University of Alberta

Kura clover (*Trifolium ambiggum*) seed production and establishment in Alberta.

by

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Prologue

Our interest in Kura clover began in 1999. Dr. Jane King was introduced species in Australia, and obtained a research grant to look at its pote Alberta. At that time, Kura clover had never been grown in western Canada. The purpose of the initial studies, begun in 1999, was to provide a foundation of knowledge that would direct further research into this plant, its management and use.

The conclusions of these tests identified the ability of Kura clover to persist in Alberta, and to be used under a variety of management strategies (Walker, M. Sc. Thesis, 2002). Annual yield was similar under a range of defoliation intensities. Quality analysis revealed that Kura clover had a high protein content, and low ADF and NDF values. Planting Kura clover with Kentucky bluegrass increased the digestibility and feed value, compared to a pure grass stand.

While several promising characteristics of Kura clover were identified, these preliminary studies also revealed two major drawbacks that would need to be overcome if Kura clover was to become a viable option for pastures in Alberta. My PhD research was designed to address these two limitations; the first being seed production of Kura clover in Alberta, and the second, establishment with high yielding grass species, particularly meadow bromegrass and orchard grass. The development of the 5 research experiments followed.

There is very limited information available on management of Kura clover grown for seed, particularly pre-harvest management. In 2002, a test was initiated to compare seed yield of two cultivars of Kura clover over a range of seeding rates and row spacings. In 2003, other experiments followed.

It is known that cover crops can have negative effects on an under-seeded perennial. We compared a range of cover crops to determine the subsequent effect on seed yield of Kura clover. For this test, we used Cossack, a cultivar developed in the United States and the most readily available for purchase.

Kura clover has slow seedling growth, consequently it can be difficult to es in mixtures with rapidly growing grass species. In order for Kura clover viable option in pastures, it is necessary that it be a meaningful contrib mixtures with high-yielding grasses. We examined the relative importance of above and below-ground competition when Kura clover was grown with meadow bromegrass.

Species composition of Kura clover – meadow bromegrass and Kura clover – orchard grass mixtures was compared when the two species were sown at the same time or when planting the grass was delayed.

The survival and growth of Kura clover seedlings was measured following planting in existing grass swards.

These experiments were designed to provide further insight into the challenges currently impeding the adoption of Kura clover for use in Alberta.

Abstract

Kura clover (*Trifolium ambiguum M. Bieb*.) is a perennial legume species has been found to have exceptional persistence in the United States, Aust and New Zealand. There are two challenges that impede the incorporatio Kura clover into pasture mixtures in Alberta. The first is the lack of available seed, and the second is poor establishment success in mixtures with highly competitive grass species. A series of experiments were conducted to (i) address the potential for seed production in a central Alberta environment and (ii) to determine alternative strategies for establishment in mixtures.

Kura clover successfully flowered and produced seed under central Alberta growing conditions. Seed production was greater from the cultivar Endura than Cossack. Kura clover seed production was not affected by row spacing however, yield was greater when clover was planted at 3 or 6 kg/ha versus 9 or 12 kg/ha. Seed yield ranged from 80 kg/ha to 350 kg/ha.

Establishing Kura clover with a cover crop reduced flowering and seed production. Corn (*Zea mays L.*) was the least competitive cover crop, followed by faba bean (*Vicia faba L.*). Canola (*Brassica napus L.*), peas (*Pisum sativum* L.), barley (*Hordeum vulgare L.*) and triticale (*X Tritosecale*) reduced Kura clover seed yield and are not recommended as cover crops.

Comparison of above and below ground competition between Kura clover and meadow bromegrass (*Bromus biebersteinii Rehm.*), indicated that below ground competition has the greatest impact on Kura clover seedling growth.

Altering seeding rate and delaying introduction of the grass species by up to two months significantly improved Kura clover establishment in mixtures.

Kura clover survival in established pastures was higher with physical than chemical sod suppression of the standing forage. Dry matter yield of Kura clover was greatest when defoliated at 6 week intervals.

Challenges still remain regarding seed production and establishment of Kura clover. However, we successfully addressed the major concerns regard potential of Kura clover in Alberta.

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List of Abbreviations

ADF	acid detergent fiber
°Cd	degree Celsius days
F	fall
H	herbicide
ha	hectare
K1	Kura clover – one true leaf
K3	Kura clover – three true leaves
KC	Kura clover
KBG	Kentucky bluegrass
kg	kilogram
MB	meadow bromegrass
NDF	neutral detergent fiber
NH	no herbicide
OG	orchard grass
S	same
SB	smooth bromegrass
SP	spring
wk	week

Chapter 1
Introduction

Forage Research in Alberta

The climate, soil and geography of Alberta are such that a large percentage of the agricultural land is dedicated to the production of grasses and legumes. There are an estimated 30,000 forage producers in Alberta, managing more than twelve million hectares of land in forage and managed range and bush (Collins, 2008). These crops provide the foundation of the beef, equine, sheep and managed wildlife industries. The Alberta forage industry also supports forage processors, suppliers, seed producers and sales companies, managers of rangeland/bush pastures and natural areas, conservation and reclamation organizations, turf grass and apiculture.

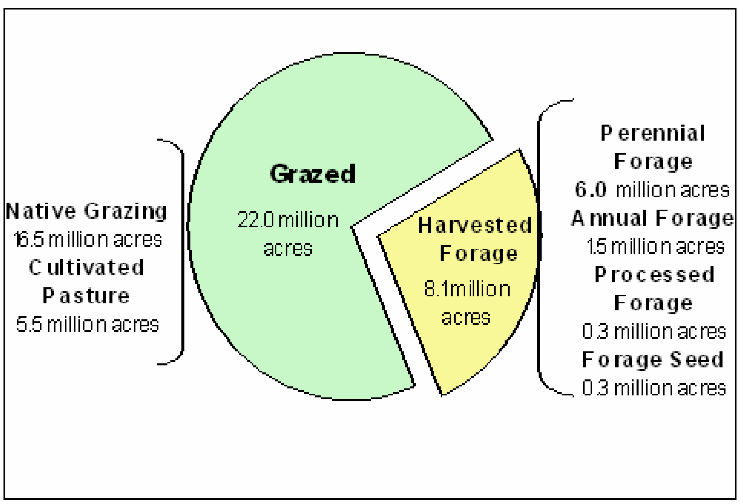

Figure 1-1. Forage Acres by Use in Alberta as reported in 2008 (Collins, 2008).

Over the past fifty years in Central Alberta, the use of native forage species has declined in favor of introduced species. Currently one-third of grazed land in Alberta relies on a large number of cultivated grass and legume species.

Legumes in Pastures

Legumes grown in mixtures with grasses for pasture offer several advantages over grasses grown alone. Legumes are able to obtain nitrogen from the atmosphere through symbiosis with N-fixing bacteria that occupy nodules on their roots, reducing the fertilizer needed to maintain pasture productivity (Moore et al., 2004). Legumes typically have higher protein content than grasses and therefore improve the nutritive value of the pasture, particularly for high-producing classes of livestock (Van Soest, 1994). The seasonal biomass accumulation of many temperate forage legumes is complementary to that of grasses (Moore et al., 2004). Despite their crucial role in western Canadian agriculture, relatively little scientific study has accompanied the use of these forage crops.

The majority of clovers inhabit temperate regions characterized by cool, moist climates (Taylor, 1989). Perennial legumes used in central Alberta are mainly alfalfa (*Medicago sp.*), red clover (*Trifolium pretense L.*), alsike clover (*Trifolium hybridum L.*), white clover (*Trifolium repens L.*) and to a lesser extent birdsfoot trefoil (*Lotus corniculatus L.*), sainfoin (*Onobrychus viciaefolia L.*) and cicer milkvetch (*Astragalus cicer L.*). Matches (1989) noted that legume use in the United States is presently limited due to lack of persistence and an inability to withstand long-term grazing. Of the legume species available for use in Alberta pastures, birdsfoot trefoil, sainfoin and red clover are short-lived, alfalfa does not tolerate heavy grazing, cicer milkvetch is difficult to establish and white clover is not productive under hot, dry conditions. Successful persistence under grazing is most likely due to complex interactions between the environment and plant characteristics (Reed et al., 1989). Success in mixed stands is determined by the legumes ability to maintain adequate plant density through both the survival of individuals, and the rate of recruitment of new individuals (Jones and Carter, 1989). Competition between grasses and legumes may result in a reduction in legume stand density, a decrease in legume plant size and yield, reduced flowering or lower seed yields (Kendall and Stringer, 1989).

Kura clover (*Trifolium ambiguum* M. Bieb.)

Trifolium ambiguum M. Bieb. was first identified by Marshal L.B.F. von Beiberstein in 1808; more precise descriptions were given by Hossain in 1961 and again in 1970 by Zohary (Bryant, 1974). Common names for *T. ambiguum* include Kura, honey, pellett or Caucasian clover. Kura clover originated in the Caucasian region of Russia; which includes Georgia, Armenia, the Crimea, Ukraine, eastern Turkey and northern Iran (Bryant, 1974; Speer and Allinson, 1985; Taylor and Smith, 1998). In its native habitat Kura clover has maintained a strong presence under intensive grazing (Lucas et al, 1997) and has been found over a wide range of elevations and environments (Speer and Allinson, 1985). Kura clover demonstrates greater productivity in cool rather than warm climates, over a wide gradient of habitats (Taylor and Smith, 1998).

Introduced into the United States in 1911, little attention was given to Kura clover as a forage legume (Taylor and Smith, 1998). The plant was named pellet clover by the bee keepers association and it was discovered to be an ideal plant in apiaries due to the short corolla tube making nectar easily available (Pellett, 1945). In 1941, four or five Kura clover plants were established from seed, fourteen months later, a dense stand filled the entire plot area (Pellett, 1945). This instigated investigations into Kura clover as a forage for grazing and hay land (Pellett 1946).

Similar to many other *Trifolium* species, Kura clover has a large taproot and crown, demonstrating rhizome development at three months after germination (Speer and Allinson, 1985). Unique to Kura clover, however, is the extensive nature of its root and rhizome system. Kura clover's initial crown growth and deep, branched rooting system is supplemented by early development of extensive rhizome growth (Forde et al., 1989). New clover plants arise from the rhizomes, progressively farther out from the parent plant. Average root: shoot ratios in Kura clover are reported to be 2.74, in comparison to white clover at 0.16 in an Australian mountain environment (Spencer et al., 1975). The massive below-ground biomass provides Kura clover adaptability to a variety of environmental conditions, persistence under grazing, and drought and cold tolerance (Genrich et al., 1998; Taylor and Smith, 1998).

In seedling Kura clover plants, vegetative production initially arises from a single meristem, located at the crown; additional meristems develop with the appearance of rhizomes (Genrich et al., 1998). Trifoliate leaves are glaucous and vary in size from 1 – 8 cm in length and 0.5 – 5.0 cm in width (Taylor and Smith, 1998). Kura clover exhibits an indeterminate growth pattern, with flowering occurring in response to long-day photoperiod (Speer and Allinson, 1985). White to pink flowers are both lateral and terminal, each stem carrying several flowers (Speer and Allinson, 1985). Plants are self-incompatible, requiring cross pollination (Taylor and Smith, 1998).

Kura clover has demonstrated a slower rate of growth and development than other commonly used legumes in Minnesota (Seguin et al., 1999). Growing point development of Kura clover is reported to be half the rate of white clover (Watson et al, 1996). Studies in Minnesota comparing establishment of several legumes found Kura clover to be highly susceptible to competition due to its slow vegetative growth in the establishment year (Cuomo et al., 2001). Black et al., (2003) demonstrated that thermal time is the primary factor in leaf appearance, especially axillary leaf development and consequently controls canopy expansion. A comparison of thermal time requirements for seedling development revealed that white clover axillary growth begins at 439°Cd (degree Celsius days) versus Kura clover at 990°Cd (Black et al, 2006).

Speer and Allinson (1985) indicated that Kura clover has three patterns of development, (i) where primarily the mother plant develops, (ii) where there is vigorous rhizome growth and daughter plant production in a dense stand over a short distance, and (iii) colonization of a wide area with few daughter plants (Speer and Allinson, 1985). Kim (1996) determined that, in Wisconsin, rhizomes appear more quickly in plants started from rhizome pieces than in plants started from seed. In Minnesota, Kura establishment was similar from seed or rhizome sprigs, when planted into chemically suppressed sod (Sheaffer et al., 2008).

Poor establishment of Kura clover has been attributed to the inability to successfully form symbiotic relationships with nitrogen-fixing bacteria (Patrick and Lowther, 1995). A high percentage of Kura clover nodules have been found to be small and ineffective (Erdman and Means, 1956). Inoculation with the commercially available *Rhizobium trifolii* has been found to be more effective in early-nodulating Kura clover plants versus plants with later nodulation (Hely, 1963; Hely, 1972). Studies in the United States have found that Kura clover nodules appear at a minimum of 50 days after seeding compared to 14 – 21 days for other legume species (Seguin et al., 2001). Furthermore, plants that were not inoculated did not nodulate, indicating the absence of an effective rhizobia native to North American soils (Seguin et al., 2001). Three strains of rhizobia that form successful relationships with Kura clover have been isolated (Beauregard, et al, 2004).

The slow establishment of Kura clover seedlings is further impacted by the effects of competition. In Alberta, Canada, Kura clover mixtures with either orchard grass (*Dactylis glomerata L.*) or meadow bromegrass (*Bromus riparius* Rehm.) had less than 13% clover (Walker, 2002). Greenhouse trials have indicated sensitivity of Kura clover to companion grass plant density (Hill and Mulcahy, 1995; Taylor and Smith, 1998). Establishment in mixed stands has been successful with the less competitive Kentucky bluegrass (*Poa pratensis L.*) and timothy (*Phleum pretense L.*) (Kim, 1996; Jeranyama et al., 2001; Walker, 2002). Studies in Quebec, Canada indicated that Kura clover was best suited to mixtures with smooth bromegrass (*Bromus inermis* Leyss.) or timothy (Seguin, 2007). The proportion of Kura clover has been found to slowly increase over several seasons when sown in mixtures with grasses (Cuomo et al, 2003; Cuomo et al., 2005; Laberge et al, 2005a; Laberge et al., 2005b; Seguin, 2007).

Kura clover can withstand frequent defoliation. Pure stands of Kura clover have similar yields when cut or grazed two, three or four times per season (Sheaffer and Marten, 1991; Peterson et al., 1994a; Walker, 2002). Under prolonged and intensive, continuous stocking however, below-ground biomass will be reduced (Lucas et al., 1997). Peterson et al. (1994b) determined that close, continuous grazing results in a reduction of above-ground growth to favor maintenance of root and rhizome biomass. Work in Minnesota suggests that Kura clover maintains productivity under defoliation better than birdsfoot trefoil, alfalfa or cicer milkvetch (Sheaffer and Marten, 1991; Sheaffer et al., 1992).

In spite of the challenges, once Kura clover has established it is a productive species with excellent nutritional quality. Kura clover has been reported to have higher crude protein and lower fiber content than alfalfa, birdsfoot trefoil, white clover, crown-vetch (*Coronilla varia L.*) and cicer milk vetch (Allinson et al., 1985; Sheaffer et al., 1992; Sleugh et al., 2000). Walker (2002) determined that the inclusion of Kura clover with Kentucky bluegrass increased crude protein content and reduced acid detergent fiber (ADF) and neutral detergent fiber (NDF) content of the forage. Kura clover – ryegrass (*Lolium perenne L.*) mixtures had higher nitrogen content than white clover-ryegrass mixtures and gave higher live-weight gains in lambs (Black et al., 2007). Kura clover-reed canary grass (*Phalaris arundinacea* L.) silage had higher yields and better nutritional characteristics than alfalfa silage (Kammes et al., 2008).

Legume Seed Production

In 2001, 3,808 Canadian farms reported income from growing forage seed crops. Of the 133,000 hectares dedicated to legume seed production, two-thirds are located in the prairie provinces (Alberta Agriculture, 2001). Specifically, forage seed production is based in northern Alberta, Saskatchewan, Manitoba and irrigated areas of Alberta and Saskatchewan (McCartney and Horton, 1997). The Canadian forage seed industry is small in comparison to other agricultural commodities. In 1994, 37 million kg of forage seed was exported generating 54 million dollars (McCartney and Horton, 1997). Alfalfa is the primary forage seed crop, while timothy, creeping red fescue (*Festuca rubra L.*), red clover, smooth bromegrass, meadow bromegrass, crested wheatgrass (*Agropyron cristatum L.*) and meadow fescue (*Festuca elatior* L.) are all produced for export (McCartney and Horton, 1997). Forage seed production is an essential part of Canadian agriculture, providing product for export as well as impacting seed processing, distribution, sales and beekeeping industries. The process of growing forages for seed is carefully monitored to maintain a high quality, saleable product. Stands of legumes and grasses must meet strict purity requirements in order to be considered for seed production. Despite the importance of the forage seed industry, Fairey and Hampton (1997) identified a general lack of knowledge regarding the plant processes involved in legume seed production.

Seedbed preparation is of utmost importance when planting small-seeded alfalfa and clovers whether for seed or forage (Rinker and Rampton, 1985). Recommended row widths range from 45 – 90 cm. Selection of row spacing is typically dependent on equipment rather than agronomic considerations (Rolston et al., 1997). In North America, legume seed crops are usually sown at 30 cm row spacing. Much variability exists between different species and environments for the optimum plant density that maximizes seed yield (Marshall et al., 1997).

Ideal seeding rates vary with species and environmental conditions. Red clover, for example, planted in rows is seeded from 0.5 – 2.2 kg/ha, and at 9 – 13 kg/ha when broadcast seeded. White clover is sown at 3 – 4.5 kg/ha. Seeding depth should be from 0.5 – 1.5 cm depending on soil type. Inoculation of legumes is mandatory to ensure vigorous growth (Rinker and Rampton, 1985).

Weed control in legume seed crops is essential. The presence of weed seeds in the harvested product will reduce seed quality, and may harbor diseases and insects, preventing certification. Planting an annual grain crop as a cover crop with the establishing clover is one common method of controlling weeds in the year of forage establishment (Miller and Stritzke, 1995). The competitive effect of the annual species, however, often reduces the establishment of slow-growing legumes (Miller and Stritzke, 1995; Fairey and Lefkovitch, 1991; Steiner and Snelling, 1994; Jefferson et al., 2005). Reducing the seeding rates of the annual crop, and harvesting early, can mitigate the negative impact of a cover crop on an establishing forage perennial.

Nearly all clovers and alfalfa must be cross-pollinated to produce seed (Rinker and Rampton, 1985). Providing bees, in close proximity to the crop, will ensure that pollination occurs. An abundance of bees, in addition to warm, dry weather, are necessary components for maximizing seed production. Two and a half to four bee hives per hectare are suggested for ladino and crimson clover seed production (Rinker and Rampton, 1985) and 4 – 5 hives per hectare for red clover and Kura clover (Bryant, 1974).

Perennial clovers can be difficult to harvest. A large proportion of seed is often not threshed out of the pod (Rinker and Rampton, 1985). Careful adjustments to the combine cylinder will improve harvest efficiency.

Many environmental and management factors can influence legume seed yield. Maximizing seed yield requires the development of strong plants prior to floral induction, exposure to the appropriate environmental conditions, successful pollination of flowers and adequate conditions for harvest (Aamlid et al., 1997). White clover yields in Oregon are highly variable and often low (Medeiros and Steiner, 2000). Medeiros and Steiner (2000) determined that adequate soil moisture was necessary for canopy development and to support initial flowering in white clover. Steiner and Alderman (2003) however, recommend harvesting spring vegetative growth to stimulate flowering. Spring biomass removal will delay flowering until later in the season, coinciding with higher pollinator activity levels and increasing the reproductive: vegetative growth ratio (Steiner, 1992). Harvesting legume seed in more than one season will maximize economic returns to the producer. Establishment costs are often high, with no economic return for a minimum of 16 months after planting (Medeiros and Steiner, 2000). Seed production in red clover is significantly lower in the second production year (Steiner and Alderman, 2003).

Boron deficiency has been associated with reduction in flower head number and fewer seeds $head^{-1}$ in red clover. Steiner and Alderman (2003) found that increasing boron in soils doubled white clover seed yield.

Kura Clover Seed Production

Similar to other legume species, little is known about the mechanisms controlling seed production in Kura clover. Seed production has been the limiting factor in the use of Kura clover (Hill and Mulcahy, 1993; Dear and Virgona, 1993). Seed production is currently limited to a small number of locations in Australia, New Zealand and the United States (Taylor and Smith, 1998). Seed yields obtained in New South Wales, Australia were well below commercially acceptable levels, ranging from 40 – 80 kg/ha (Hill and Mulcahy, 1993). Dear and Virgona (1993) indicated that seed yields as high as 300 kg/ha are possible in New Zealand. Speer and Allinson (1985) compared the three ploidy levels of Kura clover and found that diploid lines produce flowers earlier in the season than tetraploid or hexaploid lines.

The reproductive stems in Kura clover have many nodes and therefore the ability to produce multiple flower heads (Steiner, 1992). Spring removal of herbage was found to compromise flower production in Kura clover (Steiner, 1992). Steiner (1992) indicated that new stems require vernalization in order to become reproductive, and therefore no flowers are produced in the seeding year.

Coolbear et al. (1994) noted that maximizing root and crown development in Kura clover is an essential determinant in reproductive growth. Flowering stems arise only from the crown and are primarily main stems as opposed to those from daughter plants or axillary meristems. Taylor and Cornelius (1994) reported little success using phenotypic recurrent selection to develop improved flowering and seed production in Kura clover. Recent work in Tasmania has resulted in the development of a new Kura clover cultivar ‘Kuratas’. ‘Kuratas’ Kura clover is consistently higher yielding than other cultivars and a new rhizobium strain has improved establishment (Hall and Hurst, 2008). Hall and Hurst (2008) report seed yield of ‘Kuratas of 1000 kg/ha. Full commercial release of this cultivar is planned for 2011 in Australia and New Zealand.

Establishment of Grass- Legume Mixtures

Increasing pressure on managed agricultural land for non-agricultural uses has created a need for higher productivity and efficiency of grasslands. One of the most effective ways to increase forage quality and productivity in pastures, is to establish mixtures that contain legume species.

In mixtures, competition for resources plays a major role in shaping plant communities. The ways in which plants obtain and use resources, making them unavailable to neighboring plants, are mechanisms of inter and intra-specific interactions (Bazzaz, 1990). Two species sharing a space results either in exclusion of one species or co-existence of the species (Torssell and Nicholls, 1978). Competition for resources in artificial plant species mixtures can be minimized by including plants that utilize different niches in time and space (Firbank and Watkinson, 1985). Combining legumes with grass species provides more uniform seasonal distribution of yield (van Keuren and Hoveland, 1985), however, establishment of legumes in mixtures with grasses is challenging.

Grass and legume seedlings compete for light, water and nutrients (Skinner, 2005). Small seeded legumes are vulnerable to moisture deficits; legume seedlings tend to grow and develop more slowly than non-legume species, increasing their sensitivity to competition (Sheaffer, 1985). Successful establishment of forage mixtures requires matching species to edaphic and climatic conditions, proper preparation of the seed bed, adequate seeding rates, inoculation of the legumes, and planting at an appropriate time of year (Miller and Stritzke, 1995). Grasses demonstrate a large photosynthetic advantage over legumes; however, models formulated to address the complexity of relationships in grass: legume mixtures indicate that legumes have distinct advantages in growth due to their ability to fix nitrogen (Thornley et al., 1995). Success of both species is strongly influenced by soil nitrogen pools and competition for light.

Kura Clover Establishment

Kura clover seedling survival can be compromised by several factors, one of which is competition from other species. It is advantageous for seedlings to emerge early, to ensure access to light and nutrients is gained prior to competitors of the same or other species (Forbes and Watson, 1996). Several studies have suggested that the establishment success of Kura clover would be greatly improved by removing inter-specific competition during seedling development (Seguin et al., 1999; Cuomo et al., 2001; Black et al, 2006). Walker and King (2009) determined that isolation from either above or below-ground competition increased Kura clover leaf area. However, the greatest increase in leaf area occurred when Kura clover roots were separated from those of meadow bromegrass (Walker and King, 2009). Thorsted et al. (2006), observed a similar pattern in wheat – clover intercrops. They report that changes in canopy architecture may increase the effectiveness of light capture, and thus biomass production. Thorsted et al. (2006) found that the greatest proportion of resource utilization influencing plant growth is occurring below-ground.

Kura clover has numerous characteristics that make it a desirable legume for use in long-term pastures. Cuomo et al. (2003) found Kura clover has superior persistence, spread and competitive ability in cool-season grass pastures compared to alfalfa, birdsfoot trefoil and red clover. Studies in Minnesota, USA (Sheaffer and Marten 1991; Peterson et al. 1994) indicate that, once established, Kura clover is a productive and beneficial component of pasture swards with the ability to withstand a variety of defoliation intensities. To this point, however, Kura's use has been limited by establishment challenges. Hill and Mulcahy (1995) demonstrated that Kura clover root and rhizome development is greater with lower densities of companion grasses. This is similar to the findings of Hill and Hoveland (1993), who reported that Kura clover roots showed less branching when seeded with tall fescue compared to monoculture sowings. The negative effect of competition on below-ground growth is mirrored by vegetative production. White clover had greater establishment with early versus late maturing grasses (Sanderson and Elwinger, 1999). Similar results have been found for Kura clover – grass mixtures. Kura clover contributed more to biomass when sown with Kentucky bluegrass and timothy than in mixtures with highly competitive meadow bromegrass or orchardgrass (Kim, 1996; Jerenyama et al., 2001; Walker, 2002; Kim and Albrecht, 2008). Kim et al. (2006) indicate that

Kura clover establishment in mixtures is greatly improved with more lenient defoliation frequencies in the year of planting.

