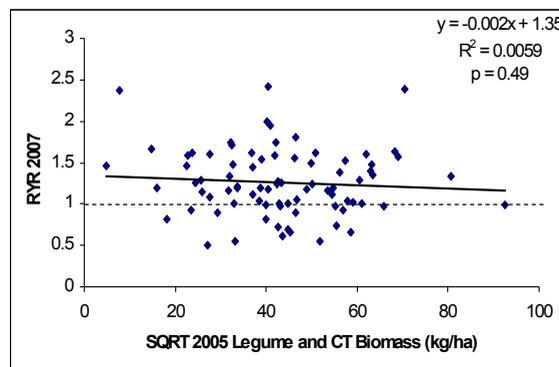
(a) Legume removed



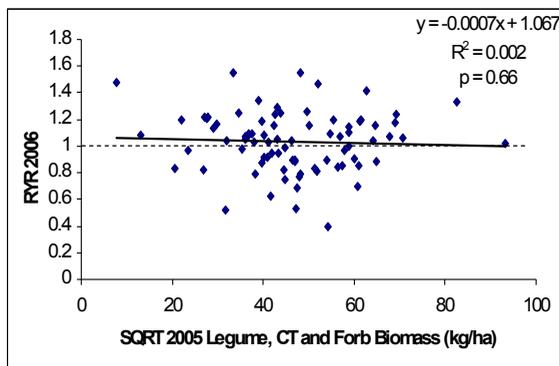
(b) Legume removed



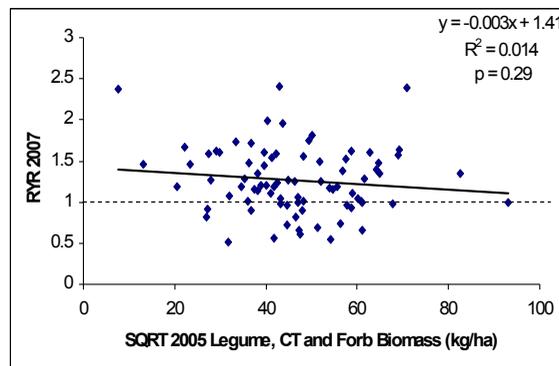
(c) Legume + CT removed



(d) Legume + CT removed

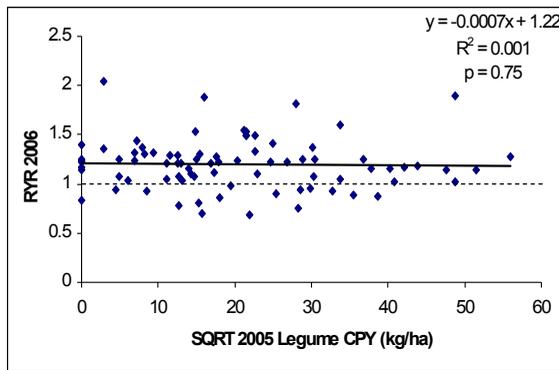


(e) Legume + CT + Forb removed

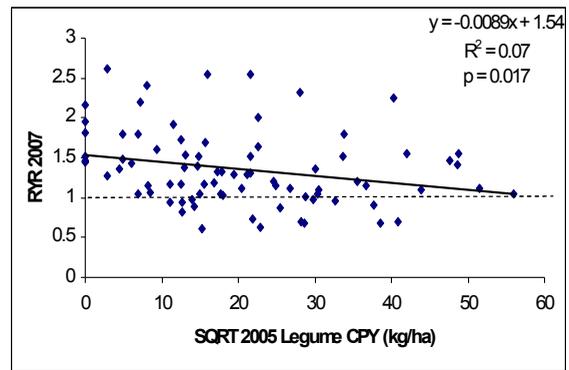


(f) Legume + CT + Forb removed

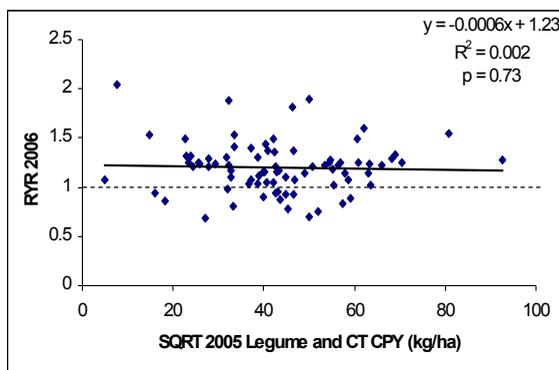
Figure 4.5 Empirical relationship between forage biomass (kg/ha) relative yield ratio (RYR) in 2006 and 2007, and biomass (kg ha^{-1} square root transformed) of the pre-spraying (2005) sward vegetation components legume, legume + CT or legume + CT + forb at the LI study site.