Studies in Eastern Canada and several states in the USA have found that Kura clover increased its contribution in mixtures over time, frequently out-competing Kentucky bluegrass and orchard grass (Seguin, 2008; Cuomo et al., 2003; Zemenchik et al., 2001). In contrast, studies in Alberta demonstrated the inability of Kura clover to compete in mixtures with high producing grass species (Walker, 2002). Sanderson et al. (2002) recommended reducing the seeding rate of the grass when establishing mixtures using brome species. Kura clover establishment may be improved by removing inter-specific competition during seedling development (Seguin et al., 1999; Cuomo et al., 2001; Black et al, 2006). Relative time of emergence has been identified as an important component in cereal crop-weed competition and in intercrops (O'Donovan et al., 1985; Tofinga et al., 1993).

The density of Kura clover and white clover stands declined in the first production year under continuous grazing after which they maintained their plant number and productivity (Brummer and Moore, 2000). Kleen et al. (2006) also found that Kura clover and white clover had similar productivity in mixtures cut for silage, or in simulated grazing systems, however, Kura clover demonstrated better tolerance to drought. However, once established, Kura clover is more persistent than white clover in a variety of management systems and environmental conditions (Brummer and Moore, 2000).

Kura Clover Establishment in Existing Stands

The introduction of legumes into existing pasture stands is a potential method to improve sward productivity, nitrogen fertility and feed quality (Awan et al., 1997). Interseeding legumes into pastures is reported to have variable results (Sheaffer, 1989; Lowther and Patrick, 1992; Awan et al., 1997) Success of seedling emergence in established stands is dependent on ground cover (Reader, 1991). Plant establishment and root weight were greater when Kura clover seed was directly drilled into sod versus surface broadcast (Moorehead et al., 1994).

In contrast, Cuomo et al., (2001) found that successful establishment occurred regardless of planting method as long as the sod was chemically suppressed. Initially, Kura clover had poor establishment when sod-seeded due to limited leaf production. However in subsequent seasons plant density increased (Cuomo et al., 2001).

Physical sod suppression through grazing or mowing has been suggested as an alternative to herbicide suppression. Biomass yield of the sward was greater, and there was less weed invasion with physical sod suppression, compared to using herbicide (Seguin et al., 2001). However, red, white and Kura clover establishment and yield were higher when the sod was chemically suppressed (Seguin et al., 2001; Laberge et al., 2005a; Laberge et al., 2005b).

Kura clover seedlings started from seed or rhizome clippings had a similar degree of spread in both smooth brome and Kentucky bluegrass pastures (Kim, 1996). Sheaffer et al., (2008) found little difference in establishment when Kura clover was introduced as seed or as rhizomes; however, size of rhizomes affected the vigor of establishing plants (Sheaffer et al., 2008).

Objectives

The goal of this thesis is to examine the two major impediments to the wide adoption of Kura clover in Alberta. Our aims were first, to determine the potential for Kura clover seed production in Alberta and to develop Kura seed production guidelines. Secondly, to investigate methods of improving Kura clover establishment in newly planted mixtures with grasses, to identify the relative importance of above versus below ground competition in Kura clover – grass mixtures, and to examine Kura clover survival and spread in existing swards.

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Chapter 2

The effect of seeding rate and row spacing on the seed yield of two cultivars of Kura clover (*Trifolium ambiguum* M. Bieb.).

A version of this chapter was submitted to Grass and Forage Science.

Hypotheses Tested

Seed yield of Kura clover cv. 'Endura' will not differ from the seed yield of Kura clover cv. 'Cossack'.

Seed yield of Kura clover will not differ when planted at 22, 30 or 45 cm row spacing.

Seed yield of Kura clover will not differ when planted at 3, 6, 9 or 12 kg/ha.

Yield components of Kura clover cv. 'Endura' will not differ as the yield components of Kura clover cv. 'Cossack'.

Introduction

Kura clover (*Trifolium ambiguum M.Bieb.*) is a perennial legume recently introduced into Canada. Native to the Caucasus region of Russia, it demonstrates great tolerance to a wide range of environmental conditions (Taylor and Smith, 1998). Through preferential partitioning of carbohydrates, Kura clover develops an extensive root and rhizome system that contributes to its longevity, productivity, and tolerance to grazing (Taylor and Smith, 1998). Prior studies in Alberta (Walker 2002) indicated that Kura clover had potential as a legume in binary pasture mixtures in central Alberta and in other parts of North America (Seguin et al., 1999; Cuomo et al., 2003).

The number of studies involving Kura clover seed production is limited, and lack of seed supplies limit use (Bryant, 1974). Studies that have examined the reproductive capabilities of Kura clover have found that diploid lines exhibit more even flowering than tetraploid or hexaploid lines (Bryant, 1974). Kura clover flowers under a long-day photoperiod, diploid cultivars flower earlier than tetraploid and hexaploid cultivars (Speer and Allinson, 1985). Seed is produced primarily on the first growth, since plants require vernalization to produce inflorescences (Taylor and Smith, 1998). Individual Kura clover plants have been found to vary widely in the number of flowers produced and subsequent seed yield (Fu et al., 1999b). Fu et al, (2001) suggested that development of secondary crowns was the main influence on number of reproductive shoots. Biomass removal, a common practice in other types of clover seed production, has been found to reduce flowering and subsequent seed yields by 90% compared to un-cut treatments, regardless of timing of biomass harvest (Steiner, 1992).

At the time of this study, there were only two commercially available cultivars of Kura clover in North America, Endura and Cossack. Endura is a hexaploid cultivar developed in New Zealand in 1998. Endura was selected from the cultivar Monaro, for improved seed yield and increased seedling vigor (Dear and Hackney, 2007). The cultivar Cossack was developed in the United States. Kura clover seed yields ranged from 100 - 300 kg ha^{-1} in the United States, and 40 – 80 kg ha^{-1} in New Zealand (Taylor and Smith, 1998; Hill and Mulcahy, 1993).

Current seed supplies are imported into Canada from the United States and New Zealand. At present, little effort has been directed toward examining seed yields of Kura clover in Canada. Potential growers have no guidelines regarding the agronomic factors impacting seed yield. The acceptance and use of Kura clover would be more rapid if seed was produced locally and available at a reasonable price.

The primary objectives of this study were to compare seed yield, and yield components of two cultivars using a range of row spacings and seeding rates, and ultimately, to determine if sufficient quantities of seed can be produced to make Kura clover a viable seed crop for central Alberta.

Experimental Design and Methodology

An experiment was initiated at the University of Alberta's Edmonton Research Station (53°25'N, 113°,33'W), Edmonton, Alberta, on a black chernozemic soil. A split-split plot randomized complete block design with four replicates was utilized to determine the effects of cultivar, row spacing and seeding rate on seed yield of Kura clover. The treatment factors consisted of cultivars 'Cossack' and 'Endura' Kura clover (sub-subplots) planted using 22, 30 or 45 cm row spacing (main plot) and sown at 3, 6, 9 or 12 kg ha^{-1} (sub-plots). Plots were established in each of three years (May 2002, 2003 and 2004). Prior to seeding, soil samples were taken to determine nutrient status (Norwest Labs, Edmonton, Alberta) and granular 11-52-0 (N-P-K) fertilizer was added at a rate of 100 kg ha^{-1} according to recommendations for legume seed production. Both Endura and Cossack Kura clover seed was pre-inoculated with rhizobia (*Rhizobium leguminosarum bv. trifolii*), however, because successful nodulation of Kura clover is known to be challenging, additional rhizobia were added at a rate of 10% Kura clover seed weight, using a thirty percent corn syrup solution as a sticking agent, just prior to seeding.

Plots were sown using a double disk press drill; individual plots measured 2 x 6m. Weeds were controlled by hand in the year of establishment. Hives of honey bees (*Apis mellifera)* were placed adjacent to the plots beginning in the year following planting, and each year thereafter, to facilitate pollination of the Kura clover.

Beginning in the year following planting, plots were harvested for seed using a field plot combine (Table 2-1). To determine seed yield components, 25 heads were collected at random from each plot (100 heads per treatment). Each head was individually examined to determine number of flowers, number of seeds and seed viability. In 2003 plots were swathed two weeks after the first killing frost to allow the plant material to dry. This was only partially successful as plant material did not completely dry down, and in subsequent years a desiccant was used to improve harvesting ease. Plots were desiccated using Reglone at a rate of 1.1 L ha^{-1} with Ag-Surf at 0.1% in 90 L (Table 2-1). Desiccant was applied when seeds located at the lower and mid position within an individual clover head were brown and upper seeds were firm but still green to brown. Two weeks following application of the desiccant, plots were harvested using a plot combine (Nurserymaster Elite, Wintersteiger, Saskatoon, Saskatchewan). Harvested material required additional processing after combining to thresh Kura clover seeds from the flowers. Collected material was processed in a scarifier then run through an aspirator to remove extraneous material. Weight of harvested seed, 1000 seed weight, and percentage germination were determined.

Because of the late maturation of the seeds, in 2004 and 2005, a one square meter sample was harvested by hand to ensure data was obtained prior to the onset of winter. Combine harvests occurred October 6, 2003; December 3 2004; and October 25, 2005 (Table 2-1). A combine harvest did not take place in 2006 due to low flower production. In both 2005 and 2006, the number of inflorescences per square meter was counted to determine if a combine harvest was justified. The trial planted in 2002 was only harvested in the first production year.

The GLM procedure of SAS (2003) was used for analysis of variance to test the effects of cultivar, row spacing and seeding rate on seed yield of Kura clover. Planting year, cultivar, row spacing and seeding rate were considered fixed effects. Treatment means were separated using a Tukey test, significance was tested at $p < 0.05$. Results for analysis of variance tests are presented in tabular form located in the Appendix (Table A-1).

Results

Overall Response

Kura clover will successfully flower and produce mature seed under central Alberta growing conditions. Reproductive growth did not occur in the year of seeding. Flowers were produced early in the season each year thereafter. Apart from the 2004 planting, the greatest seed yield was consistently produced in the first production year (year following seeding). First year harvested seed yield ranged from 2.2 – 3.5x greater than second year production, and 7-10.5x greater than third year production. Cultivar differences were present for all treatments and in all production years, Endura yielded significantly more than Cossack ($p < 0.001$ over all years).

Yield components also exhibited cultivar differences. Endura seeds were significantly larger, weighing 5.2 gm per thousand versus 3.6 gm per thousand for the cultivar Cossack ($p < 0.01$). Endura had significantly more flowers per head (97 flowers/head) than Cossack (88 flowers/head). Seed viability did not differ between cultivars. Germination was 78% for Endura and 74% for Cossack when averaged across row spacing and seeding rate treatments. Seeding rate and row spacing had no effect on the number of flowers per head, seed weight or germination.

The harvested seed yield of Kura clover varied greatly between plots established in different years; therefore data was analyzed independently for each of the trials (2002, 2003 and 2004). Commonalities between tests established in different years are discussed.

2002 Trial

Plots established in 2002 were first harvested in 2003. Plants produced an abundance of flowers early in the season. The cultivar Endura produced 1.8x more harvested seed than the cultivar Cossack. Due to a high level of variation within each treatment, there were no significant row spacing or seeding rate effects and data is therefore not presented.

2003 Trial

First production year

Plots sown in 2003 were first harvested in 2004. Endura had significantly higher harvested seed yield than the cultivar Cossack ($p<0.001$; Figure 2-1). Average seed yield in the first production year for Endura (635 kg ha^{-1}) was 156 kg ha^{-1} more than Cossack, (479 kg ha^{-1}). Altering row spacing at the time of planting had no effect on the subsequent seed yield, however, the seeding rate of the clover stand did significantly impact the harvested seed yield ($p < 0.001$; Figure 2-1). For both cultivars, harvested seed yield was greatest when seeding rate was low. Kura clover sown at 3 or 6 kg ha^{-1} yielded 119 – 196 kg ha^{-1} more than Kura clover sown at 9 (492 kg ha^{-1}) or 12 kg ha^{-1} (463 kg ha^{-1}). Harvested seed yield ranged from 464 to 659 kg ha^{-1} when averaged across cultivars (Figure 2-1).

Second production year

A significant interaction was present between cultivar and seeding rate ($p<0.001$; Figure 2-2). Differences were due to the higher rate of seed production in Endura. For both cultivars, seed production declined at higher seeding rates. Row spacing did not affect seed yield. Harvested seed yield of Endura from a 3 kg ha^{-1} seeding rate was 253 kg ha^{-1} higherthan from a 12 kg ha^{-1} seeding rate (199 kg ha^{-1}) whereas for Cossack Kura clover, seeding rate of 3 kg/ha was 82 kg/ha more than from a seeding rate of 12 kg ha^{-1} (50 kg ha^{-1}; Figure 2-2). Harvested seed yield ranged from 199 – 452 kg ha^{-1} for the cultivar Endura and from 42 – 148 kg ha^{-1} for cultivar Cossack (Figure 2-2).

Third production year

Yields in the third year of seed production (2006) were lower than the first and second year seed yields (Figure 2-1). Harvested seed yield was 10% of seed yield in first production year, and only 50% of second production year harvested yields. Reduction in yield resulted from lack of inflorescence production rather than failure to produce mature seed (Walker, visual observation). As found in years one and two, the cultivar Endura had significantly higher production than the cultivar Cossack, with 54 kg ha^{-1} more harvested seed (38 kg ha^{-1}; $p<0.001$). Row spacing did not affect harvested seed yield. Seeding rate significantly affected seed yield ($p < 0.01$). The 3 kg ha^{-1} rate produced higher seed yields than the other 3 rates. Seed yield from 3 kg/ha was 44 kg/ha greater than from the 6 – 12 kg/ha seeding rate (51 kg/ha).

2004 Trial

Kura clover plots established in 2004 did not achieve the level of seed production of those sown in 2002 and 2003. First and second year yields were less than 3% of the seed yield harvested from the plots sown in 2003.

First production year

Plots sown in 2004 were first harvested in 2005. Vegetative growth of the Kura clover was vigorous, however, harvested seed yield for both cultivars was very low. Seed maturation did occur, however, very few inflorescences were present and subsequent yield was limited. Altering row spacing at the time of planting had no effect on the subsequent seed yield (Figure 2-3). An interaction between cultivar and seeding rate was significant for harvested seed yield ($p<0.001$). The interaction resulted from differences in the rate of responses of each cultivar to seeding rate. For both cultivars, harvested seed yield was greatest when seeding rate of the stand was low. Endura sown at 3 kg ha^{-1} was 26 kg ha^{-1} greater than 6, 9 and 12 kg ha^{-1} (9 kg ha^{-1}). Cossack yielded significantly more from 3 kg ha^{-1} rate than from other seeding rates (Figure 2-3).

Second production year

Harvested seed yield was higher in the second production year (2006) than the first production year for plots established in 2004. Yields remained well below those harvested from plots established in 2002 and 2003. As in previous years, there was a significant seeding rate effect ($p<0.001$). Endura, when sown at 3 kg ha-1 produced significantly more seed (54 kg ha^{-1}) than when sown at 6, 12 kg ha^{-1} (92 kg ha^{-1}) and 9 kg ha^{-1} (30 kg ha^{-1}; Figure 2-4). Seed yield of Cossack was greatest when sown at 3 kg ha^{-1},45 kg ha^{-1} higher than a seeding rate of 6 kg ha^{-1} (46 kg ha^{-1}), 9 and 12 kg ha^{-1} (16 kg ha^{-1}; Figure 2-4).

Discussion

Seed yield obtained from the 2003 sown plots (2004 harvest) was 2 – 6x greater than harvested seed yield reported in other seed yield trials in the USA, Australia and New Zealand (Taylor and Smith, 1998; Hill and Mulcahy, 1993). Kura clover seed yields in Kentucky ranged from 113 – 289 kg/ha for the cultivar 'Rhizo' (Taylor and Smith, 1998). Hill and Mulcahy (1993) reported yields of only 40 – 80 kg/ha, well below the commercially accepted yield in New Zealand.

Our data indicates a general reduction in seed production over the three harvest years. Production in the second year was less then 50% of the harvested seed yield in the first production year. However, harvested yield in the second production year (2003 plots) was greater than reported yields in Kentucky and the northern United States (Taylor, 2002). The reduction in Kura clover seed production over time is consistent with seed yield in other *Trifolium* species. In white clover (*Trifolium repens* L.) first year seed production is greater than that obtained in the second year (Menderos and Steiner, 2000).

Seed yield is related to the number of inflorescences per plant, number of seeds per flower head and the individual seed size (Rinker and Rampton, 1985). Heads per plant in Kura clover, have been found to range from 49.9 in diploid lines to 72.6 in hexaploid lines, flowers per head ranged from 79 in tetraploid lines to 90 in diploid and hexaploid lines (Kannenberg and Elliot, 1962). Kannenberg and Elliot (1962) reported average seed weights of 2.99 gm per hundred seeds. The number of flowers per head in our study, for both Endura (97 flowers/head) and Cossack (88 flowers/head) (hexaploid) were consistent with previously reported numbers for hexaploid cultivars. Under our growing conditions however, seed weight was 20% greater in Cossack and 75% greater in Endura than the mean seed weight previously reported for hexaploid lines.

Row spacing did not affect the harvested seed yield of Kura clover in this study. The response of plants to row spacing varies with cultivar and species. Legume seed crops in North America are typically sown at 30 cm row spacing, however, much variability exists between species for the optimum plant density that maximizes seed yield (Marshall et al., 1997). Recommended row spacing for red clover (*Trifolium pratense* L.) planted for seed production is 45 to 90 cm, depending on planting equipment (Rincker and Rampton, 1985). In dry areas of Britain, narrow row spacing was found to improve seed yield in white clover (Hollington et al., 1993). Clifford (1974) noted that red clover seed production in New Zealand was maximized when sown at a 60 cm row spacing. Based on our findings, selection of row spacing should be based on equipment capabilities and environmental conditions when establishing Kura clover for seed production.

Optimum seeding rates for legume species differ when sowing stands for forage biomass versus seed production. The goal in establishing a stand for seed is to obtain strong and vigorous plants rather than a dense stand of smaller, often weaker plants (Rolston et al., 1997). In this study, when lower seeding rates were used (3 and 6 kg ha^{-1}), individual plants achieved greater size. Previous studies suggest a larger plant should have a greater number of flowers per plant and therefore seed yield should also be greater. Fu et al., (1999) determined that seed yield of Kura clover genotypes differ in their response to seeding rate. While several genotypes were unaffected by seeding rate, in some, the number of inflorescences declined as plant density increased (Fu et al., 1999a). Coolbear et al., (1994) stated that two thirds of the reproductive shoots on Kura clover arise from the main crown, with the remaining third coming from secondary crowns. The size of a plant's root/crown ratio, indicative of energy stores, is correlated with the number of flowers produced (Larcher, 2003). The dominant role of the main plant in seed production apparently carried through to the second and third years of production in our study where seed yield did not increase due to the presence of reproductive capabilities of the daughter plants. In red clover grown for seed, seeding rate is influenced by environment. Lower seeding rates are used when planting conditions are ideal and increased rates under more adverse conditions to ensure adequate plant numbers (Rincker and Rampton, 1985).

Kura clover flowers in response to long-day photoperiod (Speer and Allinson, 1985). The differences in seed yield observed in our experiment may be due to environmental conditions during the time that the Kura clover plants were initiating flowering. Cooler and cloudier spring weather may reduce the strength of the plant's physiological signal to initiate reproductive growth. In the spring of 2004 and 2005, while temperatures were within the range for ideal growth of the clover plants (Figure 2-4), most days were cloudy and this may have reduced flower initiation. Low light intensities reduce the effectiveness of the photosynthetic process, changing the rate of carbohydrate sequestration within the plant. Low light intensity has been found to reduce flowering in timothy, red clover, white clover and alfalfa (Aamlid et al., 1997). In addition to the quality of the incoming radiation, the precipitation pattern differed among the establishment years. Oliva et al. (1994) determined that seed yield of red clover was maximized when soil water availability exceeded crop requirements during peak flowering. In our study, in 2004, the month of July had more precipitation than the 30 year average, all other years of establishment showed lower precipitation during the growing season (Figure 2-6). The large volume of seed harvested in 2004 from plots sown the previous season may be due in part to the abundance of moisture available during flowering in 2004. Similarly, poor seed yields in other years may have resulted from a deficiency in soil moisture during certain periods of Kura clover development.

Kura clover is highly self – incompatible and therefore seed yield is significantly influenced by the availability of pollinators (Speer and Allinson, 1985). A density of 4 – 5 hives per acre is suggested to provide sufficient bees for successful pollination (Bryant, 1974). Based on this guideline, the number of hives that were placed adjacent to the Kura clover plots provided an adequate number of bees necessary to optimize fertilization. In all years, bees were observed actively pollinating the Kura clover plants. Low seed yields in the first and second year of production from the 2004 established plots did not result from a lack of available pollinators rather low seed yield was directly related to the failure of plants to produce inflorescences.

Economic Considerations

In recent years, Kura clover has been given attention as a pasture legume for central Alberta both in the scientific community and in the popular press. The cost, and lack of availability, of seed however, has greatly limited its adoption. Only one company in the Edmonton area had Kura clover seed available in the spring of 2007, other suppliers would order it from elsewhere if requested. The cost to producers was $16.80 per kilogram (Proven Seeds, June 2007) nearly quadruple the cost of white clover ($4.15), and many times greater than red clover ($1.99) and alsike clover ($1.49). Based on yields obtained in this trial, development of a local seed supply may be possible, however, there are some harvesting challenges that must first be addressed. Since Kura clover is an indeterminate crop, desiccation of the stand was necessary to facilitate drying of the plant material. In the short growing season of central Alberta, the timing of desiccation may need to occur before the seeds have reached an appropriate stage of maturity. Thus, producing a consistent yield of quality Kura clover seed may be difficult. In southern Alberta, where the growing season is longer, Kura clover plants may have more opportunity to produce mature seed. This would ensure a more consistent level of production from year to year. Additionally, we found that harvested material requires additional processing. Kura clover seeds are small, light and require a great deal of abrasion to remove the remaining floral parts. Obtaining a clean sample from the combine resulted in high seed losses. The material harvested from the combine was only partially threshed and needed to be put through an abrasion process to remove the seed from the flower, and then aspirated to separate the seed from the chaff. The difficulties we encountered obtaining a clean seed product are not unique to Kura clover. Rincker and Rampton (1985) stated that effective threshing of clover seeds is the biggest challenge, requiring special combine settings and careful adjustments to the type and speed of the cylinder. While processing of forages seeds is commonly practiced this will increase the cost of seed (Simon et. al., 1997).

Conclusions

Seed production has been reported to be a consistent problem in Kura clover making seed expensive to purchase and difficult to obtain. Reported seed yields from both the United States and New Zealand typically range from less than 100 kg ha^{-1} to 200 kg ha^{-1}. Kura clover, in some of our trials, exceeded the expected seed yield by up to three fold suggesting that central Alberta may have conditions favourable for seed production. Minimizing intra-specific competition for the mother plants was found to have a positive impact on the harvested seed yield. Lower seeding rates, 3 and 6 kg ha^{-1}, consistently produced higher seed yields than stands seeded at 9 – 12 kg ha^{-1}. While harvested seed yield was highest in the first production year, the amount of seed harvested in the second production year of the 2003 seeding, remained above the average recorded yields in the United States. Developing a local source of Kura clover seed would reduce the cost and increase awareness of the species, making Kura clover a more attractive alternative to producers for seeding in pastures.

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Table 2-1. Record of harvest dates for Kura clover grown for seed. Trials were established in three years, at the University of Alberta's Edmonton Research Station, Edmonton, Alberta, Canada.

Date of Planting	Date of desiccation	Date of hand harvest (m^2)	Date of combine harvest
June 4, 2002	n/a	n/a	Oct 6, 2003
May 28, 2003`	Sept 28, 2004	Oct 15, 2004	Dec 3, 2004
	Sept 12, 2005	Oct 4, 2005	Oct 27, 2005 *
		Oct 9, 2006 **	
May 20, 2004	Sept 12, 2005	Oct 5, 2005	Oct 25, 2005
		Oct 9, 2006 **	

* combine harvest was unsuccessful, plants were too short and most material was not picked up by the combine
** flower production was limited, number of heads per m^2 were counted and it was determined that production was not sufficient to warrant a harvest

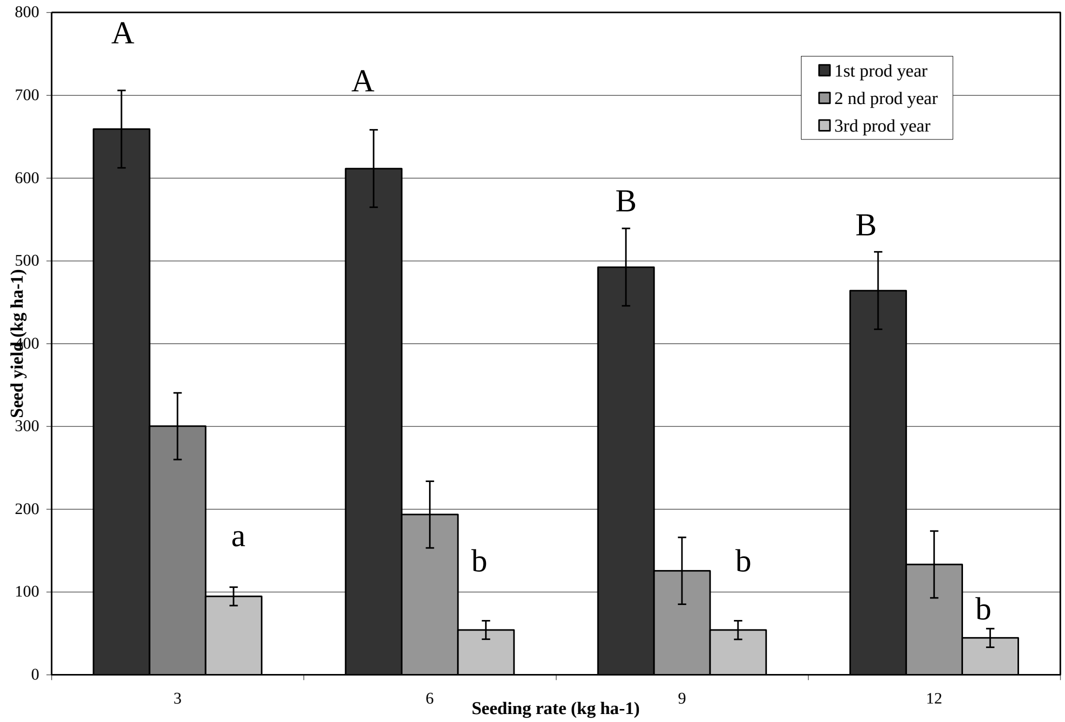

Figure 2-1. Seed yield of Kura clover from plots established in 2003, averaged across cultivars, in the first, second and third years of production (2004 - 2006) sown at 3, 6, 9 or 12 kg/ha at the Edmonton Research Station, Edmonton, Alberta.
A-B / a –b means followed with the same letters are not significantly different : $p<0.0001$ in the first production year *(A-B)* and $p<0.01$ in the third production year *(a-b)*.
*second production year had a significant interaction between seeding rate and cultivar (see Figure 2).

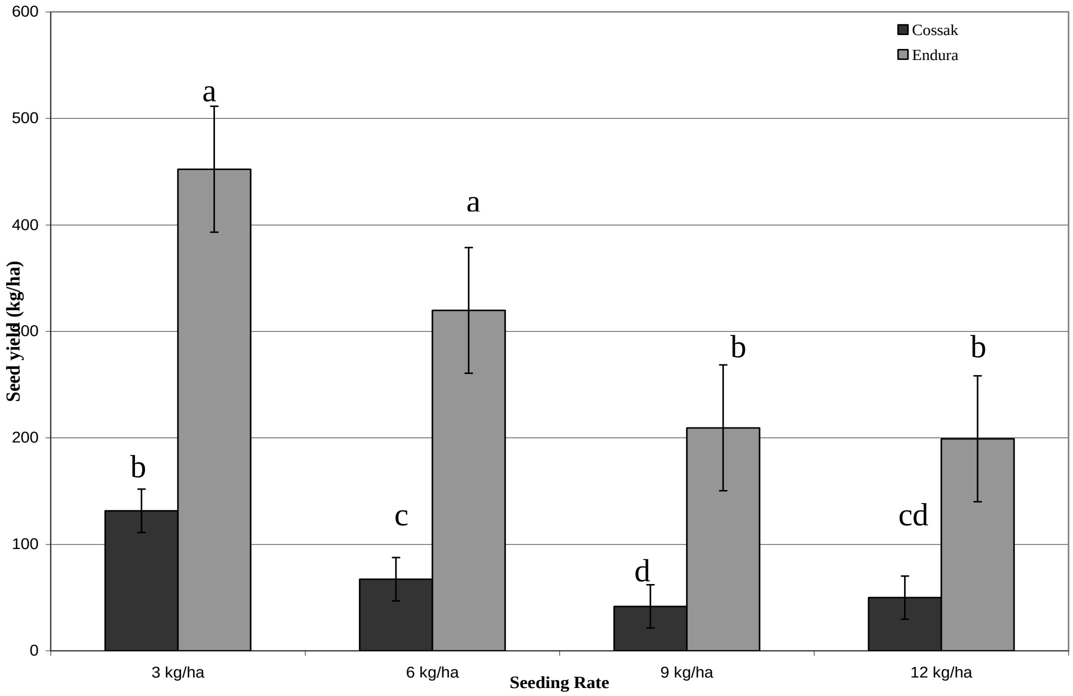

Figure 2-2. Interaction of cultivar and seeding rate ($p<0.001$) for Kura clover in the second year of seed production (2005) for plots sown in 2003 at three row spacings and four seeding rates at the Edmonton Research Station, Edmonton, Alberta.

$a - c$ means followed with the same letters are not significantly different: $p<0.001$.

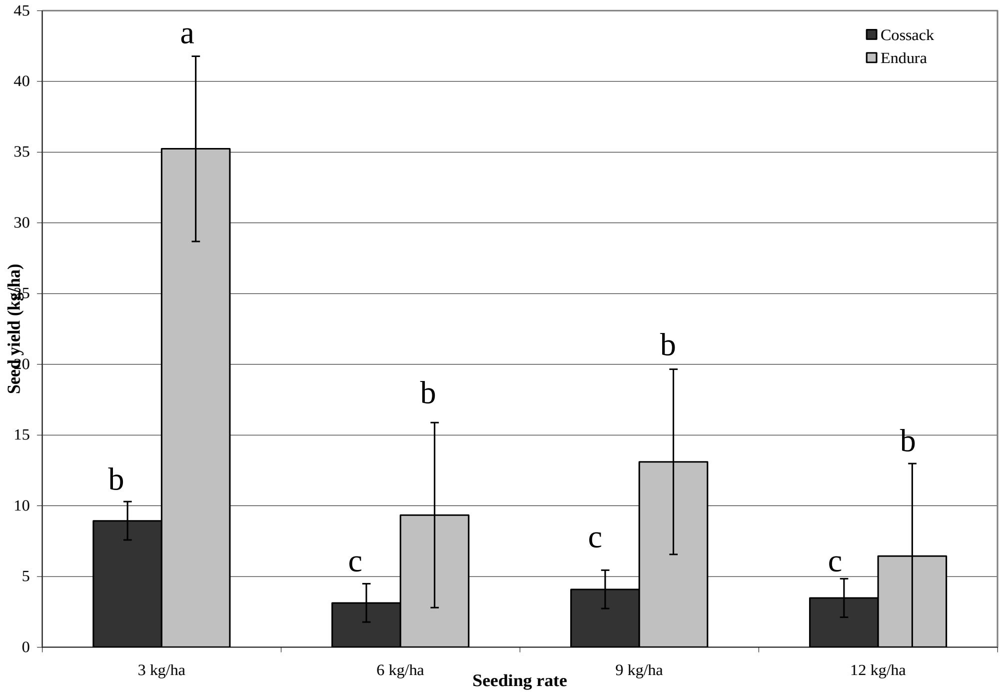

Figure 2-3. First production year seed yield (2005) of Kura clover sown in 2004, located at the Edmonton Research Station, Edmonton, Alberta, at three row spacings and four seeding rates.
a –c means followed with the same letters are not significantly different: $p<0.01$

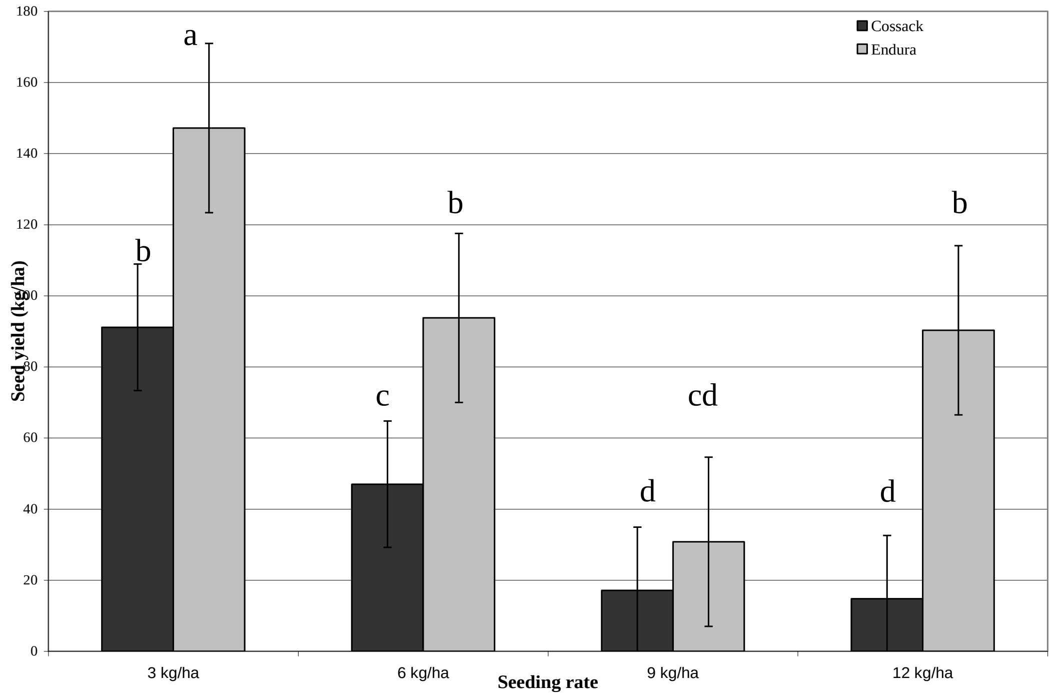

Figure 2-4. Second production year seed yield (2006) of Kura clover sown in 2004 and located at the Edmonton Research Station, Edmonton, Alberta, at three row spacings and four seeding rates.
a –c means followed with the same letters are not significantly different: p<0.01

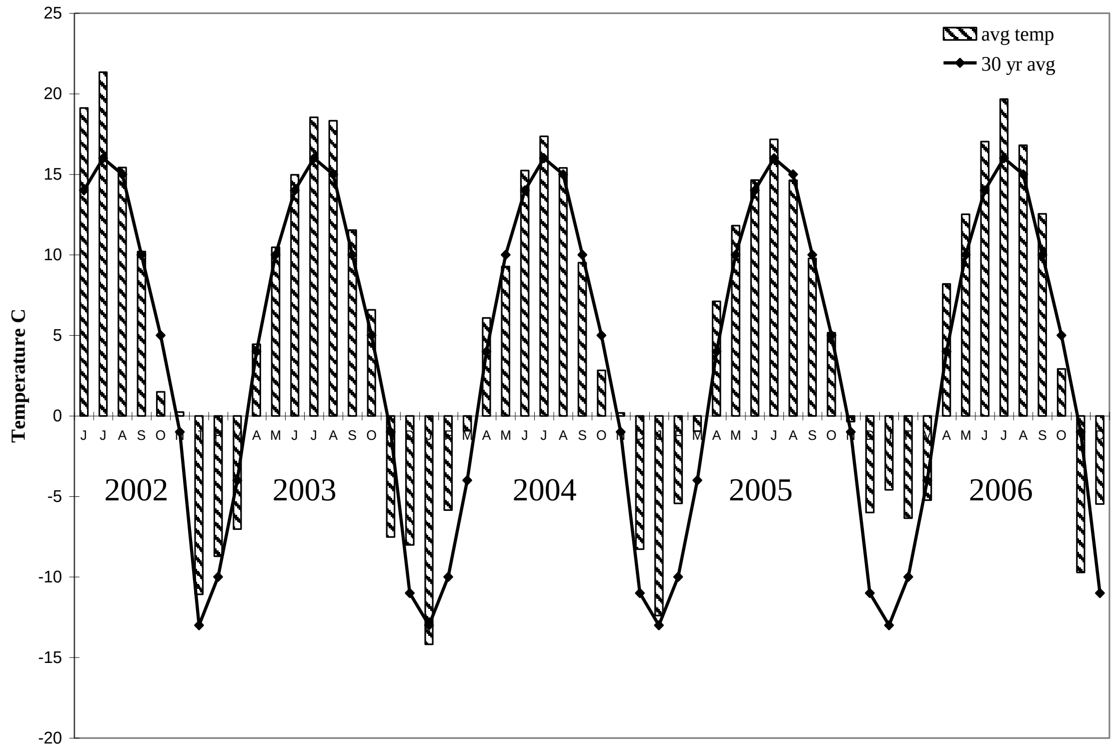

Figure 2-5. Average monthly temperature (°C) at Edmonton Research Station, Edmonton Alberta, from June 2002 to December 2006, and the 30 year average monthly temperature (°C) for Edmonton, Alberta.

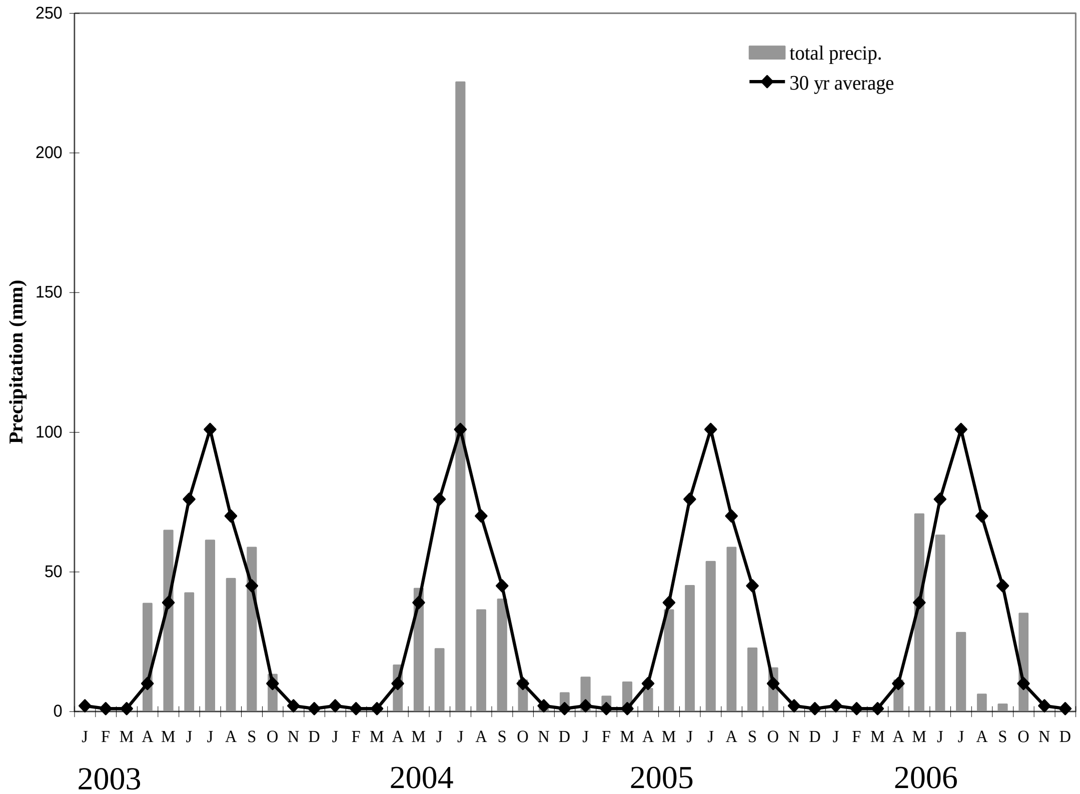

Figure 2-6. Average monthly precipitation (mm) at Edmonton Research Station, Edmonton, Alberta, from January 2003 to December 2006, and the 30 year average monthly precipitation (mm) for Edmonton, Alberta.

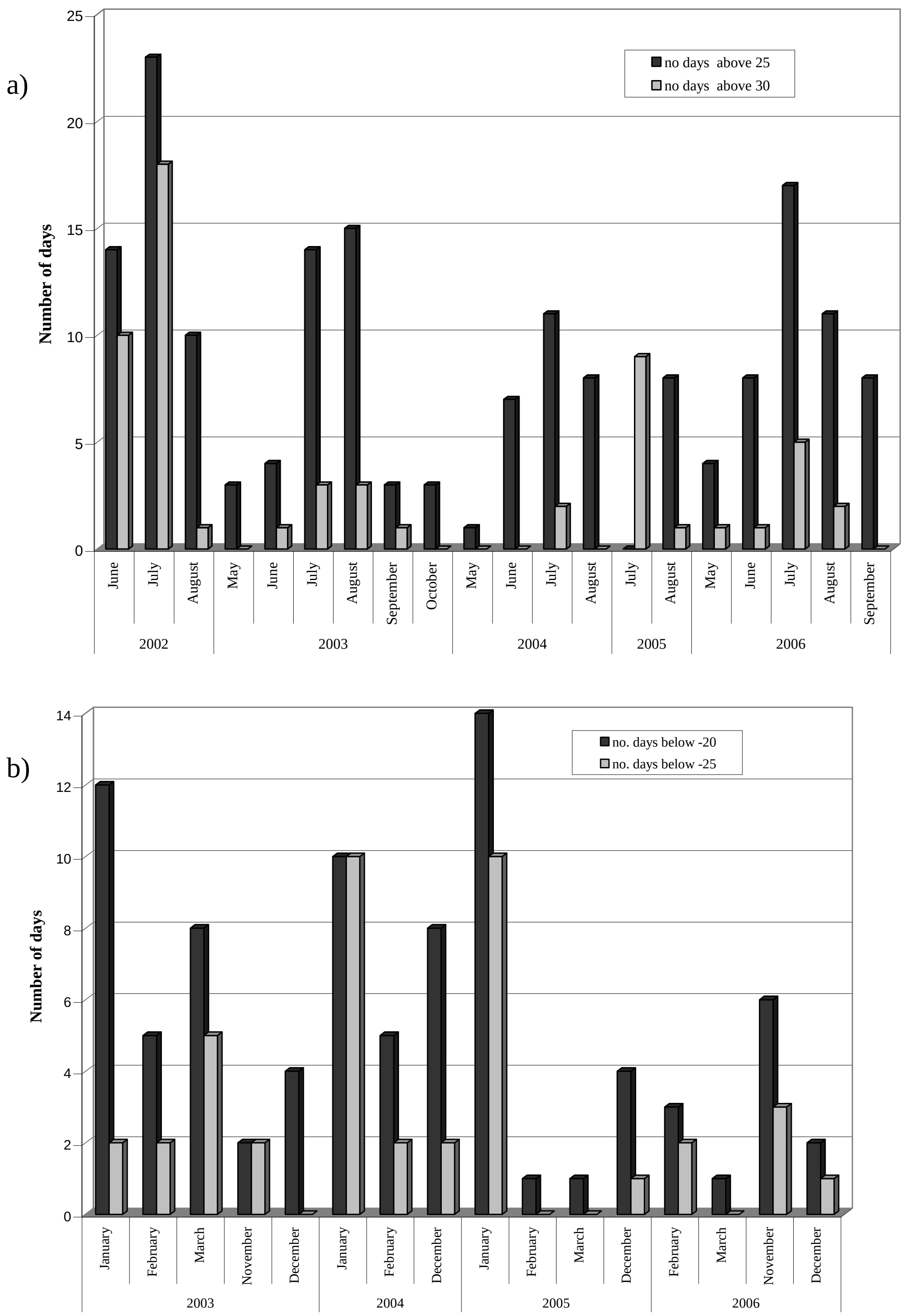

Figure 2-7. Frequency of days a) where maximum temperature reached 25 ˚C and 30˚ C and b) where minimum daily temperature reached -20˚ C and -25˚C at the Edmonton Research Station, Edmonton, Alberta, over the duration of this trial (June 2002 and December 2006).

Chapter 3

Cover crop effects on Kura clover (*Trifolium ambiguum* M. Bieb.) seed production.

A version of this chapter was submitted to Field Crops Research.

Hypotheses Tested

Harvested silage yield of barley, canola, corn, faba bean, pea and triticale will not differ.

Light availability to Kura clover will not differ when grown under a barley, canola, corn, faba bean, pea or triticale cover crop.

Ground cover the spring following planting, will not differ for Kura clover established as a monoculture or with a cover crop.

Ground cover the spring after planting, will not differ for Kura clover established under a barley, canola, corn, faba bean, pea or triticale cover crop.

Seed yield of Kura clover established with or without a cover crop will not differ.

Seed yield of Kura clover will not differ following establishment under a barley, canola, corn, faba bean, pea or triticale cover crop.

Introduction

It is a common practice in western Canada to establish a perennial forage crop in association with an annual cover crop. The annual crop provides a measure of weed control by providing quick ground cover, as well as a saleable product in a year of little or no economic return from the forage (Millar and Stritzke, 1995). Establishing perennial forages with an annual cover crop can have a negative impact on the subsequent yield of the forage. To minimize the effects of the cover crop on the forage, the cover crop is often seeded at a reduced rate and harvested as silage rather than for grain (Miller and Stritzke, 1995). Alberta Agriculture advises against the use of a cover crop unless soil erosion is of concern, or if it is essential for silage/green-feed to be produced. If a cover crop is necessary, reducing seeding rates and nitrogen fertilizer, as well as harvesting as early as possible, will reduce inter-specific competition and allow forage seedlings to successfully establish (Anon., 2007). Barley (*Hordeum vulgare L.*) is often used as a cover crop in Western Canada due to its rapid growth rate and nutritive value for silage. However, these qualities also result in an intense level of inter-specific competition between the cover crop and the perennial forage crop.
Fairey and Lefkovitch (1991) utilized cover crops of barley, oats and canola (*Brassica napus* L.), when establishing alfalfa (*Medicago sativa* L.) alsike clover (*Trifolium hybridum* L.) and red clover (*Trifolium pretense* L.). Legumes established under oats gave the highest seed yield while barley and canola reduced legume seed yields (Fairey and Lefkovitch, 1991). They found no benefit from reducing the seeding rate of the cover crop. Steiner and Snelling (1994) report an economic benefit to establishing slow growing species with a wheat cover crop (*Triticum aestivum L.*).

Limited information is available concerning the best management practices for production of Kura clover (*Trifolium ambiguum* M. Bieb.*)* seed.

The objectives of this study were to determine the effects of using a range of annual cover crops on the establishment and subsequent seed yield of Kura clover.

Experimental Design and Methodology

Plots were established at one site in 2003 and two sites in 2004 at the University of Alberta, Edmonton Research Station (53°25'N, 113°,33'W). A randomized complete block design was used to test the response of Kura clover seed yield to establishment with or without a cover crop. Six different annual crops were selected as cover crops based on differences in growth habit, morphology, and competitive ability. The six crops were barley, triticale (*X Tritosecale*), canola (*Brassica napus* L.), peas (*Pisum sativum* L.), faba beans (*Vicia faba* L.) and corn (*Zea mays* L.) (Table 3-1). Three treatments of Kura clover (cv. Cossack) seeded alone were either hand weeded, mowed to control weeds, or left weedy. Prior to seeding, soil samples of the test area were taken and their nutrient status determined at Norwest Labs, Edmonton Alberta. Nutrient deficiencies were corrected prior to sowing according to the nutritional requirements for legume seed crops, 100 kg/ha 11-52-0 (N-P-K) granular fertilizer was broadcast, incorporated first with a cultivator and then, in a different direction with a harrow.

All species were sown using a double disk press drill at 30 cm row spacing, with the exception of corn which was sown at 60 cm row spacing. Plots measured 2.4m x 6m, with the exception of corn (4.8 x 6.0 m) and consisted of 20 rows of clover and 8 rows of the cover crop species. The clover was seeded perpendicular to the cover crop species. Cover crop species were sown at half of the recommended seeding rate for cropping in monoculture and at a depth appropriate to seed size (Table 1). Kura clover was sown at 10 kg/ha and at 1 cm depth.

Data collection

Year 1 - Kura clover establishment under a cover crop

Seeding date varied from year to year based on soil temperature and moisture conditions (Table 3-1). Light interception by the cover crop canopy was measured at weekly intervals beginning 4 wk after planting. Radiation was measured above the canopy and at the height of the clover at ten locations within each plot using a point source quantum meter (LI-90 Quantum Meter, LICOR, Lincoln, Nebraska, USA). Measurement sites were located centrally within plots to remove sampling error due to edge effects.

For the mowed Kura clover alone treatment, weeds were controlled by mowing the plot area using a self-propelled sickle mower, (Swift Machine & Welding, Saskatoon, Saskatchewan) at 6 wk following planting and again at 10 wk following planting. Mowing height was dependent on the weed species present as well as the height of the Kura clover, and ranged from 5 cm to 10 cm above the soil surface. Weed biomass was removed from the plots.

A harvest for silage was taken as each crop reached the "silage stage" determined by the recommended practice for each species (Table 3-2). Prior to each harvest, 30cm was trimmed from the ends of each plot to remove edge effects. Using equipment appropriate to the species (flail mower, jerry mower, hand clippers) 0.6m x 5.4m of the plot was harvested (2 crop rows and the associated clover rows). Harvested material was dried at 60°C to constant weight and dry matter production calculated.

Detailed plant characteristics were measured on ten annual crop plants removed from random locations within each plot prior to harvest. Data recorded included the number of leaves and tillers per plant, leaf area and stem area per plant (cm^2), pod number per plant and biomass (dry weight (g) per plant) of roots, crowns, stems and leaves (Table 3-3).

Years 2 & 3 - first and second season of Kura clover seed production

In the year following establishment, Kura clover ground cover (%) was determined early in the spring. Hives of honey bees (*Apis mellifera)* were placed adjacent to the plots beginning in the year following planting, and each year thereafter, to ensure successful pollination of the Kura clover. Seed yield was determined at the end of the season.