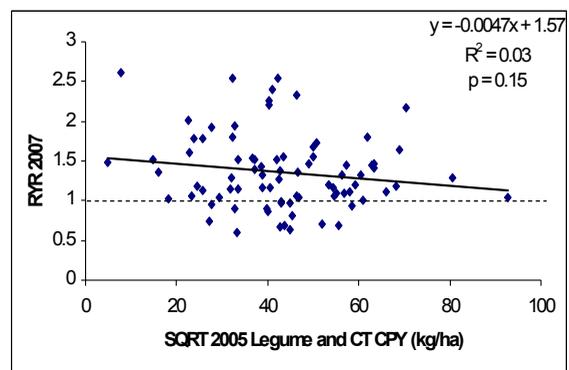
(a) Legume removed



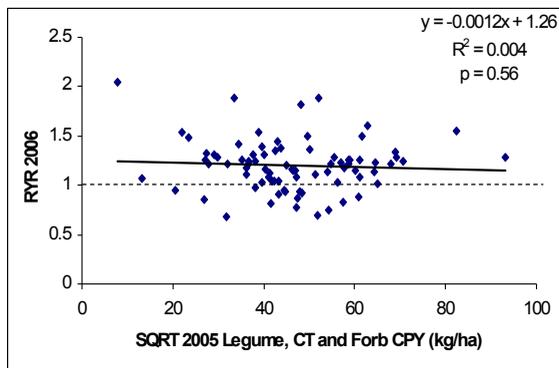
(b) Legume removed



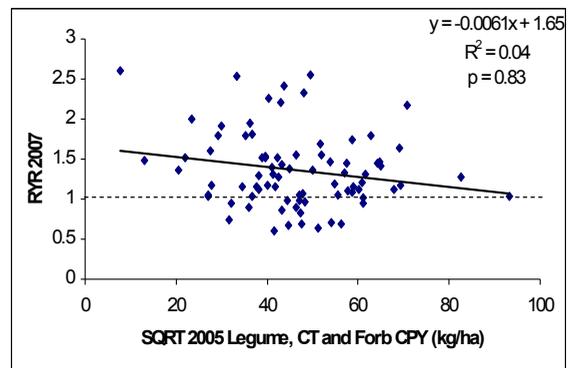
(c) Legume + CT removed



(d) Legume + CT removed



(e) Legume + CT + Forb removal



(f) Legume + CT + Forb removed

Figure 4.6 Empirical relationship between forage crude protein yield (CPY) (kg/ha^{-1} square root transformed) relative yield ratio (RYR) in 2006 and 2007 of the pre-spraying (2005) sward vegetation components legume, legume & CT or legume, CT & forb at the LI study site.

CHAPTER 5.

Synthesis and Management Implications

Demand for the establishment and maintenance of highly productive forage pastures has increased in Alberta. Factors such as the rising cost of production, competing land uses, and the development of new technologies such as biofuels have led to a greater need for increased productivity from agricultural land. As more marginal land that typically has been used in the past for forage production is put into more intensive annual cropping systems, less land is available for forage production, thereby facilitating the need to either maintain or increase productivity of existing pastures. An understanding of how mixed grass-legume sward components interact with each other and their aggregate response to herbicides for noxious weed control are important to provide producers with the tools to identify optimal conditions for which weed control or legume removal are justified.

Invasive weeds such as Canada thistle (CT) are prevalent in the majority of agronomic fields across western Canada (Wilson and Kachman 1999, Brehrens and Elakkad 1981), and are known to increase within pastures if not actively managed, potentially leading to forage yield losses. The most effective control of Canada thistle is through the integration of various management strategies, which often include broadleaf herbicides (Masters and Sheley 2001, DiTomaso 2000). However, the use of broadleaf herbicides within mixed swards containing legumes such as clover or alfalfa results in a fundamental trade-off between the desired control of the noxious weed (Canada thistle) and the removal of beneficial legumes. Previous studies such as Grekul and Bork (2004) have examined the effects of Canada thistle on grass yields. The intent of this research

study was to 1) examine the ability of mixed swards to compensate for legume removal (Chapter 3) and 2) further understand forage dynamics in legume-grass swards prior to and following weed control with herbicides, under varying levels of CT and other weeds (Chapter 4). Moreover, post-spraying pasture sward dynamics were used to determine if weed and legume thresholds could be established to assist producers in making decisions regarding when the use of broadleaf herbicides are justified within mixed forage swards.

The study designed in Chapter 3 was used to isolate the direct relationship between legume removal and subsequent grass responses within four different forage mixtures in the absence of CT and other forbs. Mixtures containing the more aggressive and highly productive legumes (i.e. alfalfa) were generally associated with greater yield losses. Swards containing meadow brome (MB) generally led to greater losses, while those with smooth brome (SB) demonstrated the ability to at least partially compensate for legume loss through increased grass production. However, none of the alfalfa or clover based experimental swards examined were able to fully compensate for the loss of high quality legumes (i.e. CPY production) during all three years post-spraying.

In clover based swards, forage yield losses and associated compensation were more poorly expressed as compared to swards containing alfalfa. Responses were also more variable due to the low intensity defoliation regime that helped facilitate the natural decline of clover, independent of herbicide treatment. Changes to a multiple defoliation management system and the timing of each harvest may have maintained the clover populations within these swards and altered the responses over the three year period after spraying. Therefore, forage quality of the species removed from a sward should be taken

into consideration along with management systems to fully understand the implications of legume removal during herbicides use.

Experimental swards in Chapter 3 tended to converge towards an equilibrium amount of legume within the sward 4-5 years after seeding, particularly within alfalfa based swards. These results suggest that a relatively 'stable' value over time was observed in the alfalfa based swards (i.e. MBALF and SBALF) and that this response remains independent of the seeding ratios, or site characteristics. This finding also highlights that within grass-alfalfa dominated swards, legume removal within seeded pastures will tend to be between 30-60% legume within swards four years or older.

Assessment of complex sward dynamics within established pasture in Chapter 4 revealed competitive and facilitative relationships among grasses and legumes in the presence of CT and other broadleaf plants (i.e. forbs), both prior to spraying, as well as responses up to two years after spraying. However, the only relationship demonstrating a significant impact on total forage production (i.e. grass + legume) prior to spraying was forb biomass. Unexpectedly, CT had little relation with total forage production at either site suggesting this weed may have been occupying ecological niches not exploited by other vegetational components. Results from in this study prior to spraying also suggested that under a low frequency defoliation regime, competition and facilitation increased between vegetational groups within the more drier upland site (i.e. Lake Isle) where resources such as water and nutrients were more limited, as compared to the more mesic lowland site (i.e. Parkland Conservation Farm).

The removal of legumes, forbs and CT due to spraying generally resulted in a net negative impact on total forage production with the exception of CT removal at the

alfalfa based site. The partial positive response to CT removal was magnified from 2006 to 2007, and suggested that while CT did not have a strong impact on total forage biomass prior to spraying, this sward responded favorably (though weakly) to weed removal. More importantly, grass regrowth following spraying was insufficient to offset the loss of alfalfa. Within clover based swards, the absence of a yield loss one year after spraying suggested that grasses in this sward were able to compensate for clover removal at this location, a process likely aided by abundant moisture. However, grass compensation remained short-lived and was followed by yield losses two years after spraying.

Thresholds calculated in association with weed and legume removal in Chapter 4 are useful due to their ability to quantify the benefits and opportunity costs resulting from weed control with broadleaf herbicides. However, results from this study indicated that thresholds were also dynamic in space and time varying between sites (i.e. forage mixes and growing conditions) as well as from the first to second year after spraying. Moreover, the presence of a threshold within swards revealed that the removal of CT and legumes did not consistently equate to a loss in forage production, but rather some swards responded positively to varying levels of legume removal depending on the site characteristics, legume species and weed or legume abundances. The use of a relative yield ratio (RYR) and the inclusion of forage quality parameters (i.e. CPY) were key methods in being able to interpret sward dynamics in these highly variable pastures and understand the cost associated with weed control within mixed swards. Although the current study represents an important first step in detecting these thresholds, future research is needed to refine the specific methods used to quantify where and when these

thresholds occur, such that producers may more readily put into practice the use of weed and legume thresholds in the management of mixed swards.

The information gathered in this study has furthered our understanding of how various vegetation components, including legumes, grasses, forb and CT, interact with one another and respond to herbicide application. In addition, this research highlighted the key impact of varying pasture sward composition and growing conditions on observed forage dynamics. Management practices, site characteristics and species composition of the forage mixes and pastures examined here played a key role in the detection of both competitive and facilitative responses to legume and weed removal. The inclusion of forage quality played a significant role in altering legume removal impacts and often changed interpretation of ecological and agronomic costs/benefits to herbicide weed control with these experimental swards. Through the development of legume removal thresholds, producers should be able to determine when and at what cost herbicide weed control is justified, including anticipated sward responses in the short-term. Producers should also take into consideration the cost of the herbicide and the application when deciding if spraying is a viable option for weed control. In this study our costs associated with weed control for chemical and application were \$38 acre⁻¹ or \$93.86 ha⁻¹. Ultimately more widespread availability of this information will enable producers to make more informed decisions about weed control in mixed pastures to maintain or improve the productivity and longevity of pasture swards in Alberta.

5.1 Literature Cited

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Appendix A

Table A.1 Summary of regression results 1 year post-spraying (2006) of net production differences between sprayed and unsprayed sub-plots (i.e. S-US) for biomass and CPY responses relative to initial legume removed due to spraying at W240.

Response	Forage Mix	Intercept ^a	Model ^a	R ²	Equation ^b
Total Forage Biomass					
	MBALF	0.431	0.1099	0.11	$y = -18.46x - 414$
	SBALF	0.0001	<0.0001	0.52	$y = -26.42x - 1088$
	MBCLR	0.188	0.0272	0.203	$y = -16.69x + 422$
	SBCLR	0.0166	0.68	0.008	$y = -5.18x - 1251$
Grass Biomass					
	MBALF	0.824	0.0035	0.33	$y = 27.08x - 87$
	SBALF	<0.0001	<0.0001	0.60	$y = 25.77x - 1034$
	MBCLR	0.124	0.0224	0.18	$y = 36.9x - 572$
	SBCLR	0.013	0.0105	0.26	$y = 34.85x - 1312$
CPY Total Forage					
	MBALF	0.0116	0.0003	0.46	$y = -3.66x - 109$
	SBALF	0.0173	<0.0001	0.76	$y = -4.98x - 66$
	MBCLR	0.899	0.434	0.028	$y = -1.2x - 9$
	SBCLR	0.952	0.137	0.097	$y = -1.98x + 3$
CPY Grass					
	MBALF	0.300	0.0009	0.401	$y = 2.87x - 37$
	SBALF	0.0041	<.0001	0.69	$y = 3.1x - 62$
	MBCLR	0.635	0.0029	0.34	$y = 5.16x - 33$
	SBCLR	0.910	0.0031	0.33	$y = 4.23x - 6$

a Regression model and intercept p-values, where $p > 0.05$

b Regression equation where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table A.2 Summary of regression results 2 years post-spraying (2007) of net production differences between sprayed and unsprayed sub-plots (i.e. S-US) for biomass and CPY responses relative to initial legume removed due to spraying at W240.

Response	Forage Mix	Intercept^a	Model^a	R²	Equation^b
Total Forage Biomass					
	MBALF	0.0649	0.0013	0.38	$y = -22.9x - 563$
	SBALF	0.837	0.647	0.01	$y = 5.63x + 108$
	MBCLR	0.717	0.99	0.0001	$y = 0.061x - 109$
	SBCLR	0.207	0.0171	0.23	$y = 25.6x + 502$
Grass Biomass					
	MBALF	0.0003	0.0002	0.47	$y = 24.06x + 1093$
	SBALF	0.1112	0.0005	0.43	$y = 803.28x + 45$
	MBCLR	0.562	0.045	0.17	$y = 19.05x - 171.07$
	SBCLR	0.378	0.0002	0.47	$y = 55.13x + 434$
CPY Total Forage					
	MBALF	0.003	0.0008	0.41	$y = -5.11x - 204$
	SBALF	0.224	0.0085	0.28	$y = -3.37x - 63$
	MBCLR	0.847	0.0058	0.30	$y = -1.89x - 5$
	SBCLR	0.0586	0.159	0.09	$y = -1.13x + 61$
CPY Grass					
	MBALF	0.123	0.0001	0.49	$y = 1.78x + 29$
	SBALF	0.157	0.0005	0.44	$y = 2.53x + 39$
	MBCLR	0.622	0.18	0.08	$y = 0.90x - 14$
	SBCLR	0.227	0.0023	0.35	$y = 3.49x + 49$

a Regression model and intercept p-values, where $p > 0.05$

b Regression equation where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table A.3 Summary of regression results 3 years post-spraying (2008) of net production differences between sprayed and unsprayed sub-plots (i.e. S-US) for biomass and CPY responses relative to initial legume removed due to spraying at W240.