Prior to harvesting, plots were desiccated using Reglone at a rate of 1.1 L/acre with Ag-Surf at 0.1% in 90 L (September 28, 2004 / September 12, 2005). Desiccant was applied when seeds located in the lower and mid position within an individual clover head were brown and upper seeds were firm but still green to brown. Two weeks following application of the desiccant, plots were harvested (Table 3-3) using a plot combine (Winter Steiger, Saskatoon, Saskatchewan,Canada). Harvested material required additional processing after combining to thresh Kura seeds from the flowers. Collected material was processed in a scarifier then run through an aspirator to remove extraneous material. Because of the late maturation of the seeds, in 2004 – 2005, prior to the machine harvest, one square meter was removed by hand to ensure data was obtained prior to the onset of winter. Combine harvests in the first production year occurred on December 3, 2004, and October 25, 2005. A combine harvest did not take place in the second and third production years (2005 or 2006) due to low inflorescence production. In both 2005 and 2006, the number of inflorescences per square meter was counted and yield estimated based on yield parameters previously established by Walker and King (2009). At two locations within each plot the number of flowers within a 1m x 1m quadrat was recorded. In the summer of 2005, Kura clover plants appeared chlorotic and unthrifty. Plant material and soil were sampled and nutritional status examined at Norwest Labs, Edmonton, to determine if low inflorescence production was due to a nutrient imbalance.

Data Analysis

All data were tested for normality using PROC UNIVARIATE (SAS Institute, 2003). Growing environment (site x year) was considered to be a random effect as sites were selected to represent the Parkland region of Alberta, Canada and climatic conditions throughout the study were typical for the area. Therefore, analyses of variance were performed using data combined across environments (site x year) with the MIXED procedure of SAS (Littell et al., 2006). Means were separated by the Tukey method at 5% significance level. Results for analysis of variance tests are presented in tabular form located in the Appendix (Table A-2).

Results

Cover crop species were selected based on a range of morphology, growth rate and projected time of harvest. Large differences in silage yield were expected.

Barley and canola cover crops had more tillers/branches per individual plant than the other cover crop species (Table 3-3). Faba beans had the highest leaf area of the six crop species, followed by peas and triticale. Despite having more stems per plant, barley and canola had the lowest leaf area per plant (Table 3-3). The barley canopy developed quickly, reaching 90% light interception 6 wk following planting. All other cover crops had light interception at 6 wk of 10 – 60% (Figure 3-1). Barley captured nearly 100% of incoming radiation by silage harvest at eight weeks following planting (Figure 3-1). Canopy closure occurred at a faster rate in canola and peas than for corn, faba beans and triticale (Figure 3-1). Peas reached 90% light interception 8 wk after seeding; triticale, faba beans and canola by 10 wk following seeding. The corn cover crops approached 90% light interception at the time of its harvest at 12 wk (Figure 3-1).

Monocot cover crop species produced a greater biomass than dicot species (Figure 3-2). Triticale yielded the greatest silage biomass (16,079 kg/ha), 1.5x greater then barley (11,109 kg/ha), ($p<0.0001$). Dry matter silage yield (kg/ha) of barley did not differ significantly from canola, corn and faba beans (Figure 3-2). Barley (11,109 kg/ha), and corn (16,079 kg/ha) produced significantly more silage biomass than peas (6,075 kg/ha) (Figure 3-2).

The presence of a cover crop reduced the ability of the clover to establish and cover the soil surface. Spring growth of Kura clover was significantly lower when seeded with a cover crop versus seeding alone (Figure 3-3). Cover estimates taken each spring indicated that establishment of Kura clover was not directly related to the yield of the cover crop. In the spring following seeding, Kura clover established alone had significantly higher percentage ground cover (40%), regardless of method of weed control, than when established under barley (16%), canola (24%), triticale (17%) or peas (15%; Figure 3-3). Kura ground cover when established using corn (31%) or faba beans (25%) as the cover species did not differ from weedy Kura monocultures (29%), however these treatments had significantly lower ground cover of Kura compared to monocultures that were hand weeded (41%) or mowed (35%) to control weeds (Figure 3-3).

Ground cover of Kura clover increased over time at different rates for each treatment (Figure 3-3). The greatest increase in Kura clover ground cover occurred in the season following establishment. Kura clover planted with a barley cover crop showed the highest percentage increase in ground cover, increasing from 16% to 47% between the first and second production years. Kura clover established under faba beans, increased from 24% to 81% ground cover between the first and second production years (Figure 3-3). Ground cover of Kura established under corn, peas, triticale and the weedy Kura increased to 80%, 68%, 57% and 81% respectively, from the first to the second production years. By the third production year, ground cover of Kura clover did not differ significantly between Kura clover established without a cover crop and Kura established with a cover crop. Percentage ground cover ranged from 80% to 95% (Figure 3-3).

Seed yield was very low for all treatments. Combine harvests occurred only in the year following seeding. In subsequent years, not enough inflorescences were present to justify a combine harvest. Seed yield was highly variable within treatments. Kura clover seed yield under no cover crop was significantly higher than Kura clover under barley or triticale ($p<0.001$; Figure 3-4). Hand weeded Kura monocultures yielded six times more harvested seed (137 kg/ha) than Kura established under a barley or triticale cover crop (Figure 3-4). When compared within the cover crop treatments, Kura seed yields established with corn as the cover crop (98 kg/ha), yielded significantly more than peas (63 kg/ha), faba bean (45 kg/ha), and canola (45 kg/ha). Barley (23 kg/ha) and triticale (21 kg/ha) yielded significantly less than the other cover crops (Figure 3-4). Fewer than 100 inflorescences per square meter were present the second and third years following planting (Figure 3-5).

Total seed yield of the three production years indicated that Kura clover grown yielded significantly more than when established without a cover crop (Figure 3-6).

Discussion

Kura clover plants flowered and produced seed in the first production year, when established with or without a cover crop. Seed yield ranged from 21 kg/ha to 137 kg/ha. Flower production in the second and third production years was minimal. Seed yields in this study, were only 25% of yields obtained in Alberta under similar conditions in 2003 and 2004 (Walker and King, personal communication) but were similar to yields reported in the United States and New Zealand (Taylor and Smith, 1998; Hill and Mulcahy, 1993).

One of the challenges of producing forage seed is controlling competition from weed species. In this study, seed yield was not significantly reduced by weed presence in the establishment year. The competitive effect of the weeds however, resulted in smaller Kura clover plants with a slower rate of spread (based on ground cover measurements) than when weeds were removed by hand. Redroot pigweed (*Amaranthus retroflexus*) dominated the plot area in the establishment year and the clover plants remained beneath the pigweed canopy (Walker, visual observation). Perhaps infestations with more competitive weed species would have shown a greater influence on Kura clover seed production. Clean seed is necessary for certification purposes however, so weed removal is recommended.

There are currently no herbicides registered for use in Kura clover. Consequently, control of weeds must involve physically removing the weeds from the clover. In this study, two alternatives were compared. The hand weeding or mowing treatments during the establishment year, did not significantly increase seed yield compared to weedy plots. Kura clover plants showed a significantly higher percentage of ground cover when weeds were removed by hand during the establishment year compared to the weedy treatment. Mowing is reported to be an effective means of weed control with select weed species (Rincker and Rampton, 1985). Relative plant height and mowing height influences the degree of weed control. Clipping weeds when they are too small to be damaged or cutting too high may result in the weed plants producing more stems and branches. For the majority of clover species, clipping close to the soil surface will not damage the clover but will successfully control the weeds (Lee, 1985). In this study, removing weed biomass by mowing in the establishment year provided adequate weed control without reducing subsequent percentage ground cover or seed yield. The seed yield of Kura clover grown without a cover crop and mowed to control weeds was consistent with yields reported in the United States (Taylor, 2002). Mowing Kura clover in seed production years however is not recommended. Steiner (1992) reported a 90% reduction in seed yield when Kura clover was cut for hay and then allowed to set seed.

In regions with adequate soil moisture cover crops can be successfully used to control weed growth, however, some annual cover crops may be more competitive than the weed species they are replacing (Lee, 1985; Tan and Erkovan, 2004). In this study, Kura clover grown with weeds produced significantly more seed than when a cover crop was present. This suggests that the effect of competition from weeds may be less damaging to developing Kura clover plants than the effect of a cover crop; however weeds differ in their competitive ability and if different weed species were present, this may not have been the case.

Cover crops were selected with different growth rates, morphologies and competitive abilities. Hill and Mulcahy (1993) found Kura clover seed yield under corn was similar to seed production levels in New Zealand. In our study, Kura clover plants established with a corn cover crop demonstrated a greater percentage of ground cover and subsequently higher seed yields than when sown with other cover crops. Corn as a cover crop, yielded the second highest silage biomass but still allowed the Kura clover to establish, resulting in the greatest Kura clover spring ground cover when compared to the other cover crops. The slower growth rate of corn, open structure of the canopy, and wider row spacing reduced the competition for light. Steiner and Snelling (1994) stated that Kura clover development was less affected when the cover crop was planted with wider row spacing. Wider row spacing used when planting corn may also play a role in reducing below-ground competition for resources by reducing total plant density. Walker and King (2009) found that reducing below-ground competition significantly increased Kura clover's vegetative production.

Barley, the traditional cover crop species in Alberta, had the third highest silage biomass and Kura clover established with barley as the cover crop showed the lowest amount of ground cover and second lowest seed yield. The barley canopy reached 90% light interception by 6 wk following planting and at the time of the silage harvest (8 wk following planting) was intercepting 98% of the incoming solar radiation. Despite a reduced barley seeding rate and early removal of the barley canopy, Kura clover establishment was significantly affected by the aggressive growth of the barley cover crop. The negative effects on clover plant size (as measured by ground cover) reached into the third season after planting.

Previous studies in Alberta found that triticale had higher silage yields than barley but was less suppressive to inter-seeded berseem clover (*Trifolium Alexandria* L.) (Ross et al., 2000). Similarly, Berkenkamp and Meeres (1987) found that triticale was less competitive in mixtures than barley. In our study, triticale gave the highest silage yields and was one of the most detrimental cover crops to Kura clover growth and seed yield. The triticale cover crop intercepted 95% of incoming solar radiation by 8 wk after planting, similar to barley, but was not harvested until 10 wk after planting. Consequently, Kura clover's photosynthetic rate and growth may have been significantly reduced during the 2 wk between triticale canopy closure and the silage harvest.

Brassica crops have several properties that indicate potential for use as cover crops. Mills et al. (2007) examined the effectiveness of rape (*Brassica napus*) as a cover crop when establishing grass pastures in the United Kingdom. Yield of the pasture mixture did not differ when establishment occurred with or without a rape cover crop; however, weed biomass was reduced by 40% (Mills et al., 2007). The reduced seed yield under barley and canola cover crops in our study, is consistent with the findings of Fairey and Letkovitch (1991). Alfalfa, red and alsike clovers established under barley or canola cover crops had significantly reduced seed production in the year following seeding (Fairey and Letkovitch, 1991). Waddington and Bittman (1984) found that alfalfa and sainfoin had poor establishment under a Polish rapeseed (*Brassica campestris* L.) cover crop. Jefferson et al. (2005) found that the effect of canola on establishment of perennial forage species varied with soil type and environment. In this study, on a black chernozemic soil, Kura clover establishment and seed yield was significantly reduced when canola was used as a cover crop.

Legumes planted for grain or silage are often less competitive than cereals, and may allow better establishment of a perennial forage crop (Strydhorst et al., 2008). The competitive effects of a pea cover crop reduced subsequent forage yields in Saskatchewan (Jefferson et al., 2005). The severity of the reduction varied with environment and Jefferson et al. (2005) found that peas were an acceptable alternative cover crop in certain soil zones. By 8 wk after planting light interception by the pea cover crop in this study, was 90%. This was due to the lodging of the pea crop that occurred between 7 and 8 wk after planting. Kura clover seedlings thus had minimal exposure to light from the time the cover crop lodged until its harvest, reducing the Kura clover ground cover in the year of planting. The negative effects of the pea cover crop on Kura clover ground cover, were only ameliorated in the third production year. Kura clover seed yield under peas was significantly lower than yields in Kura clover monoculture but was, similar to seed yield under a faba bean or canola cover crop and significantly greater than clover seed yield under barley or triticale.

In this experiment, both the pea and faba bean cover crops had one or two stems per plant, with leaf: stem ratios near 1:1. Based on this plant morphology we anticipated less competition to the seedling Kura clover, however, a significant reduction in Kura clover seed yield still occurred. Faba beans are not a traditional silage crop in central Alberta. To our knowledge, no previous studies have examined the use of faba beans as a cover crop; however, faba beans show several characteristics that might make them a suitable choice. Strydhorst et al. (2008) found that faba beans are less competitive than field pea when grown in mixtures with barley. In this experiment, silage yield of faba bean was comparable to barley, canola and peas. Canopy closure occurred more slowly in faba bean, with light interception increasing steadily until harvest. Six wk after planting, the faba bean canopy intercepted only 25% of incoming radiation, whereas barley at 6 wk, intercepted 90% of solar radiation. Barley, triticale, peas and canola initially had a rapid rate of canopy closure. Kura clover established under faba bean had significantly higher percentage ground cover than under barley, triticale or pea cover crops. Kura clover seed yield was 2x greater when established under a faba bean cover crop than under a barley or triticale cover crop.

In the second and third production years, vegetative growth of the Kura clover was slow; plants appeared small in stature and were pale in color. Estimated harvested seed yields in the second and third production years ranged from 3 – 19 g/m^2 (Figure 5), and were lower for all treatments than yields reported in the United States (Taylor, 2002) as well as those obtained in other Alberta trials (Walker and King, 2009). Soil and tissue testing determined that all the required macro and micro nutrients were present in soil and tissue. Poor plant performance did not arise from a lack of nutrients, but may have been due to some undetermined physiological stress.

In this study, Kura clover planted without a cover crop, had higher seed production in the first production year, than reported yields in New Zealand (Hill and Mulcahy, 1993) and was similar to yields in the United States (Taylor and Smith, 1998). Seed production in this study was much lower than previously reported yields in Alberta (Walker and King, in review). Low seed yields did not result from a lack of available pollinators. Bees were observed actively pollinating each growing season. In this test, low seed yield was directly related to a failure to produce inflorescences.

Askin (1990) suggests that crops with fewer leaves, small and upright in stature are more suitable for cover crops. In our study, barley and canola had the smallest number of leaves per plant and the greatest numbers of tillers/branches per plant and, along with triticale, were the most detrimental to the development of the Kura clover. Corn and faba bean had one or two stems per plant, and leaves were distributed more frequently along a single stem versus barley and canola where leaves were spaced more widely along several stems. Consequently, speed of canopy closure varied between cover crop species. Variation in cover crop morphology appears to play an important role in the success of the subsequent Kura clover stand. Kura clover seed yield under corn or faba bean was higher than when established under other cover crops.

Coolbear et al. (1994) suggested that maximizing root growth and crown development is critical for successful reproductive growth in Kura clover. Walker and King (2009) found below-ground competition from meadow bromegrass influenced above ground Kura clover production more than competition for light. Planting cover crop species at a lower seeding rate and wider row spacing may reduce the competitive pressure on Kura clover root development and allow for individual clover plants to attain a larger size.

Establishment of Kura clover with a cover crop in this study, resulted in only one season of seed production. Flower production was too low to merit a harvest in the second and third seasons. In previous Alberta trials, Kura clover established without a cover crop produced adequate seed levels in two subsequent seasons (Walker and King, 2009). Utilizing a cover crop to establish Kura clover for seed is not recommended due to low seed yields in a single season.

Conclusions

Seeding an annual crop along with perennial forage is a common practice that allows economic return in the year of forage establishment. In this experiment, the effects of competition from the cover crop resulted in smaller Kura clover plants at the start of the first production year with Kura ground cover ranging from 15% to 30%. Establishing Kura clover with a cover crop significantly decreased the subsequent seed production. Kura clover seed yield when established without a cover crop was similar to reported yields in the United States. Based on the results of this study, establishing Kura clover with any cover crop is not recommended. The relative reduction of the cover crop on seed yield was barley , triticale > canola, faba bean, peas > corn. More consistent Kura clover growth following establishment and increased seed production might be achieved in southern Alberta which has a longer season and the potential to irrigate.

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Table 3-1. Cultivar, seeding rate, seeding depth and date of planting of six annual species used as cover crops with Kura clover at the University of Alberta, Edmonton Research Station, Edmonton, Alberta for plots established in 2003 (28 May) and 2004 (20 May) at three locations.

Species	**Cultivar**	**Seeding rate**	**Seeding depth**
Barley (*Hordeum vulgare* L.)	AC Lacombe	120 plants /m2 (1 bu/acre)	2.5 cm
Canola (*Brassica napus*)	Clearfield	2.5 kg/ha	1 cm
Corn (*Zea mays*)	Dekalb	80, 000 kernels/ha	2.5 cm
Faba beans (*Vicia faba* L.)	Blitz	22 plants / m2 *	2.5 cm
Peas (*Pisum sativum* L.)	Performance 4010	3.5 plants /sq foot *	2.5 cm
Triticale (*X Tritosecale*)	Pronghorn	120 plants/m2 (1 bu/acre)	2.5 cm
Kura clover (*Trifolium ambiguum*)	Cossack	10 kg/ha	1 cm

* As recommended by Alberta Pulse Growers

Table 3-2. Description of "silage stage" for the six cover crop species used in this study according to the recommendations for ensuring proper ensiling and to maximize quality of the harvested product.

Cover crop species	Description of "silage stage"
Barley (*Hordeum vulgare* L.)	Soft dough – when individual seeds are pressed, the contents are expressed as a soft white doughy substance AlbertaAgriculturehttp://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9682 (accessed August 2007).
Canola (*Brassica napus*)	End of flowering – flowers should still be present at the top of the plant and seeds in the lower pods should be green and soft (Agriculture Saskatchewan; (www.agriculture.gov.sk.ca/Default.aspx?DN=8733c510-d266-45ae-8904-f00b02a36b04) (accessed August 2007).
Corn (*Zea mays*)	Following a frost, when the plant has reached 70% moisture, many leaves will have broken off, plant is pale in color
Faba beans (*Vicia faba* L.)	Pods on bottom of the plant have turned dark brown/black, pods at the top of the plant are green (Alberta Pulse Growers; www.pulse.ab.ca (accessed August 2007).
Peas (*Pisum sativum* L.)	Following flowering, pods on the bottom of the plant should be full, pods at the top of the plant empty (Alberta Pulse Growers; www.pulse.ab.ca (accessed August 2007).
Triticale (*X Tritosecale*)	Late milk stage – when individual seeds are pressed a milky coloured liquid is expressed) (Alberta Agriculture (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/fcd10569 (accessed August 2007).

Table 3-3. Harvest date, height, average tiller and leaf number, leaf area and dry weight per plant and average root dry weight per plant for six species used as a cover crop for establishing a Kura clover crop in the years 2003 and 2004 at three locations at the University of Alberta, Edmonton Research Station, Edmonton, Alberta.

Cover crop species	**Harvest date**	**Height (cm)**	**Tiller / stem number / plant**	**Tiller/ stem area (cm2)**	**Tiller/ stem weight (g)**	**Leaf number / plant**	**Leaf area (cm2)**	**Leaf weight (g)**	**Leaf: Stem ratio**	**Pod number/ plant**	**Pod weight (g)**	**Root weight (g)**	**Kura clover height (cm) July 23**
Barley (*Hordeum vulgare* L.)	July 30	103	4.33	209.99	6.85	18.6	294.87	1.63	1 : 4.2	n/a	n/a	1.44	8.4
Canola (*Brassica napus*)	Aug. 7	113	4.22	178.26	9.02	18.1	249.41	1.49	1: 6.1	26.72	15.23	3.08	19.5
Corn (*Zea mays*)	Oct. 17	263	1.00	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	19.9
Faba beans (*Vicia faba* L.)	Aug. 13	106	1.93	229.49	12.47	41.2	1167.44	12.55	1: 1.0	13.21	17.59	3.15	17.4
Peas (*Pisum sativum* L.)	Aug. 25	92	1.43	n/a	7.00	23.6	786.17	5.62	1: 1.3	13.33	12.23	0.38	14.2
Triticale (*X Tritosecale*)	Aug. 13	125	3.30	675.12	7.96	47.1	429.35	1.50	1: 0.5	n/a	n/a	3.81	10.9
F-test		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

N. B. Harvest for corn occurred after the killing frost, very few leaves remained on the plants
Corn and faba bean stems were too large for the leaf area machine

NS No significant effects due to variation within treatments.

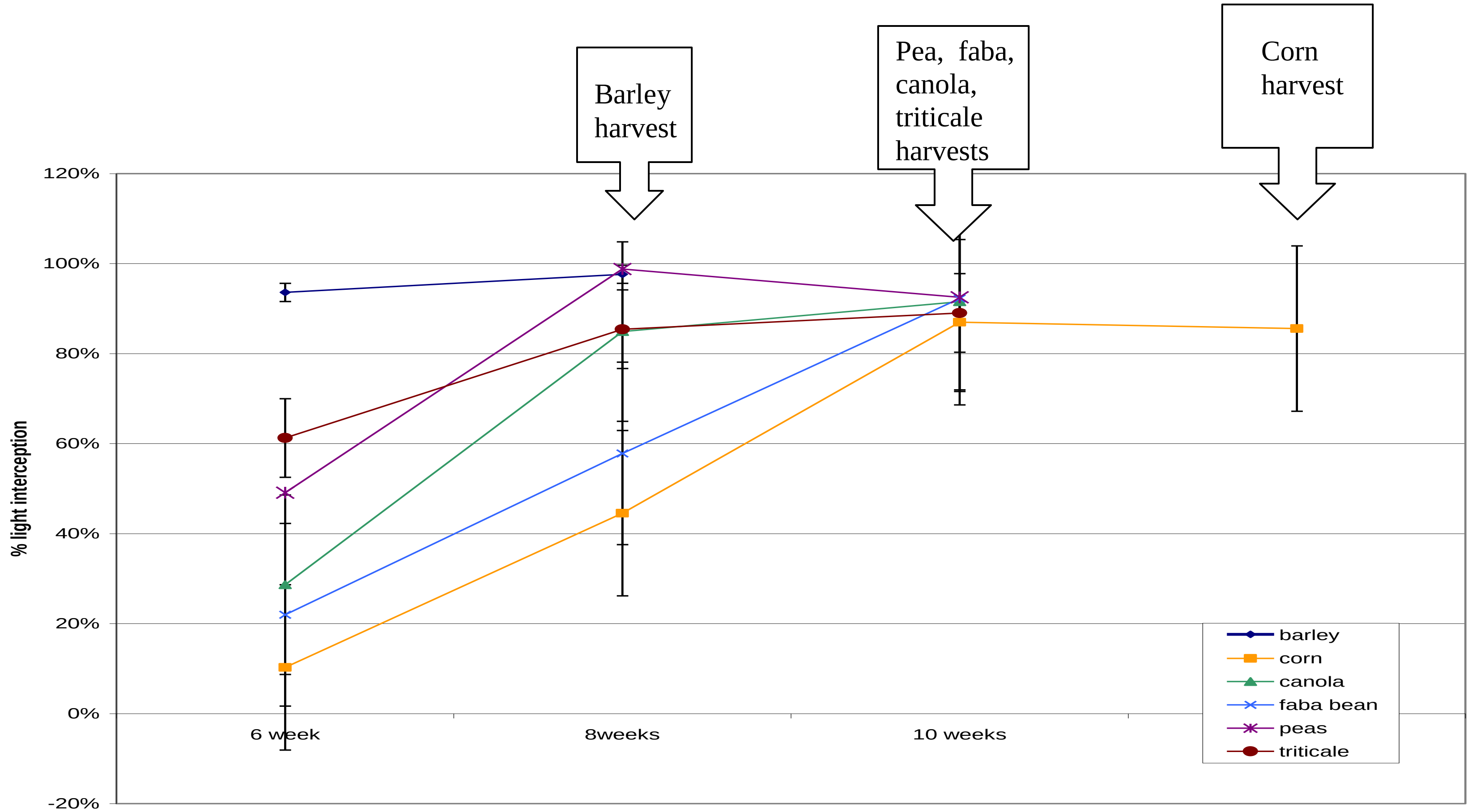


Figure 3-1. Percentage light interception by six cover crop canopies from six weeks following planting until each crop was harvested for silage. Average of plots established in 2003 and 2004 at three sites at the University of Alberta, Edmonton Research Station, Edmonton, Alberta.

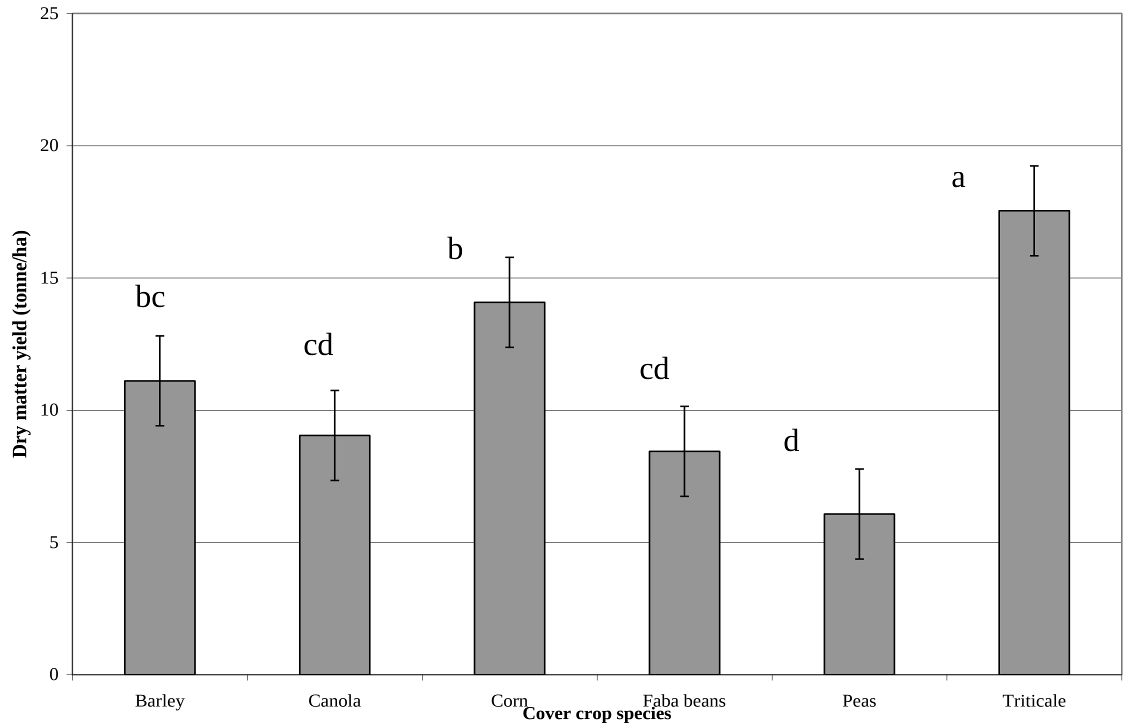
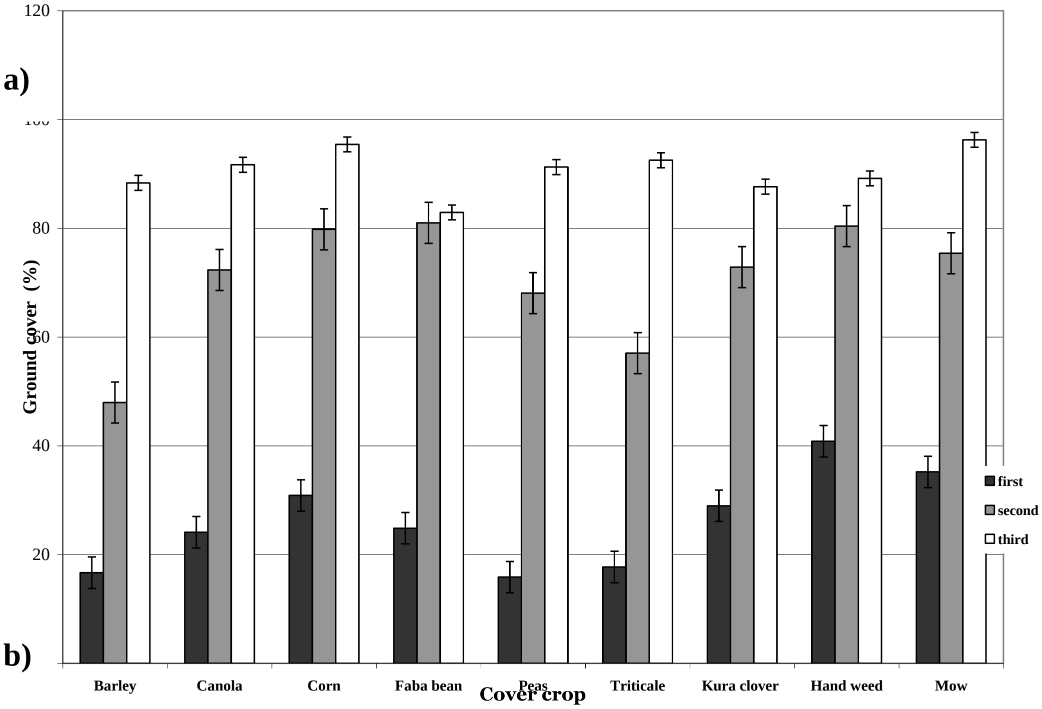


Figure 3-2. Dry matter biomass yield (tonne/ha) of six cover crop species harvested for silage at the University of Alberta, Edmonton Research Station, Edmonton, Alberta averaged over the years 2003 and 2004 and three locations. $a - d$ Columns with the same letter are not significantly different at $p < 0.05$.

a)



b)

Treatment	First production year	Second production year	Third production year
Barley	d	d	a
Canola	bc	a,b	a
Corn	b	a	a
Faba beans	bc	a	a
Peas	d	b	a
Triticale	c d	c	a
Kura clover no cover	b	a,b	a
Hand weeded Kura clover	a	a	a
Kura clover mowed	a,b	a	a

a – d means followed by same letter are not significantly different, $p<0.05$

Figure 3-3. a) Percentage ground cover of Kura clover following establishment under six cover crop species or alone taken each spring of the first, second and third production years averaged over plots established in 2003 and 2004 at three locations at the University of Alberta, Edmonton Research Station, Edmonton, Alberta. b) Mean comparisons for the percentage of Kura clover ground cover.

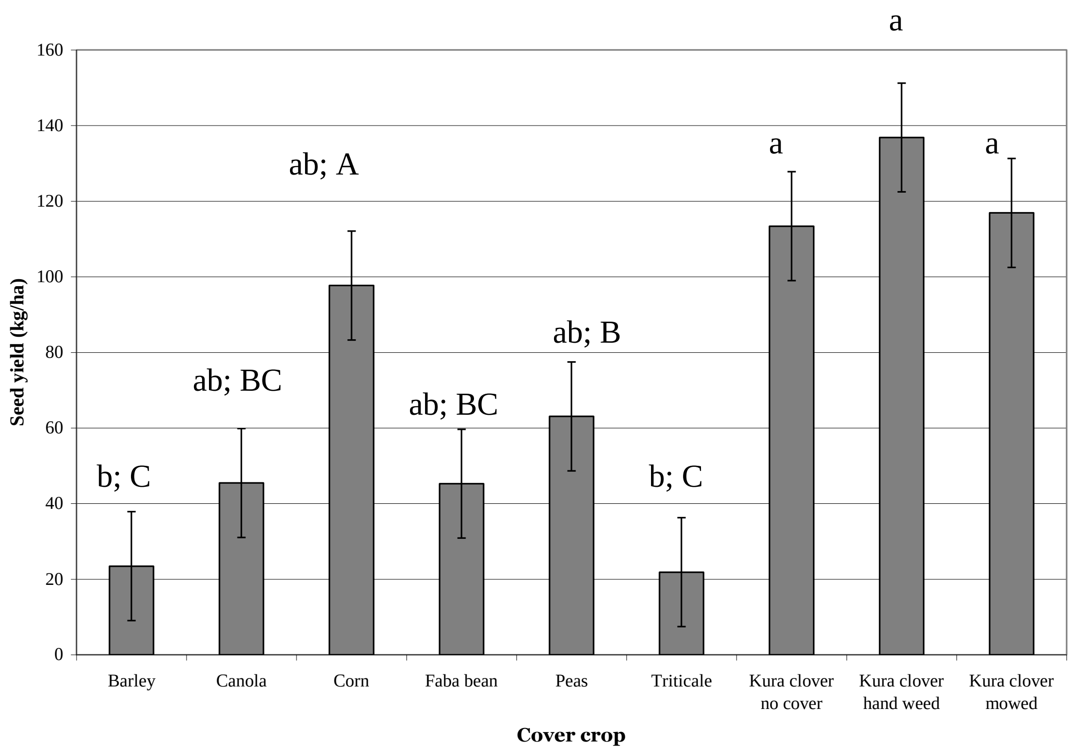
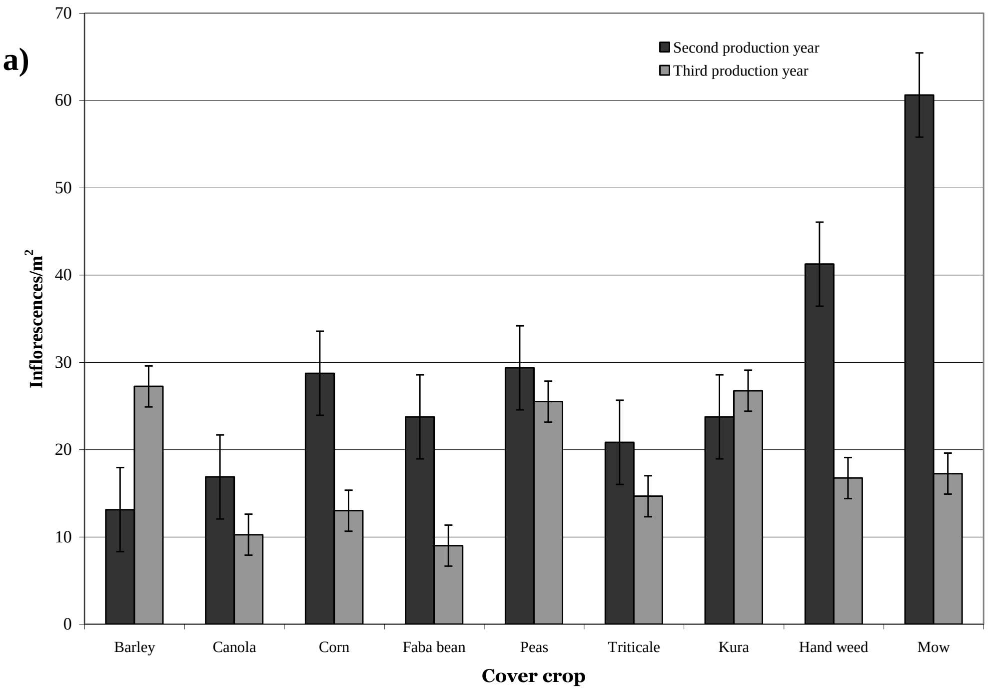


Figure 3-4. Seed yield (kg/ha) of Kura clover in the year following seeding after establishment with or without a cover crop averaged over treatments planted in 2003 and 2004 at three locations at the University of Alberta, Edmonton Research Station, Edmonton, Alberta.
$a - b$ means followed by the same letter are not significantly different, $p<0.05$. $A - C$ means followed by the same letter are not significantly different $p<0.05$.

a)



b)

Cover crop treatment	Estimated Kura clover seed yield (kg/ha) Season Two	Season Three
Barley	41	86
Canola	54	33
Corn	91	41
Faba Beans	75	29
Peas	93	80
Triticale	66	47
Kura clover no cover	75	85
Kura clover hand weed	130	53
Kura clover mowed	192	55

Figure 3-5. a) Number of Kura clover flowers per square meter in the second and third seasons following planting, established with or without a cover crop and **b)** estimated seed yield in the second and third production years for Kura clover planted with or without a cover crop averaged over the years 2003 and 2004 and three locations at the University of Alberta, Edmonton Research Station, Edmonton, Alberta.
* No significant effects due to high degree of variation within treatment.
Seed yield = no. of heads * flowers/head * weight/1000 seeds (Walker and King, in review).

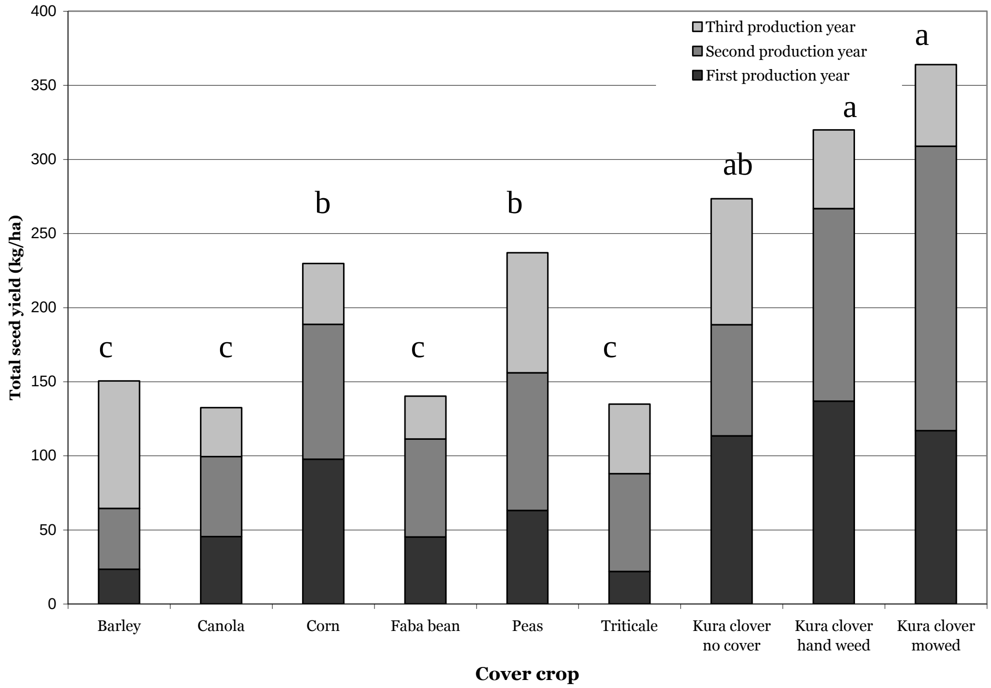


Figure 3-6. Total seed yield (kg/ha) of Kura clover in the first, second and third production years, after establishment with or without a cover crop averaged over treatments planted in 2003 and 2004 at three locations at the University of Alberta, Edmonton Research Station, Edmonton, Alberta.
a – b means followed by the same letter are not significantly different, $p<0.05$.

Chapter 4

Above and below-ground competition between Kura clover (*Trifolium ambiguum* M. Bieb.*)* and meadow bromegrass (*Bromus riparius* Rehm.*)*: a greenhouse study.

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Walker, J. A. and King, J. R. 2009. Above and below-ground competition between Kura clover (*Trifolium ambiguum)* and meadow bromegrass (*Bromus biebersteinii)*: a greenhouse study. Canadian Journal of Plant Science, 89:21 – 27.

Hypotheses Tested

Kura clover leaf number will be the same when grown with or without above or below-ground competition from meadow bromegrass.

Light availability will be the same at the top of the canopy, at the height of the clover and at the soil when Kura clover and meadow bromegrass are grown with or without above or below-ground competition.

Height of Kura clover will be the same when grown with or without above or below-ground competition from meadow bromegrass.

Leaf area of Kura clover will be the same when grown with or without above or below-ground competition from meadow bromegrass.

Root weight of Kura clover will be the same when grown with or without above or below-ground competition from meadow bromegrass.

Shoot weight of Kura clover will be the same when grown with or without above or below-ground competition from meadow bromegrass.

Introduction

Plant competition examines the relationship between plants sharing limited resources. The ability of a plant to thrive in a community is dependent on an individual's ability to adapt through various physiological changes (Tremmel and Bazzaz 1993). The ways in which plants obtain and use resources, making them unavailable to neighboring plants, can be considered mechanisms of inter and intra-specific interactions (Bazzaz, 1990). Competition for resources in artificial plant species mixtures can be minimized by including plants that utilize different niches in time and space (Firbank and Watkinson, 1985). The inclusion of a legume in a perennial forage mixture provides a source of nitrogen to non-fixing plant species, evens out biomass production over the growing season, and improves the overall nutritional quality of the plant material available to the grazing animal (van Keuren and Hoveland, 1985).

Previous studies on Kura clover have shown its potential for inclusion in managed pasture mixtures in Alberta (Walker 2002). A major factor limiting the use of Kura clover is its slow rate of growth during the seedling year, allowing aggressive grass species to dominate the mixture (Walker, 2002). Studies in Minnesota, USA (Sheaffer and Marten 1991; Peterson et al. 1994) indicate that once established, Kura clover is a productive and beneficial component of pasture swards with the ability to withstand a variety of defoliation intensities. Examination of Kura clover grown with commonly used pasture grass species would allow insight into interspecific compatibility.

Growing Kura clover with meadow bromegrass affects the growth and development of the clover plants (Walker 2002). Methods of studying above and below ground competition in plants are limited in their scope and applicability and their validity is debated within the scientific community (McPhee and Aarssen 2001). The neighborhood design, a variation of the additive design, allows the separation of above and below ground competition experienced by Kura clover plants (McPhee and Aarssen 2001). With this method, the focal species is set in the center of each pot and surrounded by a second species. Above and below ground barriers are added and removed to create varying combinations of above or below ground competition. The neighborhood design is favored for its simplicity and yields a manageable data set to evaluate whether or not competition is taking place.

Understanding interactions among species is increasingly important as we seek to optimize production from multi-species plant communities. Knowledge regarding nutrient use and niche preferences will aid in creating stability and longevity in pasture swards (Hall 1976). Creating an optimal clover and grass mixture requires a better understanding of the nature of competition, and specifically whether above or below ground competition is primarily responsible for the composition of the sward.

A greenhouse study was undertaken to examine the nature of grass – Kura clover competition, utilizing meadow bromegrass as the grass species. Compatibility of these two species is necessary for them to contribute optimally to yield in pasture swards. Our goal was to evaluate how Kura clover growth was affected when grown in a mixed stand with meadow bromegrass when moisture and nutrients were not limiting. Quantification of the relative importance of above and below ground competition during the establishment phase of a binary mixture may suggest ways to alleviate competitive pressure on the less dominant species.

Experimental Design and Methodology

Kura clover cv. Cossack and meadow bromegrass cv. Fleet were grown in pots in a greenhouse with four separation treatments. The treatments were: no separation (full competition), above ground separation (root competition), below ground separation (shoot competition), or above and below ground separation (no competition). Treatments were replicated ten times.

Greenhouse temperature was 21/18° C day/night with a 16-hr photoperiod. The experiment was replicated in time with one run occurring from February 28, 2005 to May 17, 2005 and the second run March 30, 2006 to July 2, 2006.

Plants were grown in 3½-inch square pots using Turface®, a granulated natural clay product, as the growth medium. Experimental units consisted of five pots arranged in an 'x' shape with a Kura clover plant in the center pot and four meadow bromegrass plants surrounding it (Figure 4-1, Plate 4-1a). For treatments requiring below ground separation, plants remained in individual pots, when below ground separation was not needed, the sides of the pots that were shared with another plant were removed and the five pots glued together, in the 'x' shape (Plate 4-1a). Each experimental unit was then placed in a tray and randomly arranged on the greenhouse bench, trays were rotated weekly moving from the front to the back of the greenhouse bench. Plants were watered twice daily, and a complete liquid fertilizer was given on a bimonthly basis for the first 5 wk and on a weekly basis thereafter.

Both Kura clover and meadow bromegrass seeds were sown on the soil surface, and watered frequently until germination occurred. Two weeks following planting, experimental units were thinned to one plant per pot. Plant development was monitored with leaf counts recorded every 2 wk. Six weeks following planting, above-ground barriers were installed. Barriers were made of galvanized welded iron wire mesh (hardware cloth) with 6 mm mesh and 23 gauge. Mesh was cut into a 30 by 38 cm rectangle and shaped into a free-standing square tube, open at each end. The barrier was then placed over the center pot and the Kura clover plant positioned so that all vegetative growth was enclosed within the tube and all meadow bromegrass leaves excluded (Plate 4-1b).

Plants were harvested May 17, 2005 and July 2, 2006 after 12 wk of growth. Prior to harvesting, light readings were taken using a LI-COR (LI-190 Quantum Sensor) point source light meter at three locations within each experimental unit; above the canopy, at the top of the Kura clover canopy, and at soil level. The height of tallest leaf, number of leaves per plant (Kura clover), tiller number (meadow bromegrass), and leaf area per plant were recorded. Total root mass was carefully removed from pots and placed in a tub of warm water to soak for several minutes before carefully separating into individual plants (Plate 4-1c). Vegetative and root biomass of individual plants was placed in paper bags and dried at 62° C for 5 d after which dry weights were recorded. Data were analyzed with analysis of variance and the general linear model of SAS (SAS Institute, 2003) to determine the effects of above and/or below ground competition on vegetative development, biomass and root growth of Kura clover. Significance was measured at p = 0.05, means were separated using the Tukey test (SAS Institute, 2003).

Results

Leaf Number

The presence or absence of competition did not affect meadow bromegrass rate of leaf accumulation (Figure 4-2;. Growth of meadow bromegrass plants slowed between 4 and 8 wk after which leaf accumulation accelerated at the onset of tillering.

Growth and development of Kura clover were more uniform than that of the meadow bromegrass (Figure 4-2). Two weeks following planting cotyledons were present for all treatments at 4 wk plants averaged three true leaves, with leaf number doubling by 8 wk and again by the 12-wk harvest date. Neither root nor shoot competition altered the rate of leaf development of Kura clover.

Light

Treatment did not affect the percentage of solar radiation available to the Kura clover (Figure 4-3).

Height

The leaf length of meadow bromegrass plants approached significance ($p=0.05$). A general trend was observed however, which indicated that meadow bromegrass plants were tallest when grown without competition, followed by plants with shoot competition, root competition and lastly where root and shoot competition was allowed (Table 4-1).

Height of Kura clover differed among competition treatments ($p<0.01$). Kura clover plants were tallest when only shoot or no competition with meadow bromegrass was allowed (Table 4-1).

Leaf Area

Leaf area for both meadow bromegrass ($p<0.01$) and Kura clover ($p=0.01$) was affected by the competition treatments. Greatest leaf area occurred in meadow bromegrass plants grown with no competition while for Kura clover the greatest area was found under shoot competition (Table 4-1). Leaf area of Kura clover plants grown with root competition was lower than leaf areas measured in other treatments.

Root:Shoot Ratio

Root:shoot ratios of both Kura clover and meadow bromegrass were not affected by treatment (Table 4-1).

Biomass

Root mass of meadow bromegrass and Kura clover was not affected by presence or absence of competition (Table 4-1). However, root mass appeared to differ between species (visual observation, Plate 4-1d), mean values were different although not statistically compared (Table 4-1). Meadow bromegrass produced an abundance of fibrous roots that completely filled their individual soil volume, and when root competition was allowed, reached into the other plant's rooting space. Kura clover plants had branched taproots as well as a fine root system. Kura clover roots were finer textured and less dense than those produced by the meadow bromegrass plants, however they also extended into neighboring plants' rooting space when root competition was not restricted. The competition treatments differed in vegetative biomass for both Kura clover ($p=0.02$) and meadow bromegrass ($p=0.02$; Table 4-1). Both meadow bromegrass and Kura clover produced the greatest biomass when roots were not allowed to mingle. Meadow bromegrass approximately trebled the biomass of Kura clover in all treatments. Dry weight of Kura clover leaves grown without root competition were double those of plants grown with root competition.

Discussion

Leaf appearance in our study occurred at a greater rate than previously reported. Unifoliate and first trifoliate leaves of Kura clover have been found to appear 3 – 4 wk after planting, with second and third trifoliate leaves appearing 5 wk after planting (Genrich et al., 1998). Black et al. (2006) concluded that carbohydrate partitioning in seedling Kura clover may only partially explain slow establishment. The inability to expand leaf area rapidly and the slow rate of leaf appearance may be responsible for establishment failure when grown with competitive grasses (Black et al., 2006). The faster rate of leaf accumulation found in our study when compared to previous work might have occurred due to the nutritional status of the plants. Pots were fertilized at regular intervals with complete macro and micro nutrients for the entire duration of our study whereas studies by Black et al., (2006) made no mention of fertilization taking place. This may indicate that Kura establishment could be accelerated by ensuring high nutrient levels are present.

The presence of the mesh barriers did not affect light availability to the clover for treatments where roots were allowed to compete and shoots were isolated. In all treatments 74 to 81% of incoming radiation was available to the Kura clover, with only 24 to 32 % of incoming radiation passing through the entire canopy. The presence of above ground barriers was intended to remove competition for light. One may conclude that the treatment was ineffective because no difference was measured in light availability to Kura clover when comparing the presence or absence of shoot competition,. However, in treatments where only shoots were allowed to compete (i.e. full competition or shoot competition), Kura clover was 11 and 34% taller than Kura clover plants experiencing no competition and root competition respectively. This suggests that Kura clover plants respond to light competition by placing their leaves higher in the canopy, increasing their access to incoming radiation. Plant stature has been found to have a major impact on the ability to compete for light (Rhodes and Stern, 1976). Small variations in height confer large advantages, especially during leaf development. In clovers, much evidence exists to show that petiole length is associated with ability to compete for light (Rhodes and Stern 1976). When shoot competition was removed, light was allowed to penetrate further in the canopy and reduce the amount of energy required by the plants to grow to the light, therefore increasing levels of carbohydrates available for other components of growth. The purpose of above-ground partitions is to prevent light competition without affecting the light intensity (Wilson 1988). Transparent partitions do not always eliminate all competition for light (Wilson 1988) and as such, the wire cages utilized to separate the meadow bromegrass leaves from the Kura clover may not have completely prevented aerial interaction.

This study prevented potential treatment effects of soil nutrients and water. Under field conditions, soil nutrients and water are typically limiting, potentially causing different results. Competition studies that focus on establishing communities have found that root competition is generally predominant during seedling growth (Rhodes and Stern 1976). This is confirmed in our experiment where eliminating root competition appears to play a larger role in the morphology of Kura clover leaves than does removal of shoot competition. While isolation from either above or below-ground competition positively affected leaf area, the greatest increase occurred when Kura clover roots were separated from those of meadow bromegrass. Thorsted et al. (2006), observed a similar pattern when investigating the above and below ground effects of wheat – clover intercrops. While changes in canopy architecture may increase the effectiveness of light capture, and thus biomass production, it is also possible that each species can effectively utilize light resources and thus the greatest proportion of resource utilization, positive or negative, occurs below the soil surface (Thorsted et al., 2006). A comparison of Kura clover and white clover found that although white clover had more than three times the number of leaves, leaf area between the two species did not differ (Black et al., 2003). The ability of Kura clover to intercept solar radiation is comparable to the faster establishing white clover, and suggests that competition for light does not significantly impact establishment of Kura clover. This further supports our findings that removal of shoot competition does not significantly affect biomass accumulation.