Y	Forage Mix	Intercept ^a	Model ^a	R ²	Equation ^b
Total Forage Biomass					
	MBALF	0.014	0.185	0.08	$y = -14.36x + 490$
	SBALF	0.0021	0.334	0.04	$y = 8.02x - 1225$
	MBCLR	0.302	0.1223	0.105	$y = 6.03x + 175$
	SBCLR	0.0985	0.900	0.0007	$y = -1.17x + 625$
Grass Biomass					
	MBALF	0.328	0.0525	0.16	$y = 10.86x + 247$
	SBALF	0.0516	0.0003	0.46	$y = 31.57x - 648$
	MBCLR	0.204	0.124	0.10	$y = 5.91x + 213$
	SBCLR	0.114	0.941	0.0002	$y = 0.72x + 624$
CPY Total Forage					
	MBALF	0.0022	0.0165	0.23	$y = -3.56x - 221$
	SBALF	0.0017	0.0741	0.14	$y = -1.75x - 144$
	MBCLR	0.650	0.245	0.061	$y = 0.37x + 6$
	SBCLR	0.181	0.171	0.084	$y = -1.19x + 45$
CPY Grass					
	MBALF	0.884	0.230	0.06	$y = 0.55x + 3$
	SBALF	0.0504	0.0012	0.39	$y = 1.98x - 47$
	MBCLR	0.339	0.248	0.06	$y = 0.33x + 12$
	SBCLR	0.221	0.348	0.04	$y = -0.88x + 45$

a Regression model and intercept p-values, where $p > 0.05$

b Regression equation where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table A.4 Summary of regression results 1 year post-spraying (2006) of net production differences between sprayed and unsprayed sub-plots (i.e. S-US) for biomass and CPY responses relative to initial legume removed due to spraying at E2.

Response	Forage Mix	Intercept^a	Model^a	R²	Equation^b
Total Forage Biomass					
	MBALF	0.519	<0.0001	0.52	$y = -48.07x - 877$
	SBALF	0.0076	0.0006	0.42	$y = -35.55x - 1299$
	MBCLR	0.387	0.128	0.10	$y = -24.29x - 589$
	SBCLR	0.890	0.0841	0.13	$y = -19.83x + 62$
Grass Biomass					
	MBALF	0.0532	0.319	0.05	$y = 7.71x + 829$
	SBALF	0.978	0.1074	0.11	$y = 14.4x + 11$
	MBCLR	0.372	0.0954	0.12	$y = 26.27x - 596$
	SBCLR	0.877	0.049	0.16	$y = 22.82x + 70$
CPY Total Forage					
	MBALF	0.98	0.0018	0.36	$y = -0.53x - 211$
	SBALF	0.167	0.0001	0.50	$y = -7.02x - 107$
	MBCLR	0.19	0.0299	0.197	$y = -3.73x - 94$
	SBCLR	0.737	0.0268	0.204	$y = -3.95x + 23$
CPY Grass					
	MBALF	0.0616	0.457	0.03	$y = 0.513x + 74$
	SBALF	0.243	0.275	0.05	$y = 0.91x + 49$
	MBCLR	0.119	0.0118	0.26	$y = 3.93x - 101$
	SBCLR	0.713	0.141	0.096	$y = 2.47x + 25$

^a Regression model and intercept p-values, where $p > 0.05$

^b Regression equation where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table A.5 Summary of regression results 2 years post-spraying (2007) of net production differences between sprayed and unsprayed sub-plots (i.e. S-US) for biomass and CPY responses relative to initial legume removed due to spraying at E2

Response	Forage Mix	Intercept^a	Model^a	R²	Equation^b
Total Forage Biomass					
	MBALF	0.176	0.728	0.006	$y = 6.42x - 1405$
	SBALF	0.391	0.191	0.076	$y = -15.65x + 508$
	MBCLR	0.41	0.03	0.20	$y = 21.97x - 348$
	SBCLR	0.714	0.248	0.26	$y = 20.92x + 268$
Grass Biomass					
	MBALF	0.128	0.0019	0.33	$y = 46.91x + 1160$
	SBALF	0.002	0.0628	0.15	$y = 22.31x + 2001$
	MBCLR	0.457	<0.0001	0.51	$y = 37.45x + 257$
	SBCLR	0.763	0.0099	0.27	$y = 50.82x + 220$
CPY Total Forage					
	MBALF	0.046	0.790	0.0033	$y = -0.59x - 256$
	SBALF	0.912	0.0008	0.405	$y = -5.38x - 8$
	MBCLR	0.15	0.82	0.002	$y = -0.26x - 75$
	SBCLR	0.403	0.057	0.11	$y = -2.78x + 48$
CPY Grass					
	MBALF	0.112	0.0123	0.25	$y = 2.62x + 87$
	SBALF	0.0031	0.976	0.00	$y = 0.037x + 206$
	MBCLR	0.999	0.0017	0.37	$y = 2.68x + 0.01$
	SBCLR	0.496	0.1074	0.06	$y = 2.33x + 40$

a Regression model and intercept p-values, where $p > 0.05$

b Regression equation where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table A.6 Summary of regression results 3 years post-spraying (2008) of net production differences between sprayed and unsprayed sub-plots (i.e. S-US) for biomass and CPY responses relative to initial legume removed due to spraying at E2.

Response	Forage Mix	Intercept^a	Model^a	R²	Equation^b
Total Forage Biomass					
	MBALF	0.0724	0.408	0.031	$y = -15.39x - 1895$
	SBALF	0.316	0.714	0.006	$y = 6.53x - 903$
	MBCLR	0.364	0.526	0.02	$y = -9.33x - 583$
	SBCLR	0.135	0.315	0.046	$y = -8.93x + 553$
Grass Biomass					
	MBALF	0.815	0.374	0.036	$y = 8.37x + 123$
	SBALF	0.223	0.106	0.11	$y = 15.39x + 572$
	MBCLR	0.748	0.87	0.001	$y = -1.46x - 129$
	SBCLR	0.119	0.708	0.007	$y = 3.03x + 532$
CPY Total Forage					
	MBALF	0.0205	0.0205	0.22	$y = -5.49x - 297$
	SBALF	0.0558	0.0761	0.14	$y = -3.13x - 170$
	MBCLR	0.303	0.441	0.03	$y = -1.38x - 81$
	SBCLR	0.143	0.0713	0.14	$y = -2.04x + 67$
CPY Grass					
	MBALF	0.691	0.712	0.0063	$y = 0.38x - 23$
	SBALF	0.254	0.353	0.04	$y = 0.89x + 55$
	MBCLR	0.602	0.89	0.0009	$y = 0.11x - 18$
	SBCLR	0.120	0.897	0.0008	$y = -0.12x + 64$

a Regression model and intercept p-values, where $p > 0.05$

b Regression equation where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Appendix B.