The presence of below ground barriers, for treatments without root competition, reduced the available rooting volume of individual plants to 20% of available volume when no partitions were used. As no reduction in root weight was measured we can conclude that there were adequate levels of nutrient provided to support optimal root development. Despite the lack of differences in weight of roots produced, when roots were not isolated, both Kura clover and meadow bromegrass roots grew into the rooting space of the surrounding plants.

Greenhouse and field studies have determined that by the end of the seedling year, Kura clover typically has a root:shoot ratio of 2.0 – 3.0. White clover root:shoot ratios have been found to be 0.2, however, white clover has ten times greater vegetative biomass (DeHaan et al., 2001). Kura clover root:shoot ratios in the present study, were lower than those previously reported. Root:shoot ratios in this study were measured 12 wk after planting whereas DeHaan et al. (2001) and Genrich et al., (1998) measured root:shoot ratios at the end of the seeding year.

When experiencing below ground competition, it was the vegetative components of the clover plant that responded rather than the below ground components. Bryant (1974) observed that Kura clover preferentially partitions carbohydrates toward root development. This was supported by our observation that vegetative biomass declined when resources were reduced by competition from other plants, while root development was not affected. Treatment differences may be greater in situations where water or nutrients are limited, such as typically occurs under field conditions. The relative importance of competition indicates that root competition has greater effects than shoot competition (Wilson 1988). Greenhouse studies in Australia determined that root and crown mass of Kura clover was critical to establishing successful mixed stands (Hill and Mulcahy, 1995). Data from our study supports this conclusion. As vegetative biomass is of primary concern to a grazing animal, minimizing the competition from the grass component in a grass – legume mixture, becomes critical for maximizing the species balance of the sward. Innovative seeding practices that isolate Kura clover from meadow bromegrass plants may serve to maximize early growth potential. Seeding species in alternate rows, in different directions, or delaying seeding of the grass component to allow the slower developing Kura clover plants to achieve a greater size prior to the onset of interspecific competition, would have the potential to improve the compatibility of these two species. Additionally, establishment of Kura clover may be improved by nitrogen fertilization as rhizobial inoculation does not appear to affect growth until late in the seedling year (Seguin et al., 2001).

Conclusions

Although root development did not appear to be compromised when experiencing competition, shoot production was reduced. This research appears to emphasize the importance of root access to soil volume on above ground growth when nutrients and water are not limited. By ensuring that seeding rates are adjusted to allow for a balance between adequate plant density and minimal overlap of rooting zones, especially during early establishment, Kura clover vegetative production could be increased. Managerial steps taken to reduce the intensity of competition below ground, would likely result in greater Kura clover biomass production as evidenced by the results of this greenhouse trial.

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Table 1. Vegetative and root biomass of meadow bromegrass (MB) and Kura clover (KC) plants grown with root competition, shoot competition, no competition and full competition.

	Height of tallest leaf (cm)		**Leaf Area (cm²)**		**Root: shoot ratio**		**Dry weight roots (g)**		**Dry weight leaves per plant (g)**	
Treatment	MB	KC	MB	KC	MB	KC	MB	KC	MB	KC
Full competition	60	16*b*	439*b*	108*bc*	1 : 0.93	1 : 1.14	4.09	0.83	3.79*b*	0.95*b*
Root competition	64	17*b*	327*b*	61*c*	1 : 1.01	1 : 1.41	3.62	0.63	3.66*b*	0.89*b*
Shoot competition	71	25*a*	485*b*	194*a*	1 : 1.03	1 : 1.47	3.67	1.29	3.79*b*	1.90*a*
No competition	76	22*a*	661*a*	117b	1 : 1.06	1 : 1.91	4.79	0.85	5.07*a*	1.62*a*

a-b Effects within columns are significant at $p<0.05$, means followed by the same letter are not significantly different

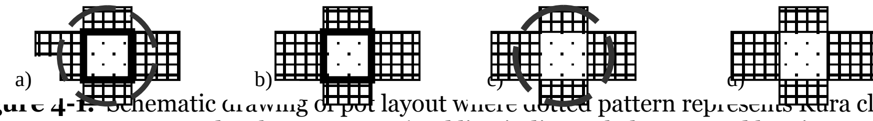

Figure 4-1. Schematic drawing of pot layout where dotted pattern represents Kura clover and grid pattern represents meadow bromegrass. (Red line indicates below ground barrier, green line indicates above ground barrier.) a) above and below ground barriers present = no competition b) below ground barriers present = shoot competition c) above ground barriers present = root competition d) no barriers = full competition.

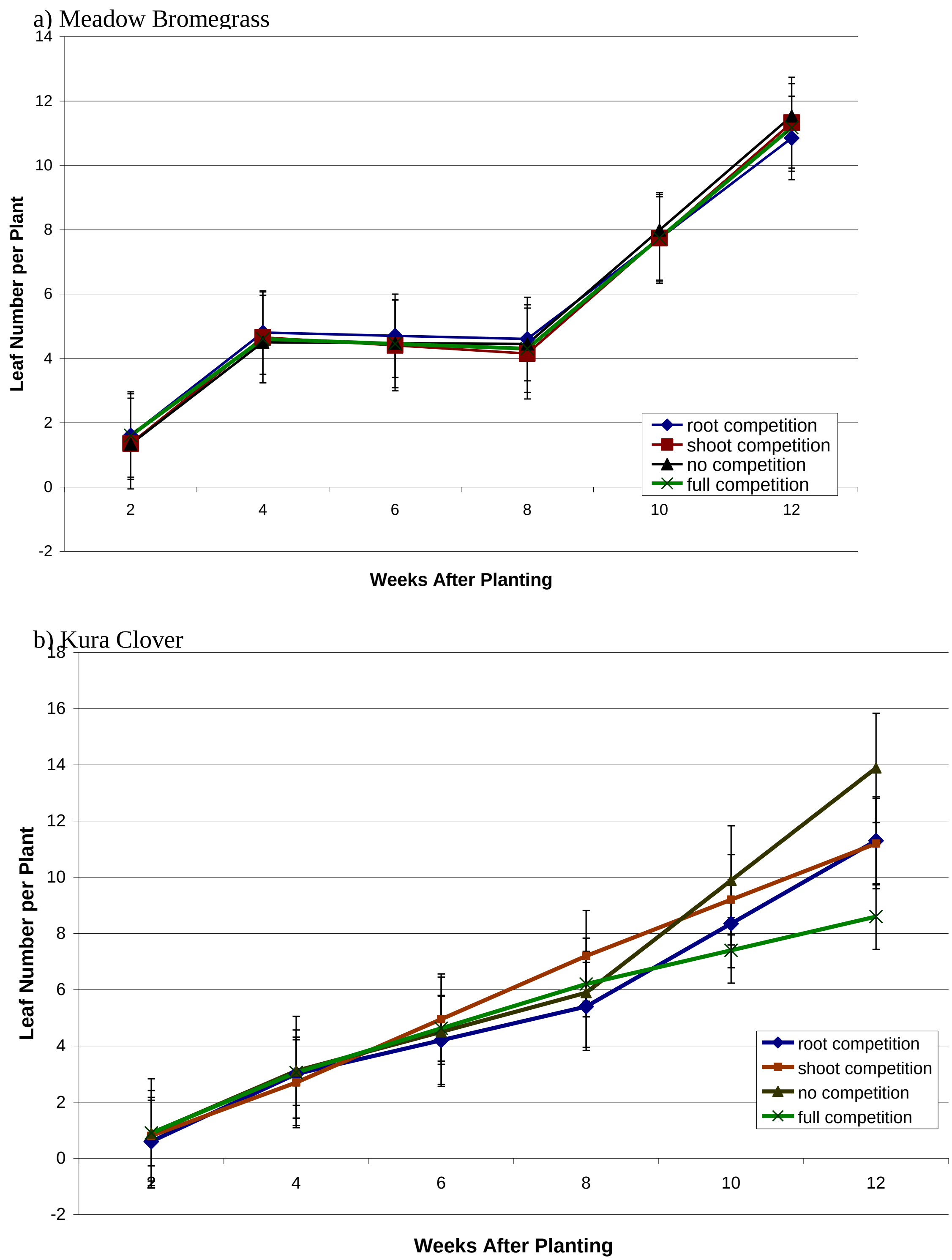

Figure 4-2. Leaf accumulation of (a) meadow bromegrass plants and (b) Kura clover plants over twelve weeks when grown with no competition (△), shoot competition (□), root competition (◇) and full competition (X). Error bars indicate one standard error of the mean.

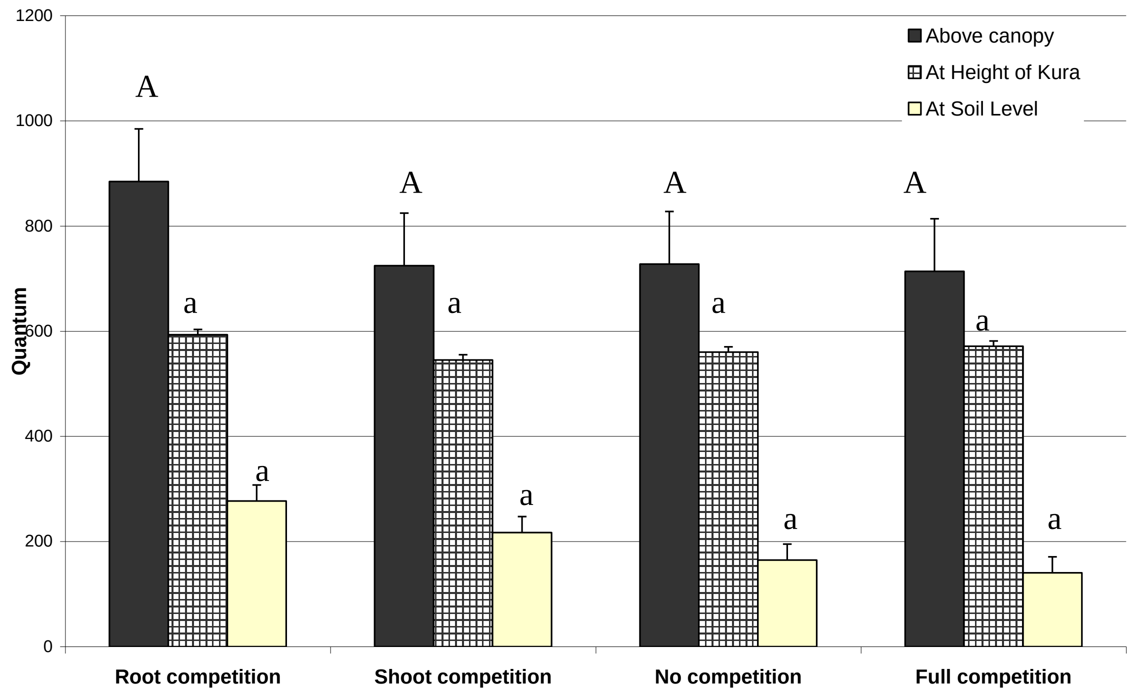

Figure 4-3. Light availability at the top of the canopy, at the height of the Kura plants and at ground level twelve weeks following planting for Kura plants grown with root competition, with shoot competition, in the absence of competition and with full competition.
Treatment did not significantly affect light availability above the canopy, at the height of the Kura clover or at soil level. Error bars indicate one standard error of the mean.
a/A means with the same letter are not significantly different when $p<0.05$.

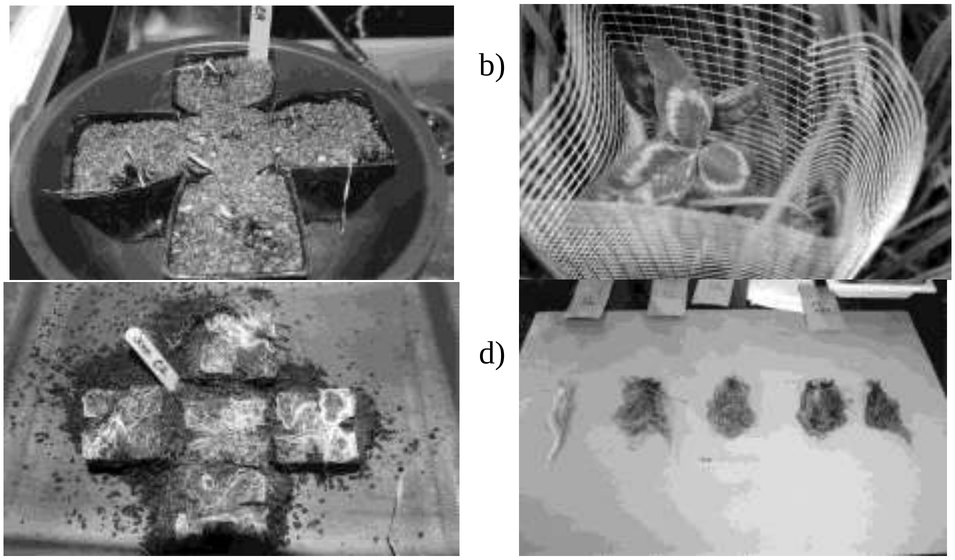

Plate 4-1. a) Pot configuration, sides removed to allow roots to interact (similar configuration without removal of common sides for treatments requiring root separation. b) Wire cage used for above ground separation. c) Inverted pots showing root growth and mingling between pots d) root mass of Kura clover (left) and four meadow brome plants grown in the absence of competition.

Chapter 5

Does relative time of emergence affect stand composition and yield in a grass: legume mixture?
Kura clover (*Trifolium ambiguum* M. Bieb.) – meadow brome grass (*Bromus riparius* Rehm.) and Kura clover – orchard grass (*Dactylis glomerata* L.) mixtures

A version of this chapter was submitted to Grass and Forage Science

Hypotheses Tested

Harvested yield of Kura clover – meadow bromegrass mixtures will be the same when planted at the same time or when the planting of grass is delayed until the clover has one leaf, three leaves, in the fall, or the following spring.

Species composition of Kura clover – meadow bromegrass mixtures will be the same when planted at the same time or when the planting of grass is delayed until the clover has one leaf, three leaves, in the fall, or the following spring.

Harvested yield of Kura clover – orchard grass mixtures will be the same when planted at the same time or when the planting of grass is delayed until the clover has one leaf, three leaves, in the fall or the following spring.

Species composition of Kura clover – orchard grass mixtures will be the same when planted at the same time or when the planting of grass is delayed until the clover has one leaf, three leaves, in the fall or the following spring.

Introduction

Kura clover (*Trifolium ambiguum M. Bieb.*) is a perennial clover species that demonstrates potential for use in pastures in western Canada. Studies in New Zealand, Australia and the United States found that Kura clover can be an excellent contributor to mixed species pastures, withstanding frequent grazing and tolerating a broad range of environmental conditions (Taylor and Smith, 1998; Peterson et al., 1994; Bryant 1974). The most unique feature of Kura clover, and probably the reason for its longevity and adaptability, is the development of a large root and rhizome system. Carbohydrates are preferentially allocated to root growth over the first two years of the plant's life (Taylor and Smith, 1998), thus above-ground, vegetative growth is initially slow. It is not until the third season that vegetative growth potential is maximized (Taylor and Smith, 1998). Planting Kura clover in mixtures with productive and fast growing grass species compromises the ability of the slow growing Kura clover seedling to compete, resulting in grass dominated swards (Walker, 2002).

Plant-plant interactions during establishment are critical to determining the composition of the stable pasture community. The presence of neighbors affects the environment of a plant and may result in changes in growth rate or morphology (Harper, 1977). Turkington et al., (1995) found different grasses have varying growth constraints on establishing clovers. Models formulated to address complexity of relationships in grass: legume mixtures indicate that, under certain conditions, legumes have distinct advantages in growth due to the ability to fix nitrogen (Thornley et al., 1995). Thornley et al. (1995) further states that grasses demonstrate a large photosynthetic advantage compared to legumes. Success of both species is strongly influenced by soil nitrogen pools and competition for light. Skinner (2005) studied differences in monoculture, binary and complex mixtures of four species and found mixture complexity had a significant negative effect on seedling emergence and survival.

Previous studies in Alberta have shown that the establishment of Kura clover grown in mixtures with meadow bromegrass (*Bromus riparius* Rehm.) or orchard grass (*Dactylis glomerata* L.) is significantly reduced compared to its establishment in monocultures or with slower growing grasses (Walker, 2002). Studies in Minnesota comparing establishment of several legumes found Kura clover to be highly susceptible to competition due to its slow vegetative growth in the establishment year (Cuomo et al., 2001). Both meadow bromegrass and orchard grass are desirable pasture species with high levels of productivity and rapid re-growth following defoliation. The addition of Kura clover to a stand of meadow bromegrass or orchard grass could increase the nutritive quality of the pasture, reduce the need for synthetic fertilizers, and improve the stand longevity and seasonal distribution of yield. Analysis of sward compositional changes at a constant level of biomass production (Askin, 1990) revealed that increasing the amount of legume in a stand significantly increased the weight gain of the grazing animal. Askin et al, (1987), showed that increases in the percentage of white clover (*Trifolium repens* L.), to over 30% of the available biomass significantly improved gain on lambs, and increased the carrying capacity of the paddock. It is generally accepted that at least 30% legume is needed for a mixed stand to be self-sufficient in nitrogen. There is an increased risk of bloat when legumes comprise more than 50% of a stand. Based on these two guidelines, pasture managers would ideally like between 30 and 50% legume in a mixed stand.

Kura clover's use is limited by slow establishment resulting in low yield in the establishment and first production year (Seguin et al., 1999). Seedlings of Kura clover are susceptible to environmental stresses, and especially sensitive to competition (Steiner and Snelling, 1994). Hill and Mulcahy (1995) postulated that root and rhizome development of Kura clover is crucial during the establishment period and enhanced when the density of companion grasses is low. They hypothesized that initially establishing pure stands of Kura clover and then direct drilling the desired grass component into the clover might improve the ability of Kura clover to compete against the aggressive nature of the companion grasses (Hill and Mulcahy, 1995).

Using the principles of relative time of emergence common to weed science, we developed an experiment to manipulate the competitive effects of meadow bromegrass or orchard grass on seedling establishment of Kura clover. Our objective was to determine if a favorable mixture of Kura clover and meadow bromegrass, or Kura clover and orchard grass, could be obtained by removing inter-specific competition during the initial stages of Kura clover development by delaying the timing of the grass seeding relative to the developmental stage of the clover.

Experimental Design and Methodology

Two experiments were established in 2003, 2004 and 2005 using a randomized complete block design, with four replications, to determine the effect of manipulating seeding time of two grasses relative to Kura clover. One test used meadow bromegrass as the focal grass species and the second test used orchard grass. Tests were planted at the University of Alberta, Edmonton Research Station, Edmonton, Alberta, Canada (53°25'N, 113°,33'W), on a black chernozemic soil, in 2003, 2004 (2 sites) and 2005.

Prior to seeding, soil samples were taken at 0 – 6" and 6 – 12" and analyzed for levels of macronutrients, electrical conductivity and pH at Norwest Labs, Edmonton, Alberta. A granular fertilizer was subsequently applied to the plot area at the levels recommended for establishing grass/legume pasture (100 kg/ha of 11-52-0 (N-P-K) actual product).

Plots measuring 2.4m x 6.0 m, were sown using a double disk press drill at 30 cm row spacing. Clover was seeded at 10 kg/ha at a depth of 1 cm. Meadow bromegrass was sown at 4 kg/ha and orchard grass at 3 kg/ha (half the recommended seeding rate for pure stands), and 1 cm depth.

The five establishment treatments were either meadow bromegrass or orchard grass seeded into establishing Kura clover at several stages of development. The grasses were either sown at the same time as the clover (MB-S/OG-S), when the clover had one true leaf (MB-K1/OG-K1), when clover had three true leaves (MB-K3/OG-K3), in the fall of the establishment year (MB-F/OG-F), or in the spring (MB-SP/OG-SP) of the following year (Table 5-1). In 2004 and 2005, a sixth treatment, Kura clover monoculture (K) was added.

In the year of establishment, a single year end harvest (Table 5-2) was taken to determine biomass yield and species composition. In the first, second and third production years, harvests occurred monthly, from June through September (Table 5-2) for a total of four harvests per growing season. In 2006, when insufficient re-growth occurred following the second harvest due to poor moisture conditions, the third harvest was delayed until the end of the season (total of 3 harvests). Plots were harvested for yield using a self-propelled flail mower, removing a 60 cm x 5.4 m area. In addition, a 50 cm x 50 cm quadrat was harvested by hand and the grass and clover components separated to determine species composition. Harvested material was dried to a constant weight at 60 ˚C. Following each harvest, the biomass remaining on each plot was mowed and removed.

Data were tested for normality using PROC UNIVARIATE (SAS Institute, 2003). Growing environment (site x year) was considered to be a random effect as sites were selected to represent the Parkland region of Alberta, Canada and climatic conditions throughout the study were typical for the area. Therefore, repeated measures analysis of variance were performed using data combined across environments (site x year) with the MIXED procedure of SAS (Littell et al., 2006). Mean separation was done using the Tukey method. Significance were tested at $p<0.05$. Results from the analysis of variance are presented in tabular form located in the Appendix (Table A-3 through A-5).

Results

Climatic Conditions

Temperatures at each site were slightly above the 30 year average in June, July and August in 2003, 2004 and 2005 (Figure 5-1). The winter of 2005 – 2006 was considerably warmer than the 30 year average. Growing season precipitation (May – September) at each site was much lower than the average. Rainfall was 57 mm, 115 mm and 161 mm below average in 2003, 2005 and 2006 respectively (Figure 5-2). In 2004, 60% of the growing season precipitation fell during July, 2.2x greater rainfall than normally experienced in July. Based on a comparison of 30 year averages and the rainfall data over the years of the study, moisture was a limiting factor in three of the four growing seasons.

Meadow bromegrass

Establishment year (2003 – 2005)

Species Composition

In the year of establishment, treatment had a significant effect on the percentage of clover biomass in the Kura clover– meadow bromegrass mixtures ($p<0.01$; table 5-3). When Kura clover and meadow bromegrass were planted at the same time, the grass dominated the sward and Kura clover contributed significantly less to harvested biomass (31%) than other treatments (Table 5-3). When the planting of meadow bromegrass was delayed until the clover had one (MB-K1) or three true leaves (MB-K3), the grass established and contributed to harvested biomass. Kura clover however, dominated these stands, accounting for 82% and 93% of the yield for MB-K1 and MB-K3 respectively. Meadow bromegrass failed to establish when introduced in the fall (MB-F) or the following spring (MB-SP). Kura clover thus comprised one hundred percent of yield in monoculture, when meadow bromegrass was fall (MB-F) or spring seeded (MB-SP).

Biomass Yield

Total yield in the establishment year was reflective of the effects of the rapid establishment and growth of the meadow bromegrass. Yield did not vary between treatment with the exception of MB-S, which yielded significantly higher then MB-SP ($p<0.01$; Table 5-4). Dry matter yield in the establishment year ranged from 2,492 kg/ha for MB-SP treatment which was 100% clover, to 6,753 kg/ha for MB-S treatments with only 31% Kura (Table 5-3; Table 5-4).

First Production Year (2004 – 2006)

Species Composition

Species composition in the first production year was significantly affected by treatment and harvest. When Kura clover and meadow bromegrass were planted at the same time (MB-S), grass continued to dominate the sward (Table 5-3). Delaying the sowing of the meadow bromegrass until the clover had one true leaf, allowed Kura clover to dominate, contributing 62% of the yield when averaged across the growing season. Planting meadow bromegrass at the third leaf of Kura clover resulted in a significantly higher proportion of Kura clover (80%) than treatments where meadow brome was introduced earlier in the season (MB-S (31%) and MB-K1 (62%)), and significantly less Kura clover than when the grass was introduced later than the third true leaf stage of the Kura clover (MB-F (93%) or MB-SP (93%); Table 5-3). When introduction of the grass occurred in the fall of the establishment year (MB-F) or the following spring (MB-SP), meadow bromegrass did not contribute to harvested yield however, the presence of grass in the stand did increase over four harvests in the first production year (Figure 5-3). Both the fall and spring sown meadow bromegrass increased their contribution to harvested yield from zero to seven percent by the end of the first production year (Figure 5-3).

Biomass Yield

Treatment significantly affected total annual dry matter yield (kg/ha) in the first production year ($p<0.01$; Figure 5-3) with MB-S out yielding all other treatments. Total annual yield ranged from 7,765 kg/ha dry matter (KM) to 11,093 kg/ha dry matter (MB-S).

Second Production Year (2005 – 2006)

Species Composition

By the second production year meadow bromegrass sown at the same time as the clover (MB-S), or planted when Kura had one leaf (MB-K1) or three leaves (MB-K3) exhibited similar proportions of clover in the mixture; 46%, 52%, and 63% respectively, when averaged across the growing season (Table 5-3). Fall and spring sown meadow bromegrass had significantly more Kura clover in the harvested biomass than other mixtures (80 – 82%), but did not differ from each other. Percentage of Kura clover declined by approximately 10% between the first and second production year when sowing of grass was delayed by one (MB-K1) and two (MB-K3) months (Table 5-3) indicating higher meadow bromegrass growth in the second season.

Percentage of Kura clover in each treatment varied significantly across the growing season ($p<0.001$; Figure 5-3). When Kura clover and meadow brome were planted at the same time (MB-S) and when meadow bromegrass was planted when Kura clover had one true leaf (MB-K1), percentage of Kura was greatest at the second and third harvests when re-growth of meadow bromegrass was slower (Figure 5-3). This differed from MB-K3 and MB-F treatments where Kura clover content declined at each harvest (with the exception of the fourth harvest in MB-F treatment).

Biomass Yield

Total annual yield (dry matter) in the second production year ranged from 7,300 (MB-F) kg/ha to 9,546 kg/ha (MB-K1; Table 5-4). Total yield was significantly affected by treatment ($p<0.001$; Table 5-4). A significant treatment x harvest interaction was present ($p<0.001$). Average yield across all harvests was 1,975 kg/ha dry matter.