Table B.1 Summary of regression results 1 year post-spraying (2006) of sprayed and unsprayed sub-plots for biomass and CPY responses relative to initial legume removed due to spraying at W240.

Response	Forage Mix	Sprayed			Unsprayed		
		P-value	R ²	Equation [†]	P-value	R ²	Equation [†]
Total Forage Biomass							
	MBALF	<0.0001	0.55	$y = -35.23x + 6826$	0.013	0.25	$y = -16.77x + 7240$
	SBALF	<0.0001	0.69	$y = -47.13x + 7080$	0.007	0.28	$y = -20.71x + 8168$
	MBCLR	0.0007	0.41	$y = -46.7x + 8808$	0.0404	0.18	$y = -30.01x + 8386$
	SBCLR	0.0007	0.42	$y = -52.76x + 7791$	<.0001	0.55	$y = -47.58x + 9042$
Grass Biomass							
	MBALF	<0.0001	0.55	$y = -33.77x + 6567$	<0.0001	0.85	$y = -60.86x + 6654$
	SBALF	<.0001	0.69	$y = -47.08x + 7072$	<.0001	0.82	$y = -72.85x + 8105$
	MBCLR	0.0007	0.41	$y = -46.7x + 8808$	<0.0001	0.55	$y = -72.90x + 8555$
	SBCLR	0.0007	0.42	$y = -52.76x + 7791$	<.0001	0.78	$y = -87.61x + 9102$
CPY Total Forage							
	MBALF	0.058	0.15	$y = -1.36x + 498$	0.004	0.32	$y = 2.3x + 607$
	SBALF	0.006	0.30	$y = -1.98x + 516$	0.0008	0.40	$y = 3.0x + 582$
	MBCLR	0.86	0.001	$y = -0.24x + 615$	0.49	0.02	$y = 0.95x + 624$
	SBCLR	0.24	0.063	$y = -1.93x + 647$	0.96	0.0001	$y = 0.05x + 644$
CPY Grass							
	MBALF	0.03	0.196	$y = -1.57x + 496$	<0.0001	0.75	$y = -4.45x + 534$
	SBALF	0.006	0.29	$y = -1.97x + 514$	<.0001	0.70	$y = -5.07x + 576$
	MBCLR	0.87	0.0013	$y = -0.24x + 615$	0.0006	0.42	$y = -5.41x + 648$
	SBCLR	0.24	0.063	$y = -1.93x + 646$	<.0001	0.66	$y = -6.16x + 652$

[†] Linear regression where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table B.2 Summary of regression results 2 years post-spraying (2007) of sprayed and unsprayed sub-plots for biomass and CPY responses relative to initial legume removed due to spraying at W240.

Response	Forage Mix	Sprayed			Unsprayed		
		P-value	R ²	Equation [†]	P-value	R ²	Equation [†]
Total Forage Biomass							
	MBALF	0.04	0.17	$y = 12.01x + 4672$	0.0002	0.47	$y = 34.91x + 5235$
	SBALF	0.91	0.0006	$y = 0.96x + 7101$	0.6602	0.0089	$y = -4.67x + 6993$
	MBCLR	0.18	0.079	$y = 7.56x + 4574$	0.289	0.051	$y = 7.59x + 4682$
	SBCLR	0.52	0.019	$y = 6.03x + 7081$	0.0028	0.34	$y = -19.56x + 6579$
Grass Biomass							
	MBALF	0.19	0.075	$y = 7.11x + 4656$	<0.0001	0.52	$y = -16.94x + 3563$
	SBALF	0.94	0.0002	$y = -0.61x + 7108$	0.0001	0.49	$y = -46.21x + 6304$
	MBCLR	0.184	0.079	$y = 7.65x + 4574$	0.172	0.083	$y = -11.4x + 4745$
	SBCLR	0.522	0.019	$y = 6.04x + 7080$	<0.0001	0.65	$y = -49.09x + 6646$
CPY Total Forage							
	MBALF	<0.0001	0.57	$y = 2.04x + 294$	<0.0001	0.52	$y = 7.15x + 498$
	SBALF	0.0445	0.17	$y = 1.31x + 345$	<0.0001	0.54	$y = 4.69x + 408$
	MBCLR	0.488	0.022	$y = 0.24x + 313$	0.0058	0.298	$y = 2.14x + 318$
	SBCLR	0.0661	0.14	$y = 1.66x + 366$	<0.0001	0.74	$y = 2.8x + 306$
CPY Grass							
	MBALF	0.0041	0.32	$y = 1.22x + 293$	0.116	0.108	$y = -0.55x + 264$
	SBALF	0.0887	0.13	$y = 1.07x + 346$	0.0027	0.34	$y = -1.45x + 307$
	MBCLR	0.488	0.022	$y = 0.24x + 313$	0.353	0.039	$y = -0.65x + 327$
	SBCLR	0.0659	0.15	$y = 1.66x + 366$	<0.0001	0.51	$y = -1.82x + 317$

[†] Linear regression where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table B.3 Summary of regression results 3 years post-spraying (2008) of sprayed and unsprayed sub-plots for biomass and CPY response relative to initial legume removed due to spraying at W240.

Response	Forage Mix	Sprayed			Unsprayed		
		P-value	R ²	Equation [†]	P-value	R ²	Equation [†]
Total Forage Biomass							
	MBALF	0.016	0.24	$y = 13.34x + 2741$	0.0096	0.27	$y = 27.07x + 4048$
	SBALF	0.1336	0.099	$y = 11.92x + 3669$	0.573	0.015	$y = 3.89x + 4894$
	MBCLR	0.0017	0.37	$y = 14.51x + 2823$	0.0095	0.27	$y = 8.47x + 2649$
	SBCLR	0.246	0.061	$y = 10.8x + 4329$	0.0437	0.17	$y = 11.98x + 3703$
Grass Biomass							
	MBALF	0.0367	0.18	$y = 10.93x + 2751$	0.983	0.00	$y = 0.06x + 2504$
	SBALF	0.1209	0.106	$y = 12.14x + 3644$	0.0067	0.22	$y = -19.43x + 4293$
	MBCLR	0.0017	0.37	$y = 14.51x + 2823$	0.0072	0.29	$y = 8.6x + 2610$
	SBCLR	0.246	0.061	$y = 10.8x + 4329$	0.0877	0.13	$y = 10.08x + 3704$
CPY Total Forage							
	MBALF	0.0032	0.33	$y = 1.33x + 184$	0.0018	0.36	$y = 4.9x + 405$
	SBALF	0.0222	0.22	$y = 1.3x + 231$	0.0009	0.403	$y = 3.05x + 375$
	MBCLR	0.0004	0.45	$y = 1.32x + 182$	0.0029	0.34	$y = 0.95x + 176$
	SBCLR	0.153	0.09	$y = 1.09x + 288$	0.0012	0.39	$y = 2.29x + 243$
CPY Grass							
	MBALF	0.0108	0.26	$y = 1.01x + 185$	0.1803	0.08	$y = 0.45x + 182$
	SBALF	0.0192	0.23	$y = 1.29x + 231$	0.0959	0.12	$y = 0.68x + 278$
	MBCLR	0.0004	0.45	$y = 1.32x + 182$	0.0007	0.42	$y = 0.98x + 169$
	SBCLR	0.153	0.09	$y = 1.09x + 288$	0.0037	0.32	$y = 1.98x + 243$

[†] Linear regression where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table B.4 Summary of regression results 1 year post-spraying (2006) of sprayed and unsprayed sub-plots for biomass and CPY responses relative to initial legume removed due to spraying at E2.

Response	Forage Mix	Sprayed			Unsprayed		
		P-value	R ²	Equation [†]	P-value	R ²	Equation [†]
Total Forage Biomass							
	MBALF	<0.0001	0.69	$y = -52.96x + 7774$	0.661	0.009	$y = -4.3x + 8725$
	SBALF	<0.0001	0.52	$y = -58.81x + 9552$	0.0217	0.22	$y = -23.25x + 10851$
	MBCLR	0.0087	0.27	$y = -45.56x + 8775$	0.124	0.104	$y = -21.26x + 9363$
	SBCLR	<0.0001	0.72	$y = -75.01x + 10269$	<0.0001	0.602	$y = -55.18x + 10206$
Grass Biomass							
	MBALF	<0.0001	0.70	$y = -53.67x + 7775$	<0.0001	0.74	$y = -61.19x + 6946$
	SBALF	<0.0001	0.52	$y = -58.85x + 9553$	<0.0001	0.61	$y = -73.26x + 9541$
	MBCLR	0.009	0.27	$y = -45.38x + 8758$	0.0006	0.42	$y = -71.65x + 9354$
	SBCLR	<0.0001	0.72	$y = -74.98x + 10264$	<0.0001	0.82	$y = -97.81x + 10194$
CPY Total Forage							
	MBALF	<0.0001	0.54	$y = -3.37x + 578$	0.0102	0.26	$y = 4.4x + 749$
	SBALF	0.0036	0.33	$y = -3.65x + 687$	0.0313	0.194	$y = 3.37x + 794$
	MBCLR	0.0939	0.12	$y = -2.43x + 672$	0.189	0.077	$y = 1.3x + 767$
	SBCLR	0.0015	0.37	$y = -5.05x + 817$	0.363	0.038	$y = -1.09x + 794$
CPY Grass							
	MBALF	<0.0001	0.56	$y = -3.47x + 588$	<0.0001	0.57	$y = -3.99x + 505$
	SBALF	0.0036	0.33	$y = -3.65x - 687$	0.0004	0.44	$y = -4.56x + 638$
	MBCLR	0.0975	0.12	$y = -2.41x + 670$	0.0003	0.46	$y = -6.34x + 771$
	SBCLR	0.0015	0.38	$y = -5.04x + 817$	<0.0001	0.64	$y = -7.52x + 792$

[†] Linear regression where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table B.5 Summary of regression results 2 years post-spraying (2007) of sprayed and unsprayed sub-plots for biomass and CPY responses relative to initial legume removed due to spraying at E2.

Response	Forage Mix	Sprayed			Unsprayed		
		P-value	R ²	Equation [†]	P-value	R ²	Equation [†]
Total Forage Biomass							
	MBALF	0.104	0.12	$y = 21.94x + 4934$	0.352	0.039	$y = 15.52x + 6339$
	SBALF	0.197	0.074	$y = -23.73x + 9335$	0.571	0.015	$y = -7.99x + 8826$
	MBCLR	0.193	0.076	$y = 11.72x + 4721$	0.0857	0.13	$y = -10.24x + 5069$
	SBCLR	0.406	0.032	$y = -14.99x + 7992$	0.067	0.14	$y = -35.92x + 7724$
Grass Biomass							
	MBALF	0.1055	0.11	$y = 21.84x + 4933$	0.0005	0.43	$y = -25.07x + 3773$
	SBALF	0.194	0.075	$y = -23.73x + 9327$	0.0067	0.29	$y = -4604x + 7326$
	MBCLR	0.193	0.076	$y = 11.72x + 4721$	0.0021	0.36	$y = -25.72x + 4464$
	SBCLR	0.406	0.032	$y = -14.99x + 7992$	0.02	0.36	$y = -65.82x + 7772$
CPY Total Forage							
	MBALF	0.428	0.029	$y = 0.78x + 422$	0.545	0.017	$y = 1.83x + 679$
	SBALF	0.303	0.048	$y = -2.09x + 656$	0.0106	0.26	$y = 3.74x + 665$
	MBCLR	0.193	0.076	$y = 1.15x + 381$	0.0954	0.12	$y = 1.42x + 456$
	SBCLR	0.444	0.027	$y = -1.53x + 546$	0.464	0.02	$y = 1.24x + 497$
CPY Grass							
	MBALF	0.428	0.029	$y = 0.78x + 422$	0.006	0.296	$y = -1.83x + 335$
	SBALF	0.298	0.049	$y = -2.1x + 655$	0.09	0.13	$y = -2.14x + 449$
	MBCLR	0.193	0.076	$y = 1.15x + 381$	0.0597	0.15	$y = -1.52x + 381$
	SBCLR	0.444	0.027	$y = -1.53x + 546$	0.0259	0.21	$y = -3.86x + 506$

[†] Linear regression where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Table B.6 Summary of regression results 3 years post-spraying (2008) of sprayed and unsprayed sub-plots for biomass and CPY responses relative to initial legume removed due to spraying at E2.