Third Production Year (2006)

Species Composition

When averaged over the growing season, percentage clover in the Kura clover – meadow bromegrass mixtures was similar to the previous year. When meadow bromegrass was sown earlier in the growing season it comprised a significantly larger portion of biomass than when sown later in the season. Percentage of Kura clover in MB-S, MB-K1 and MB-K3 treatments was still significantly lower than MB-F, MB-SP and KM (Table 5-3).

Variation in species composition at each harvest was present ($p<0.001$; Figure 5-3). In the MB-F and MB-SP treatments, Kura clover percentage declined by 21% and 22% respectively over the growing season (Figure 5-3).

Biomass Yield

Annual dry matter yield in the third production year did not differ significantly between treatments and ranged from 4,922 kg/ha to 6,023 kg/ha (Table 5-4). Due to the slow re-growth of both Kura clover and meadow bromegrass, under the dry conditions of 2006, only three harvests were taken over the growing season. Numerically, greatest annual yield was produced by MB-K1 (6,023 kg/ha) followed by MB-F (5,872 kg/ha), MB-K3 (5,786 kg/ha), MB-SP (5,487 kg/ha) and lastly MB-S (4,922 kg/ha).

Orchard Grass

Establishment Year (2003 – 2005)

Species Composition

Species composition was significantly affected by treatment in the year of planting ($p<0.01$; Table 5-5). Orchard grass planted at the same time as the Kura clover (OG-S) had a significantly lower proportion of clover than other treatments (Table 5-5). Delaying planting of the orchard grass until the Kura clover had one true leaf, three leaves or in the fall resulted in swards dominated by the clover, 81%, 92%, 99% respectively (Table 5-5). Grass establishment the following spring was not successful.

Biomass Yield

Yield in the establishment year was measured by a single, end of season harvest (Table 5-6). The rapid establishment of orchard grass positively impacted the biomass production. Mixtures where planting of grass and clover occurred at the same time (14.4% clover) had significantly higher yields than treatments where planting of the orchard grass was delayed (>80% clover; $p<0.001$; Table 5-5). Establishment year production ranged from 870 kg/ha dry matter (OG-SP) to 2,313 kg/ha dry matter (OG-S) (Table 5-6).

First Production Year (2004 – 2006)

Species Composition

The greatest percentage of Kura clover occurred in treatments where the sowing of the grass was delayed ($p<0.01$; Table 5-5). When orchard grass was sown in the fall or spring (OG-F and OG-SP) significantly more Kura clover contributed to biomass than when grass was planted at the same time or at the first leaf stage of the clover (OG-S or OG-K1; Table 5-5). The proportion of Kura clover in binary mixtures with orchard grass when planting occurred at the same time (OG-S), increased from 14% in the establishment year, to 27%, in the first production year (Table 5-5). In all other treatments, with the exception of the Kura monoculture, percentage of Kura clover decreased from the establishment to the first production year (Table 5-5). The percentage of Kura clover in the stand was greatest at the first harvest and decreased over the growing season (Figure 5-4). This pattern differed from that of Kura clover – meadow bromegrass mixtures where the greatest percentage of Kura was found at the second harvest of the growing season (Figure 5-3).

Biomass Yield

Treatments did not significantly impact yield in the first production year. Total annual yield ranged from 7,088 kg/ha dry matter (KM) to 10,688 kg/ha dry matter (OG-S; Table 5-6). Yield in the OG-S treatment averaged 2,672 kg/ha dry matter at each harvest. In comparison, Kura clover monocultures (KM) produced an average of 1,772 kg/ha dry matter at each harvest (Table 5-6).

Second Production Year (2005 – 2006)

Species Composition

Species composition in the second production year was significantly affected by the interaction of the main effects ($p<0.0$. Species composition at each harvest over the growing season differed between treatments. When orchard grass was planted into more established stands of Kura clover (OG-K3, OG-F, OG-SP) the percentage of Kura clover in the stand decreased from harvest one to harvest two, and again from harvest two to three (Table 5-5; Figure 5-4). An increase in the proportion of clover occurred at harvest three. Treatments where orchard grass was planted at the same time or when Kura clover had one true leaf (OG-S, OG-K1), saw a slight decline in the percentage of Kura in harvests two and three, and a large decrease in Kura clover contribution to yield at harvest 4 (Table 5-5; Figure 5-4). The percentage of Kura in the sward ranged from 42% when both species were planted at the same time (OG-S) to 77% when orchard grass was introduced in the fall (OG-F) averaged across all harvests ($p<0.01$; Table 5-5). In all treatments, except OG-S and KM, the proportion of Kura clover was lower in the second production year than the first production year. The decrease in the amount of clover in the second production year resulted in a more even balance between the two species in the mixture (Table 5-5).

Biomass Yield

Total annual yield (kg/ha dry matter) ranged from 6,573 kg/ha (KM) to 8,762 kg/ha (OG-K3) (Table 5-6). Yield was significantly affected by both the main effects and the interaction of treatment and harvest ($p<0.001$; Table 5-6). For all treatments, except OG-SP, the first and last harvests of the season gave significantly higher yields. The spring sown treatment (OG-SP) was the exception, where the second and last harvests yielded significantly more than the first and third harvests (Table 5-6).

Third Production Year (2006)

Species Composition

Species composition was significantly affected by treatment and the interaction of treatment and harvest ($p<0.01$; Table 5-5). For all treatments, the proportion of Kura clover in the mixture significantly decreased over the season with the exception of OG-S, where the percentage of Kura clover increased from the first to last harvest (Figure 5-4). When orchard grass and Kura clover were planted at the same time (OG-S), Kura clover comprised 44% of the harvested biomass (Table 5-5). The contribution of Kura clover to harvested yield ranged from 54% when orchard grass was planted at Kura clover one leaf (OG-K1) to 74% when orchard grass was planted in the fall of the establishment year (OG-F) (Table 5-5).

Biomass Yield

Total annual yield (kg/ha dry matter) was much lower in the third production year than in previous seasons possibly due to moisture stress (Figure 5-2) and warm temperatures (Figure 5-1). Greatest biomass was produced when orchard grass was sown at the third leaf of Kura clover (6,156 kg/ha) and lowest when the two species were sown at the same time (3,448 kg/ha) (Table 5-6). Yield decreased at each harvest for all treatments with the exception of OG-S where biomass at each harvest increased (Table 5-6).

Discussion

In previous studies (Walker, 2002), when mixtures of Kura clover were established with meadow bromegrass or orchard grass sown at higher seeding rates (MB at 8 kg/ha, OG at 6 kg/ha), Kura clover contributed less than 10% of biomass yield. In this study, the reduced seeding rate of the grass resulted in Kura clover seedlings having a greater ability to compete during the establishment period. This resulted in a three-fold increase in the percentage of clover when grass and clover were seeded at the same time (MB-S / OG-S) Sanderson et al. (2002) recommended reducing the seeding rate of the grass when establishing mixtures using brome species.

The sensitivity of Kura clover to competition, found in treatments where Kura was sown at the same time as the meadow bromegrass (MB-S) or orchard grass (OG-S) was consistent with studies in Minnesota which demonstrated that when seeded with grasses, Kura clover comprised less than 30% of the sward (Peterson et al., 1994). Walker (2002), found that when meadow bromegrass or orchard grass were sown in mixtures with Kura clover, the Kura contributed less than 10% or 5% of the yield respectively. In this study, where seeding of the grass was delayed by up to two months, (MB-K1, MB-K3, OG-K1, OG-K3), allowed the Kura clover seedlings to develop free of competition from the grass for several weeks while still allowing the grass to establish successfully creating a mixed stand. This resulted in an increased presence of Kura in the stand while maintaining the grass component. Delaying the start of inter-specific competition may have allowed seedling root development of Kura clover, to occur more rapidly, thus providing greater resilience once grass species were introduced. Hill and Mulcahy (1995) stated that root and rhizome development of Kura clover is crucial during the establishment period and is enhanced when the density of companion grasses is low. Walker and King (2009) found below-ground competition from meadow bromegrass influenced above ground Kura clover production more than competition for light.

Under Alberta growing conditions, Kura clover emerged from the soil after 7 – 14 days after planting, and remained at the cotyledon stage for nearly two weeks, the first true leaf appearing four weeks following planting (Walker, personal observation). In contrast, meadow bromegrass and orchard grass, once emerged (7 – 10 days following planting), rapidly develop leaves. In a greenhouse study that tracked leaf accumulation over twelve weeks for both Kura clover and meadow bromegrass, meadow bromegrass showed rapid leaf development between the second and fourth week after planting while the Kura plants accumulated leaves more slowly (Walker and King, 2009). Sanderson et al., (2002) noted that bromegrasses and orchard grass have rapid leaf development and large seedlings in both greenhouse and field studies. Bromegrasses were found to have larger seedlings than orchard grass but orchard grass developed tillers at a faster rate (Sanderson et al., 2002). When sown with grasses whose development advances more rapidly, Kura clover plants are at a disadvantage because of their slow rate of leaf development.

In this study, when Kura clover was allowed to growth without competition for three months prior to grass seeding, Kura clover tended to out compete the grass. Casler et al. (1999) found that orchard grass showed superior establishment than smooth brome, timothy and reed canary grass. When orchard grass was seeded into established alfalfa in Wisconsin, orchard grass contributed half of the harvested yield by the end of the first production year (Undersander et al., 2001). In our study, meadow bromegrass or orchard grass seeded into Kura clover in the fall, took three seasons to establish a consistent grass presence in the mixture. At the end of the third production year, orchard grass contributed 25% to biomass yield whereas meadow bromegrass contributed only 20%. The proportion of grass in the stand did increase over the three seasons of this trial, and may further increase in subsequent years. Reader (1991) concluded that ground cover density significantly affects seedling emergence in an established stand. Increasing the seeding rate of the orchard grass and meadow bromegrass in our study, especially in later sod seeded treatments, increased the contribution of the grasses to yield. The results of this study suggest that under Alberta growing conditions delaying planting of the grass species increases the Kura clover component of the mixture. If the grass is planted the following season, Kura clover appears to have too great a competitive advantage over the grass resulting in reduced sward biomass.

In this test there was only a single harvest in the establishment year. Grazing management can be employed as a tool to reduce the effects of aggressive growth of one species on another. Frequent defoliation for the first six months following sowing was shown to improve establishment of slower growing species, particularly legumes (Askin, 1990). Thus, early grazing might be used as a tool to alleviate the stress on the Kura when sown with meadow brome or orchard grass (MB/OG-S), allowing the clover to establish a greater presence in the sward. This approach might be most successful with meadow bromegrass since orchard grass re-grows very rapidly following defoliation whereas meadow brome shows delayed re-growth. An understanding of the different re-growth characteristics of various grass species may be beneficial in obtaining a desirable species mixture.

It is generally accepted that 30 and 50% legume in a pasture mixture is desirable. This range of legume can support the nitrogen needs of the sward however, higher proportion of legumes also increase the risk of bloat to the grazing animal. In this study, treatments resulted in species composition between 47 - 80% clover when Kura clover was sown with meadow bromegrass and between 44 – 74% clover in Kura clover - orchard grass mixtures. We were able to successful establish stands that would be self-sufficient in nitrogen. However, delaying planting of the grass until fall or spring resulted in stands with a high proportion of Kura clover, increasing the risk of bloat.

Conclusions

The manipulation of competitive effects by varying seeding rate and relative time of emergence can significantly affect establishment success. This makes it possible to produce a 50:50 mixture of species with varying competitive abilities. Successful manipulation of the sward components, to favor the slower growing clover was achieved by either reducing the grass seeding rate or by delaying seeding of the grass by up to two months. Delaying grass seeding beyond two months resulted in poor grass establishment. Sod-seeding grass into an established Kura clover stand is thus not likely to be successful.

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Table 5-1. Seeding dates of Kura clover (*Trifolium ambiguum M. Bieb.*) and meadow brome grass (MB; *Bromus riparious Rehm.*) or orchard grass (OG; *Dactylis glomerata L.*) for plots established at the University of Alberta Edmonton Research Station, Edmonton, Alberta in the years 2003 through 2005.

Year of Establishment	**KM***	**Kura clover**	**MB/OG-S***	**MB/OG –K1***	**MB/OG –K3***	**MB/OG –F***	**MB/OG –SP***
2003 (1 site)	n/a	May 28	May 28	June 26	July 23	Sept 30	May 20, 2004
2004 (2 sites)	May 20	May 20	May 20	June 18	July 22	Oct 8	May 2, 2005
2005 (1 site)	May 2	May 2	May 2	June 21	July 18	Sept 30	May 19, 2006

***KM**= Kura clover monocultures, **MB/OG-S** = grass sown at same time as clover, **MB/OG-K1** = grass sown at Kura clover 1st leaf, **MB/OG-K3** =grass sown at clover's third leaf, **MB/OG-F**= grass sown in fall, **MB/OG-SP** = grass sown following spring

Table 5-2. Harvest dates of Kura clover (*Trifolium ambiguum M. Bieb.*) and meadow brome grass (MB; *Bromus riparius Rehm.*) or orchard grass (OG; *Dactylis glomerata L.*) in the establishment year, first, second and third production years. Plots were established at the University of Alberta Edmonton Research Station, Edmonton, Alberta in 2003 through 2005.

		Harvest date		
Year of Planting	**Establishment Year**	**First production year**	**Second production year**	**Third production year**
2003	Sept 11, 2003	May 27, 2004	May 27, 2005	June 7, 2006
		June 24, 2004	June 27, 2005	July 11, 2006
		July 22, 2004	July 25, 2005	Sept 7, 2006
		Sept 28, 2004	Sept 23, 2005	
2004	Oct 1, 2004	May 30, 2005	June 6, 2006	
site 1		June 28, 2005	July 12, 2006	
		July 25, 2005	Sept 6, 2006	
		Sept 26, 2005		
2004	Sept 30, 2004	May 31, 2005	June 6, 2006	
site 2		June 28, 2005	July 13, 2006	
		July 25, 2005	Sept 30, 2006	
		Sept 30, 2005		
2005	Sept 22, 2005	June 7, 2006		
		July 12, 2006		
		Sept 7, 2006		

Table 5-3. Average species composition of Kura clover – meadow brome mixtures in the establishment, first, second and third production years. Meadow brome was sown at the same time as the clover (MB-S), when the Kura clover had one true leaf (MB-K1), when Kura clover had three true leaves (MB-K3), in the fall (MB-F) or in the spring of the following year (MB-SP). Plots were established at the Edmonton Research Station, Edmonton, Alberta in the years 2003 – 2005.

	Percentage Kura clover			
Treatment	**Establishment Year (2003, 2004, 2005)**	**First Production Year (2004, 2005, 2006)**	**Second Production Year (2005, 2006)**	**Third Production Year (2006)**
K-M	100% ab	100% a	100% a	100% a
MB-S	31% c	31% d	46% c	47% c
MB-K1	82% b	62% c	52% c	52% c
MB-K3	93% b	80% b	63% c	62% c
MB-F	100% ab	93% a	80% b	80% b
MB-SP	100% ab	93% a	82% b	78% b
ANOVA				
Treatment F-Test(n=6)	**	**	**	**
SE‡ treatment	.3	0.1	0.4	0.2

a-c means with the same letter within columns are not significantly different $p<0.05$

*, **, *** Significant at the 0.05, 0.01, 0.001 probability levels, respectively.

† NS, nonsignificant at the 0.05 probability level.

‡ Standard error of the difference of two least-squares means. Standard errors have been backtransformed where necessary.

Table 5-4. Dry matter yield (DMY kg/ha) in the establishment, first, second and third production years (2003 – 2006) for Kura clover –meadow bromegrass mixtures. Treatments consisted of Kura planted in monoculture (KM), or in mixture with grass when grass was planted at the same time as the clover (MB-S), when the clover had one trifoliate leaf (MB-K1), when the clover had three trifoliate leaves (MB-K3), in the fall (MB-F) or in the spring of the following year (MB-SP). Plots were seeded at the University of Alberta, Edmonton Research Station, Edmonton, Alberta in the years 2003 – 2005.

Treatment	Establishment Year DM kg/ha	First Production Year DM kg/ha	Second Production Year DM kg/ha	Third Production Year DM kg/ha
KM	2939 ab	7765 b	7670 ab	4922
MB-S	6754 a	11093 a	8104 ab	6023
MB-K1	3332 ab	8706 b	9546 a	5787
MB-K3	4014 ab	8801 b	8655 ab	5872
MB-F	4519 ab	7847 b	7301 b	5847
MB-SP	2493 b	7909 b	7750 b	
ANOVA				
Treatment F test (n=6)	***`	**	**	NS
SE‡ treatment	67	327	179	32

a-c means with the same letter within columns are not significantly different p<0.05

*, **, *** Significant at the 0.05, 0.01, 0.001 probability levels, respectively.

† NS, nonsignificant at the 0.05 probability level.

‡ Standard error of the difference of two least-squares means. Standard errors have been backtransformed where necessary.

Table 5-5. Average species composition of Kura clover – orchard grass mixtures in the establishment, first, second and third production years. Orchard grass was sown at the same time as the clover (OG-S), when the Kura clover had one true leaf (OG-K1), when Kura clover had three true leaves (OG-K3), in the fall (OG-F) or in the spring of the following year (OG-SP). Plots were established at the Edmonton Research Station, Edmonton, Alberta in the years 2003 – 2005.

	Percentage Kura clover					
Treatment	**Establishment Year**		**First Production Year**		**Second Production Year ***	**Third Production Year***
K-M	100%	a	100%	a	100%	100%
OG-S	14.4	b	26.2	c	41.6	43.6
OG-K1	81.3	a	62.6	b	51.7	54.3
OG-K3	91.8	a	81.9	ab	62.2	64.2
OG-F	98.9	a	93.7	a	76.8	74.3
OG-SP	100.0	a	96.3	a	72.2	66.9
ANOVA						
Treatment F-Test(n=6)	**		**		**	**
SE‡ treatment	0.3		0.1		0.3	0.2

* Significant interaction of treatment * harvest ($p<0.0001$)

a-c means with the same letter within columns are not significantly different $p<0.05$

*, **, *** Significant at the 0.05, 0.01, 0.001 probability levels, respectively.

† NS, nonsignificant at the 0.05 probability level.

‡ Standard error of the difference of two least-squares means. Standard errors have been backtransformed where necessary.

Table 5-6. Dry matter yield (DMY kg/ha) in the establishment, first, second and third production years (2003 – 2006) for Kura clover–orchard grass mixtures. Treatments consisted of Kura planted in monoculture (KM), or in mixture with grass when grass was planted at the same time as the clover (OG-S), when the clover had one trifoliate leaf (OG-K1), when the clover had three trifoliate leaves (OG-K3), in the fall (OG-F) or in the spring of the following year (OG-SP). Plots were seeded at the University of Alberta, Edmonton Research Station, Edmonton, Alberta in the years 2003 – 2005.

Treatment	Establishment Year DM kg/ha	First Production Year DM kg/ha	Second Production Year DM kg/ha	Third Production Year DM kg/ha
KM	1339 b	7088	6573 b	
OG - S	2159 a	10688	6585 b	3448 b
OG-K1	893 b	9627	7473 ab	5140 a
OG-K3	1432 ab	8492	8762 a	6155 a
OG-F	1202 b	7792	7454 ab	5757 a
OG-SP	870 b	7929	7585 ab	5917 a
		ANOVA		
Treatment F-Test(n=6)	**	NS	**	***
SE‡ treatment	697	29	184	59

a-b means followed by the same letter are not significantly different p<0.05.

*, **, *** Significant at the 0.05, 0.01, 0.001 probability levels, respectively.

† NS, nonsignificant at the 0.05 probability level.

‡ Standard error of the difference of two least-squares means. Standard errors have been backtransformed where necessary.

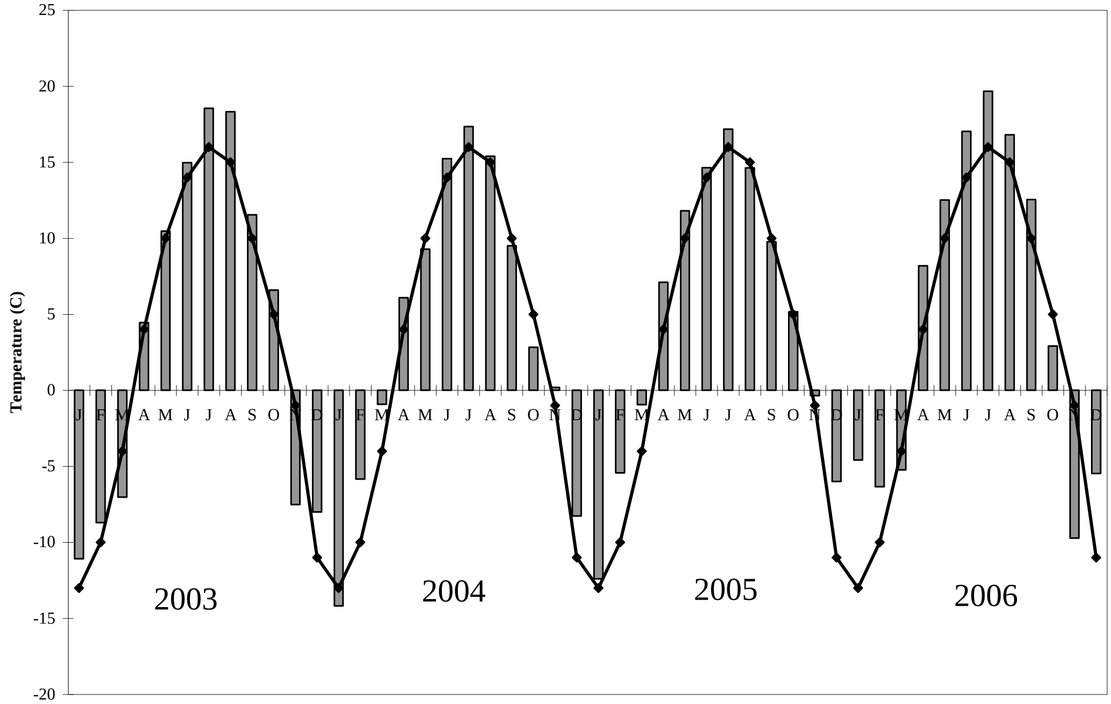

Figure 5-1. Average monthly temperature (C), indicated by the columns, at University of Alberta, Edmonton Research Station, Edmonton Alberta, from January 2003 through December 2006, and the 30 year average monthly temperature (C), indicated by the line, for Edmonton, Alberta.

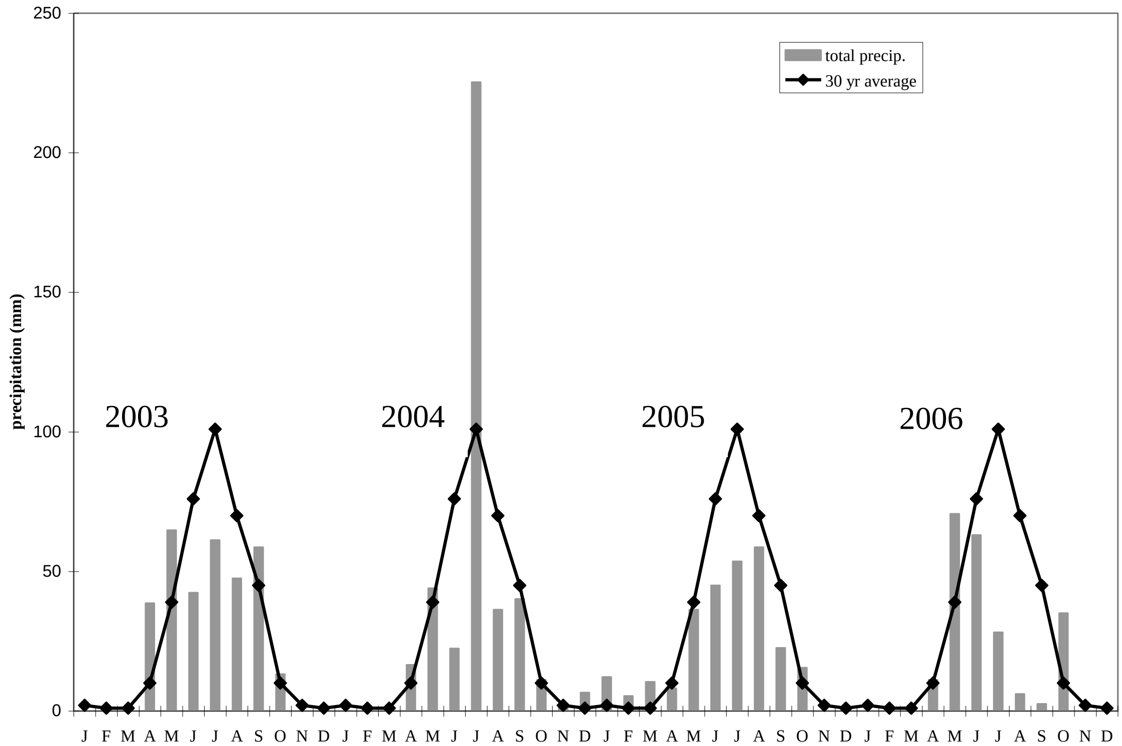

Figure 5-2. Average monthly precipitation (mm), indicated by the columns, at University of Alberta, Edmonton Research Station, Edmonton Alberta, from January 2003 through December 2006, and the 30 year average monthly precipitation (mm), indicated by the line, for Edmonton, Alberta.