Response	Forage Mix	Sprayed			Unsprayed		
		P-value	R ²	Equation [†]	P-value	R ²	Equation [†]
Total Forage Biomass							
	MBALF	0.0562	0.16	$y = 16.93x + 2593$	0.032	0.19	$y = 32.32x + 4488$
	SBALF	0.895	0.0008	$y = -1.94x + 5656$	0.504	0.021	$y = -8.47x + 6559$
	MBCLR	0.219	0.068	$y = 8.28x + 2592$	0.196	0.07	$y = 17.61x + 3174$
	SBCLR	0.603	0.013	$y = -5.88x + 5297$	0.747	0.0048	$y = 3.05x + 4745$
Grass Biomass							
	MBALF	0.154	0.09	$y = 10.17x + 2524$	0.825	0.0023	$y = 1.6x + 2401$
	SBALF	0.242	0.062	$y = -14.69x + 5811$	0.0062	0.29	$y = -30.08x + 5239$
	MBCLR	0.219	0.068	$y = 8.28x + 2592$	0.218	0.068	$y = 9.74x + 2721$
	SBCLR	0.603	0.013	$y = -5.88x + 5297$	0.334	0.042	$y = -8.92x + 4766$
CPY Total Forage							
	MBALF	0.1066	0.11	$y = 1.15x + 190$	0.0058	0.30	$y = 6.65x + 487$
	SBALF	0.408	0.031	$y = -1.09x + 485$	0.250	0.060	$y = -2.03x + 655$
	MBCLR	0.0782	0.13	$y = 1.02x + 195$	0.171	0.08	$y = 2.41x + 278$
	SBCLR	0.753	0.0046	$y = -0.4x + 454$	0.0954	0.12	$y = 1.63x + 387$
CPY Grass							
	MBALF	0.1066	0.11	$y = 1.15x + 190$	0.352	0.04	$y = 0.76x + 213$
	SBALF	0.408	0.031	$y = -1.09x + 485$	0.0543	0.16	$y = -1.98x + 430$
	MBCLR	0.0782	0.13	$y = 1.02x + 195$	0.198	0.074	$y = 0.91x + 214$
	SBCLR	0.753	0.0046	$y = -0.4x + 454$	0.745	0.0049	$y = -0.28x + 390$

[†] Linear regression where x is equal to the percentage of legume initially removed from within the stand due to spraying in 2005

Appendix C

Table C.1 Species composition within established pasture prior to spraying in 2005 at PCF and LI.

Site	Common Name	Scientific Name	Number of Plots with Species [†]	Average Plot Abundance
LI	Dandelion	<i>Taraxicum officinale</i> F.H. Wigg	100	33.7
	American vetch	<i>Vicia americana</i> Muhl. Ex Willd	17	4
	Common yarrow	<i>Achillea millefolium</i> L.	10	0.2
	Cow parsnip	<i>Heracleum lanatum</i> L.	5	0.2
	Hemp nettle	<i>Galeopsis tetrahit</i> L.	4	0.1
	Common plantian	<i>Plantago major</i> L.	3	0.1
PCF	Danelion	<i>Taraxicum officinale</i> F.H. Wigg	100	14.2
	Chickweed	<i>Stellaria media</i> L.	97	2.4
	Common plantian	<i>Plantago major</i> L.	58	1.6
	Perennial sow thistle	<i>Sonchus arvensis</i> L.	50	5.5
	Hemp nettle	<i>Galeopsis tetrahit</i> L.	42	1.3
	Wild Buckwheat	<i>Polygonum convolvulus</i> L.	12	1
	Narrow leaved hawk's beard	<i>Crepis tectorum</i> L.	6	1

[†]Each site contain 100 plots

Table C.2 Harvest dates for peak biomass within cross-seeding and pasture study field experiments for 2006-2008 at all sites.

Year	Site			
	W240	E2	LI	PCF
2006	July 4-10	July 10-17	July 18-19	July 20-21
2007	July 15-17	July 18-22	July 22-23	July 24-25
2008	July 5-15	July 16-28	--	--

Appendix D

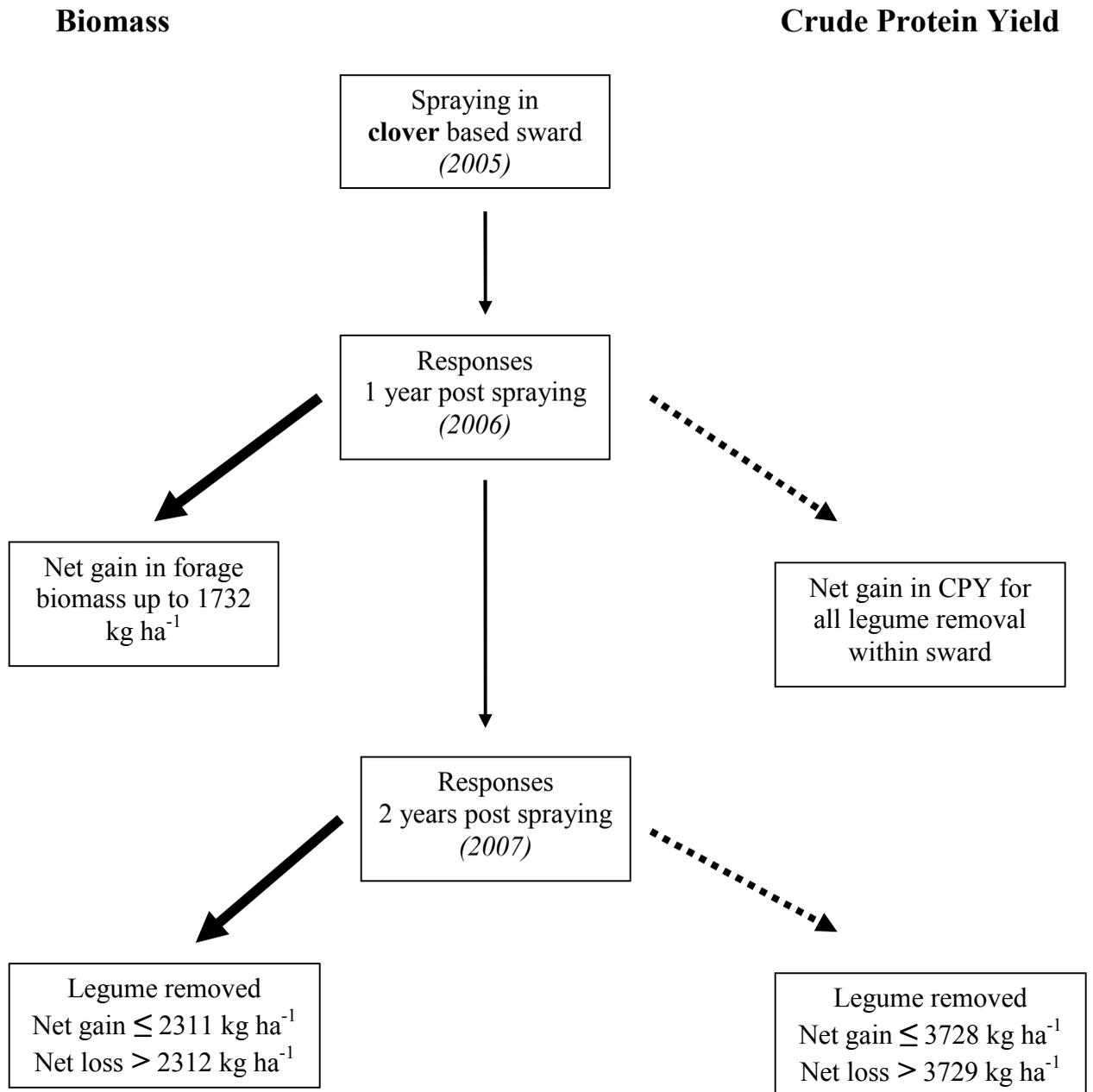


Figure D.1 Legume removal thresholds within an established clover based sward one (2006) and two (2007) years after spraying.

Biomass

Crude Protein Yield

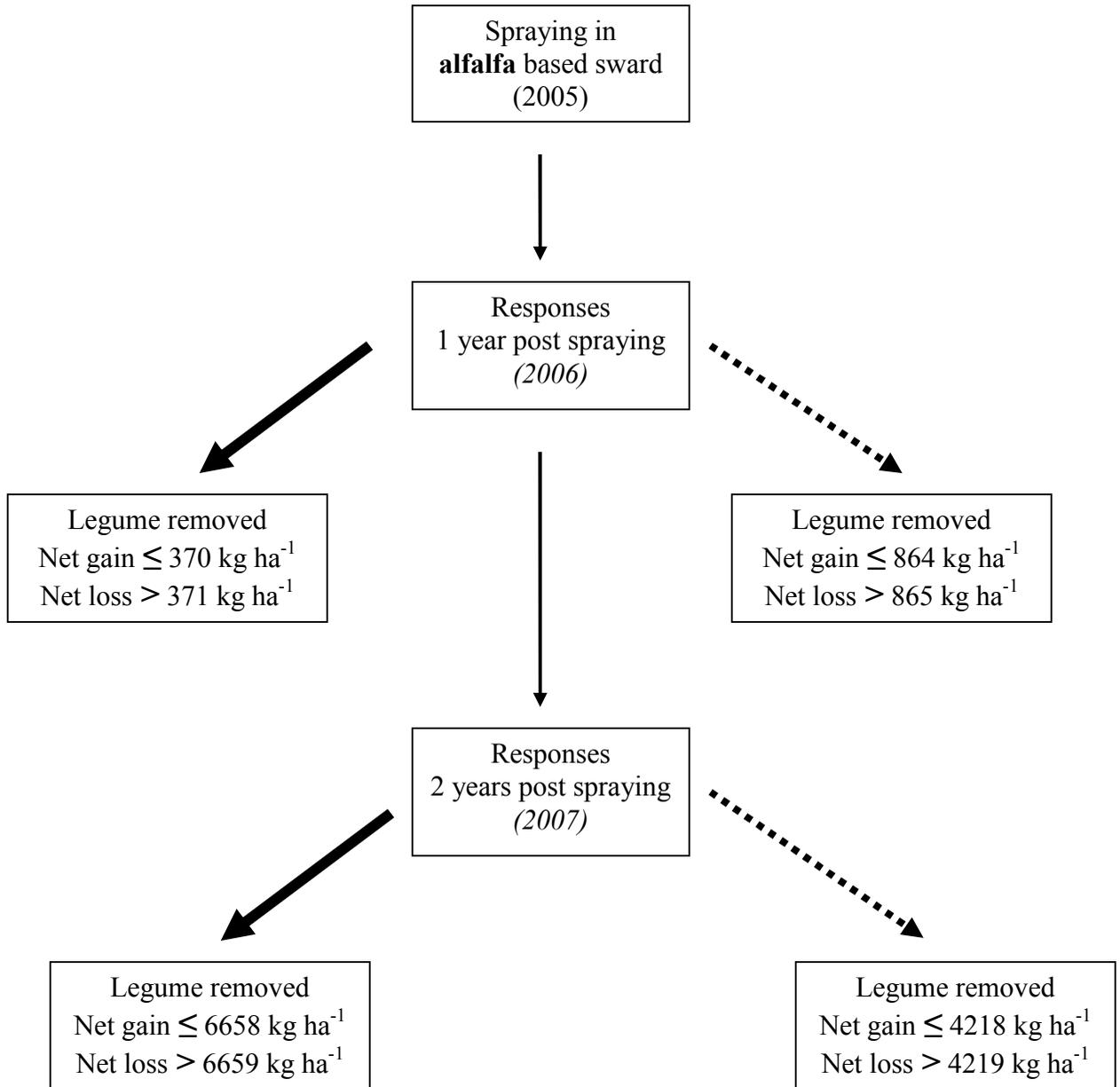


Figure D.2 Legume removal thresholds within an established alfalfa based sward one (2006) and two (2007) years after spraying.