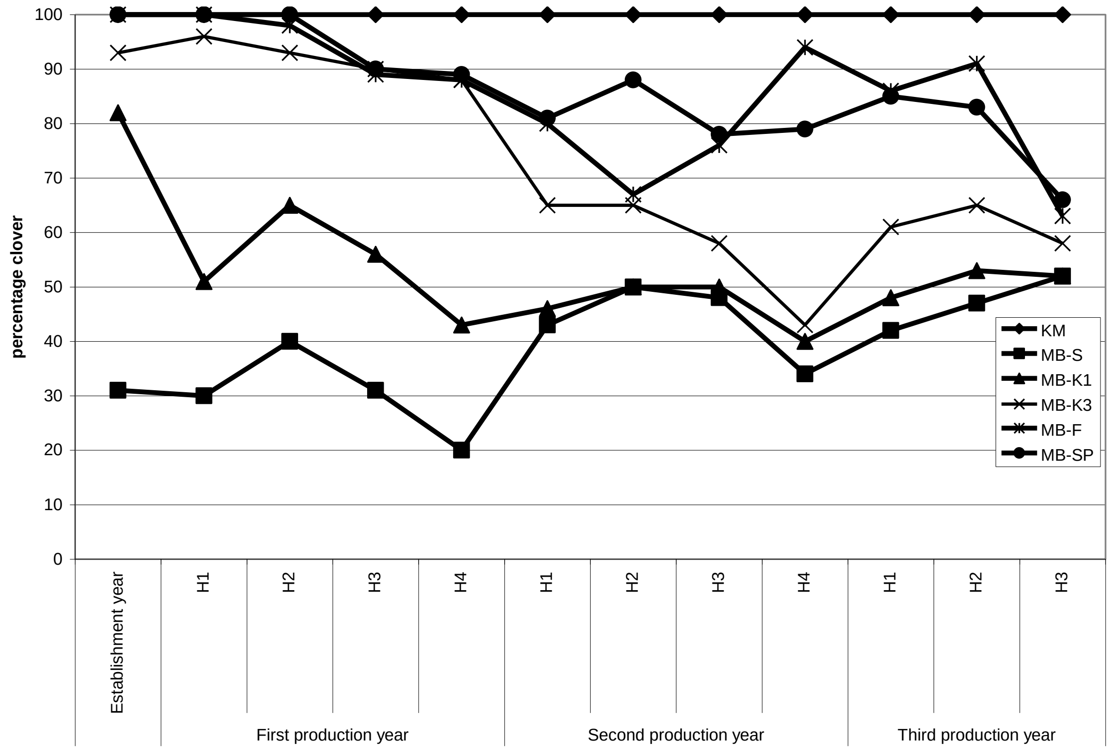

Figure 5-3. Species composition (percentage clover) of Kura clover – meadow brome mixtures over the establishment, first, second and third production years. Treatments consisted of a Kura monoculture (KM) and mixtures with meadow brome planted at the same time as the Kura (MB-S), when Kura has one true leaf (MB-K1), when Kura has three true leaves (MB-K3), in the fall (MB-F) or in the spring of the following year (MB-SP). Plots were planted at the University of Alberta Edmonton Research Station, Edmonton Alberta; data is averaged across plots established in the years 2003 – 2005.

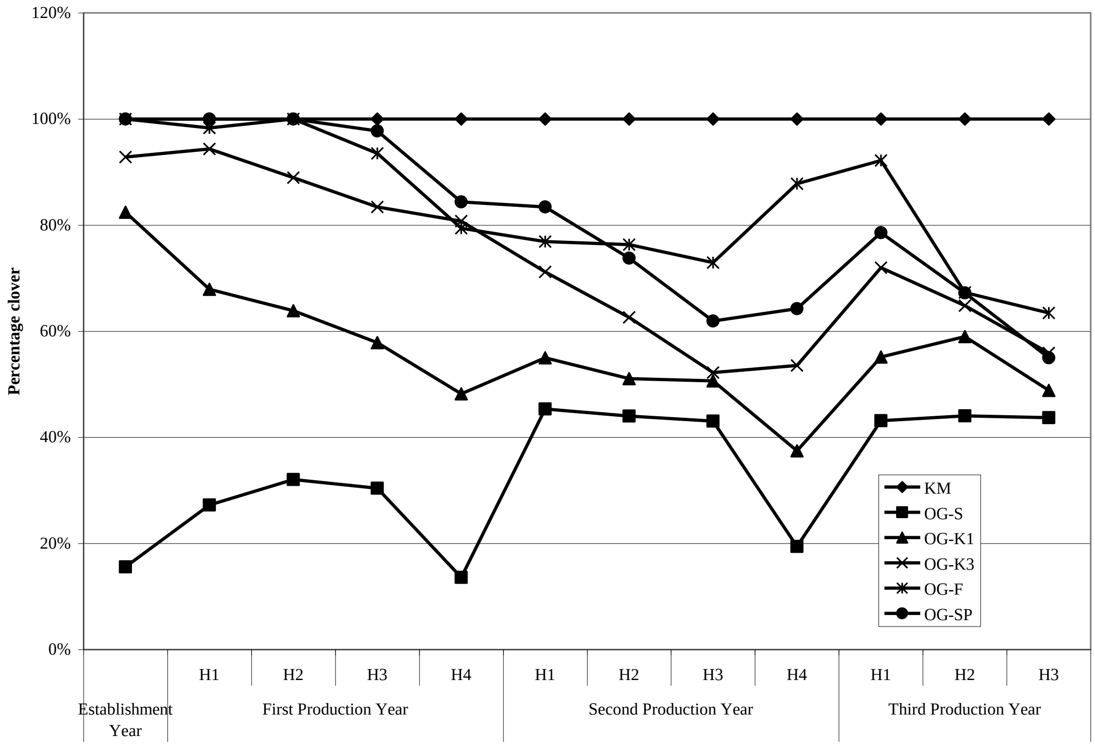

Figure 5-4. Species composition (percentage clover) of Kura clover – orchard grass mixtures at each harvest in the establishment, first, second and third production years. Treatments consisted of a Kura monoculture (KM) and mixtures with orchard grass planted at the same time as the Kura (OG-S), when Kura has one true leaf (OG-K1), when Kura has three true leaves (OG-K3), in the fall (OG-F) or in the spring of the following year (OG-SP). Plots were planted at the University of Alberta Edmonton Research Station, Edmonton Alberta; data is averaged across plots established in the years 2003 – 2005.

Chapter 6

Examining the survival and spread of seedling Kura clover (*Trifolium ambiguum* M. Bieb.) introduced into an existing grass sward.

A version of this chapter was submitted to Canadian Journal of Plant Science.

Hypotheses Tested

Kura clover survival will be the same when planted in sod with or without herbicide suppression.

Kura clover survival will be the same when harvested every four weeks, every six weeks or once, at the end of the growing season.

Kura clover leaf number will be the same when planted in sod with or without herbicide suppression.

Kura clover leaf number will be the same when harvested every four weeks, every six weeks or once, at the end of the growing season.

Dry weight of harvested Kura clover will be the same when planted in sod with or without herbicide suppression.

Dry weight of harvested Kura clover will be the same when harvested every four weeks, every six weeks or once, at the end of the growing season.

Introduction

Kura clover is a perennial legume species with several major qualities that make it a desirable species for use in long-term pastures. Originating in the Caucasian region of Russia, it has been grown in Australia, New Zealand, the United States and Canada (Taylor and Smith, 1998). Prior studies have found Kura clover to be persistent and productive for 30 years when managed under rotational grazing systems in New Zealand (Bryant, 1974). A major factor contributing to Kura clover's longevity is its extensive root and rhizome system. For at least two seasons after planting, carbohydrate partitioning favors root versus shoot development (Taylor and Smith, 1998). Consequently, Kura appears to be very slow growing during establishment. Once established, however, Kura can significantly contribute to biomass yield and has been found to improve animal weight gain (Mourino et al., 2002). In Minnesota, Kura clover was persistent under intermittent grazing, demonstrating the ability to compete and spread in mixed stands (Cuomo et al., 2003). Trials in Quebec, Canada and Minnesota, USA determined that Kura clover can establish when sod-seeded into grass pastures following a glyphosate application (Laberge, et al., 2005a). In the year following seeding, Kura clover biomass was lower than other legumes, however, Kura clover production increased over time (Laberge et al., 2005b). Studies in Wisconsin, USA found Kura clover seedlings started from seed or rhizome clippings had a similar degree of spread in both smooth brome and Kentucky bluegrass pastures (Kim, 1996). Sheaffer et al., (2008) examined the establishment success of planting rhizomes and crown sprigs in existing grass swards. They found little difference in establishment when Kura clover was introduced as seed or as rhizomes; however, the size of rhizomes affected the vigor of establishing plants (Sheaffer et al., 2008). Seguin et al. (2001) found that Kura clover yields were higher when the existing sod was suppressed chemically rather than by physical suppression. Sanderson and Elwinger (1999) looked at the compatibility of white clover with various grass species and found more clover in mixtures with early maturing grasses. Similar results for white clover were found in Europe (Rochon et al., 2004). Trials in Alberta have examined various aspects of establishing new plantings of Kura – grass mixtures (Walker, 2002; Walker and King, 2009). Walker (2002) determined that Kura clover established better in mixtures when planted early in the spring as opposed to late summer. Kura clover contributed to harvested yield and increased forage quality in mixtures with Kentucky bluegrass (*Poa pratensis L.*) relative to pure grass stands. Establishment was poor in mixtures with timothy (*Phleum pretense L.*), orchard grass (*Dactylis glomerata L.*) or meadow bromegrass (*Bromus riparius Rehm.*). Walker and King (in review) found that Kura clover established and contributed nearly half of harvested biomass when orchard grass or meadow bromegrass were planted up to two months after the clover.

Little research has been published in western Canada, on using Kura clover in the renovation of existing grass pastures as a means of improving productivity, quality and longevity.

Our objective was to determine the effect on survival and growth of Kura clover seedlings transplanted into existing grass pastures. We compared survival and productivity of Kura clover seedlings planted into killed and live pasture swards, and examine subsequent effects of variable defoliation frequency.

Experimental Design and Methodology

The experiment was conducted in Central Alberta at three locations. Two sites were established in 2004, and the third in 2005 (53°25'N, 113°,33'W). Site one was located in a high traffic pasture which had been continuously stocked for thirty years. A rotational grazing system was introduced five years prior to the start of this experiment. In the year of planting, site one was dominated by Kentucky bluegrass, other species present included quackgrass (*Elytrigia repen*s L.) and smooth brome grass (*Bromus inermis L.*). Forbs comprised less than 5% of the available forage and were predominantly yarrow (*Achilla millifolia L.*) and white clover (*Trifolium repens L*). Soil condition at the time of planting was dry; a high proportion of sand was noted. Annual biomass production was estimated to be 2500kg/ha DM using the pasture stick method (Alberta Forage Council/Western Forage Beef Group).

The second site was seeded with an unknown pre-mixed blend of grass species fifteen years prior to the initiation of this trial, and was located in a mesic environment with loamy soil. This land had been managed using a rotational grazing system with stocking rates ranging from 0.4 – 0.6 AU/acre depending on the season. In the year of planting, site two was dominated by smooth bromegrass and quackgrass with a small proportion of creeping red fescue (*Festuca rubra*), there were no forb species present. Annual biomass production was estimated (using the pasture stick, AFC/WFBG) to be 7200 kg/ha DM.

Site three was a pure stand of meadow bromegrass (*Bromus riparius Rehm.)* cv. Fleet, planted in 2004, the year prior to the start of the experiment.

At each site, the experimental design was a split-plot randomized complete block design with twelve replications. Main plot treatments were killed (H) or live sward (NH). Sub plots consisted of three harvest frequencies, with harvests occurring at 4 or 6 wk intervals or a single season-end harvest. Individual plots measured 1.5 x 1.5 m, with measurements taken within the center 1 x 1 m to remove edge effects. A 50 cm gap was left between plots treated with and without herbicide to account for drift and to allow access to plots.

Eight weeks prior to field planting, Kura clover was sown into root trainers (2.5 cm square x 15.0 cm long) and placed in a greenhouse. Greenhouse temperature was 21/18° C (day/night) with a 16 hour photoperiod. Two weeks after planting, individual root trainers were thinned to one plant per pot. Beginning six weeks following planting, mid-April, 2004 and 2005, plants were hardened by moving them outside.

One week prior to field planting, glyphosate (2.5 L/ha) was applied to the herbicide treatment field plots. One day prior to planting, the entire area was mowed to 5 cm and the vegetative biomass removed. Four Kura clover seedlings were transplanted into each plot. A quadrat, measuring 1.5 x 1.5 m, with cross wires at 50 cm intervals, was positioned over each plot and the four intersecting points were sprayed with paint, indicating the location where each clover seedling was to be planted (Plate 6-1). Holes were made at each of these points using a punch constructed for this task. Due to the dry soil conditions in the Kentucky bluegrass site (site 1), creation of the holes was difficult. Consequently, the insertion sites were watered prior to digging. Leaves of each Kura clover seedling were counted at planting. Following planting, seedlings were watered twice a day for one week to facilitate establishment. One week after planting, clover seedlings were cut to the height of the surrounding vegetation (approximately 5 cm). Harvest treatments were initiated four and six weeks following this initial defoliation.

At each harvest, the number of Kura clover leaves per plant was counted. Each Kura clover plant was cut by hand to 5 cm, the remaining plot was also harvested to 5 cm and the biomass collected. Beginning in the second season (2005 and 2006), harvested material from four randomly selected blocks was further separated into Kura, grass and forb components. Harvested material was dried at 60°C to a constant dry weight.

Transplanted Kura clover plant survival was measured at the end of each season. The number of live plants in each plot was recorded. Percentage survival in season two was calculated using the number of live plants at the end of season one as the denominator, thus accounting for losses over-winter as well as over the second growing season.

Plots were harvested over two growing seasons (2004 and 2005 – Site 1 & 2, 2005 and 2006 – Site 3). Detailed species information on each plot was also collected mid-season of each year. In the spring of 2006, over-winter survival of Kura clover plants in sites one and two, were noted. Additionally, plots were visually assessed to determine if the effects of the herbicide were still evident.

Data were analyzed using the GLM procedure to perform repeated measures analysis of variance (SAS, 2003). Site, harvest frequency and herbicide treatment were considered fixed effects. Where appropriate, means were separated using a Tukey test. Significance was measured at $p<0.05$.

Results

Climatic Conditions

Temperatures at each of the three sites were slightly above the 30 year average in June, July and August in 2004 and 2005 (Figure 6-5). The winter of 2005 – 2006 was considerably warmer than the 30 year average. Growing season precipitation (May – September) at each site was much lower than the average. Rainfall was 115 mm and 161 mm below average in 2005 and 2006 respectively (Figure 6-6). In 2004, 60% of the growing season precipitation fell during July, 2.2 x the amount of precipitation normally experienced in July. Moisture was a limiting factor across two of the three growing seasons.

Site One – Kentucky bluegrass (KBG)

Kura Clover Survival

Transplanted seedling survival across all treatments, averaged 94% at the end of the first season (2004) and 95% at the end of season two (2005; Table 6-1). In plots where the existing sward was treated with glyphosate (H), dandelion, white clover and yarrow quickly re-colonized the area. Grass encroachment into the plots, was observed near the end of the first growing season.

Leaf Number and Daughter Plant Production

Kura clover seedlings averaged 11.0 leaves at the time of planting for all treatments (Table 6-2). At the end of the first season, plants where no herbicide (NH) was applied had significantly more leaves (31.1) than clover plants in H plots (11.5; $p<0.001$; Table 6-1). Kura clover leaf number in NH plots began the second growing season with an average of 36.6 leaves per plant. Leaf number per plant declined over the second growing season in all harvest treatments, with the decline being most pronounced in plots with a 4 wk harvest frequency (Table 6-2). Kura clover plants in H treatments maintained leaf number over the first growing season, with a slight increase in leaf number under the 6 wk harvest frequency (Table 6-2). Kura clover plants in H treatment had 50% more leaves at the start of the second season than season one. Leaf number declined over the second growing season in plots with a 4 wk harvest frequency. Daughter plants were observed in both H and NH treatments (Table 6-2). The smallest number of daughter plants occurred in the deferred treatment. A significantly greater number of ramets were present in NH plots.

Harvested Biomass

Kura clover

Kura clover yield with Kentucky bluegrass was significantly influenced by both herbicide application and harvest frequency ($p<0.0001$). NH treatments resulted in significantly larger Kura clover plants producing between 3 and 4.8 times the harvested biomass of Kura clover plants in H plots in season one (Figure 6-2a). Kura clover plants in NH-4wk produced significantly greater harvested biomass than all other treatments (Figure 6-2a). In the second season, individual Kura plants were smaller than in year one but were significantly larger in NH versus H plots at all defoliation frequencies (Figure 6-2a; $p<0.0001$).

Other species

Total harvested biomass of the other species in season one was greatest in H-4wk treatment (Figure 6-2b), significantly higher than all other treatments. In the second growing season, harvested yield of the other species was significantly higher in the H–4wk and H-deferred plots, 1.4 – 2.3x greater yield than all other treatments (Figure 6-2b). In both season one and season two, biomass in H treatment was observed to be predominantly yarrow, dandelion and white clover.

Third Season Observations

Visual assessment at the beginning of the third season revealed no observable differences between H and NH treatments. Over-winter survival of Kura clover was 100% for all treatments. Grass species were dominant in all plots and winter annual species were no longer present. A greater proportion of forbs, primarily yarrow and white clover, were present in all plots when compared to species composition in the first season (approximately 15%). Variation in harvest frequency effects remained apparent however, with deferred and 6 wk frequency treatments having a greater degree of biomass carry-over than plots with a 4 wk harvest frequency.

Site Two – Smooth bromegrass /mixed grass (SB)

Kura Clover Survival

Transplanted Kura clover survival was excellent (>90%) over the first season (Table 6-1). Survival of Kura clover plants in the second season was negatively affected at this site by small herbivores which selectively defoliated Kura clover as well as consumed clover roots. Control of rodent populations may have improved survival and productivity of Kura clover at this location.

Leaf Number and Daughter Plant Production

Seedling Kura clover plants had 10 – 16 leaves at the time of planting (Table 6-2). At the end of the first season Kura clover plants in NH plots were significantly larger than plants in H plots ($p<0.001$; Table 6-2). Seedling clover plants in the NH treatment increased their number of leaves over the growing season, ranging from 31 to 51 leaves per plant at the end of the first growing season. In the second production year in all treatments, plants had fewer leaves at the end of season than at the beginning. Loss in leaf number ranged from 75 to 93% of spring values (Table 6-2). Leaf number did not differ between herbicide or harvest frequency treatments at the end of season 2 except for H-deferred which had significantly lower leaf number. Only three daughter plants were identified. All were found in plots where glyphosate was not applied and with a single, end of season harvest (Table 6-2). Herbivory from small rodents may have prevented establishment of daughter plants.

Harvested Biomass

Kura clover

Individual Kura clover seedlings had significantly greatest yields in the NH – 6wk treatment in season one ($p<0.001$; Figure 6-3a). Lowest Kura clover yield was harvested in H-deferred and NH-deferred treatments (Figure 6-3a). In the second season, Kura clover plants in NH treatments had significantly higher harvested biomass than plants in H plots ($p<0.0001$; Figure 6-3a).

Other species

The total biomass yield of all other species over the first growing season was significantly influenced by the interaction of herbicide and harvest frequency (p<0.001). When harvested at 4 or 6 wk intervals, biomass was significantly greater when herbicide was previously applied to control the growth of the grass. In the first season total annual biomass (without clover) was 285 and 271 kg/m² in H-4wk and NH-4wk treatments respectively (Figure 6-3b).

Third Season Observations

Visual assessment at the beginning of the third season revealed no differences between treatments due to herbicide application. Grass species were dominant in all plots, however, plots that had been treated with glyphosate showed invasion by dandelion. Variation in response to harvest frequency remained apparent however, with deferred and 6 wk frequency treatments having more dense stands and a greater degree of biomass carry-over than plots with a 4 wk harvest frequency.

Site 3 – Meadow Bromegrass (MB)

Kura Clover Survival

Meadow bromegrass was established in 2004 on a highly fertile, black chernozemic soil that had previously been fallow for 2 years. Kura clover seedlings were introduced in 2005, the year following meadow bromegrass establishment. Kura clover transplant survival was >95% in the first season (Table 6-1). A large number of Kura clover plants did not successfully over-winter between the first and second growing seasons in the NH treatment. As a result no biomass data was taken from the NH treatment in the second production year. Although no weed species were present in the meadow brome sward at the time of planting, Canada thistle (*Circium arvense*) invaded plots where herbicide was applied. By the end of the second season, the majority of harvested biomass in these plots was thistle.

Leaf Number and Daughter Plant Production

Kura clover plants had significantly more leaves (3 – 5x) at the end of the first season when glyphosate was used to suppress the meadow bromegrass versus no chemical suppression (Table 6-2). Clover seedlings in herbicide plots, increased in size over the first growing season, as measured by leaf number. Plants in H-6wk and H-deferred treatments increased leaf number by 3 x, and number of

Kura clover leaves doubled in the H-4wk treatment. In contrast, Kura clover seedlings planted into a live stand of meadow bromegrass (NH), decreased in size over the first growing season, average number of leaves per plant dropped from 4 at planting to 2.5 at the end of season. There were no daughter plants observed in any treatment.

Biomass

Kura clover

Kura clover plants had significantly more biomass yield in H plots compared to NH clover plants in season one (Figure 6-4a). Within herbicide treatment, yield of Kura clover plants did not differ with harvest frequency (Figure 6-4a). In season two, the effect of harvest treatment was more pronounced; Kura clover plants in H-6wk plots had 2x greater biomass yield than plants in H-4wk or H-deferred treatments (Figure 6-4a).

Other species

Total annual biomass yield of the other species, was comprised of the sown meadow bromegrass and any weedy species that colonized the plots. NH plots were 100% grass and yielded significantly more biomass than H treatments. Biomass ranged from 385 (NH-4wk) to 426 g/m^2 (NH-6wk). When glyphosate was applied to suppress the grass sward prior to introduction of the Kura clover seedlings, plots were colonized firstly by annual weeds and later in the season by thistle and dandelion. In the second production year, 20% greater yield was obtained in H-6wk compared to H-4wk (Figure 6-4b).

Discussion

The results demonstrate that Kura clover seedlings were successfully transplanted into a 30+ year old pasture sward dominated by Kentucky bluegrass and a 15 year old smooth bromegrass/mixed grass pasture. In order for a transplanted seedling to survive, rapid development of roots must coincide with expansion of leaf area (Sanderson, et al., 2002; Hill and Mulcahy, 1995). In this study, applying herbicide to suppress the growth of companion grasses did not significantly affect transplant survival. Hill and Mulcahy (1995) found that root and rhizome biomass of Kura clover decreased as the density of grass surrounding the clover increased. Unlike Hill and Mulcahy (1995), we found that defoliation of both grass and clover plants increased survival of transplants at both site one and two. The exception to this was found at site one in the second season where more intensive harvesting (every 4 weeks) was detrimental to Kura clover survival. Kura clover plants and all other species in the Kentucky bluegrass and smooth brome/mixed grass dominated sites had lower yields in the second season than the first. This was most likely influenced by a hot, dry growing season which added additional stress to the Kura clover plants. At site 1, Kura clover plants also had a greater number of leaves but lower dry weight when harvested at 4 week intervals. This may have resulted from clover plants producing leaves below the cutting height as a response to defoliation frequency. Brummer and Moore (2000) noted that white clover and Kura clover have exceptional persistence under continuous grazing, maintaining high stand densities primarily because of a prostrate growth habit.

The effect of harvest frequency on survival of Kura clover seedlings was more pronounced at the smooth brome/mixed grass site where higher productivity of grasses increased the competitive pressure on the Kura clover. Under these conditions, Kura clover showed a greater ability to survive in a smooth brome/mixed grass sward, when the grass canopy was removed at 4 week intervals versus once per season. Casler and Carlson (1996) indicate that smooth bromegrass exhibits two distinct annual production peaks, growing points are elevated and consequently re-growth is initiated from meristems below-ground. This results in a delay in vegetative growth following defoliation (Casler and Carlton, 1996). In our study, the slow re-growth of smooth bromegrass under 4 week harvest frequency reduced the competitive pressure on the Kura clover allowing it to establish and grow. In contrast, meadow bromegrass has more rapid re-growth following defoliation, many growing points remain near the soil surface and are generally not removed with defoliation (Casler and Carlson, 1996). The fast rate of re-growth in meadow bromegrass is advantageous for maximizing biomass, but it also makes it more competitive in mixtures. This was demonstrated in this study, as Kura clover seedlings were unable to survive over winter unless the meadow bromegrass was killed with glyphosate.

Kura clover is an out-crossing species (Taylor and Smith, 1998). This results in a high degree of genetic variation within the species. Forde et al., (1989) state that clonal species display high levels of phenotypic plasticity, a characteristic which enables plants to withstand various defoliation regimes and environmental stresses. In our study, variation in leaf number and size was present among all treatments. The variation observed among Kura clover plants may be the result of phenotypic plasticity, inherent genetic variation, or a combination of the two. Turkington (1989) emphasized the importance of phenotypic plasticity in the success of white clover in grass pastures. Species which spread vegetatively, via stolons or rhizomes, experience a different microclimate with each ramet, the adjustment of the individual plant allows survival in patchy environments (Turkington, 1989).

Successful introduction of Kura clover into existing pasture swards is dependent on the ability of the parent plant to maintain itself and to spread through vegetative propagation of new individuals (Jones and Carter, 1989). White clover has been reported to spread 13 cm per year via stolon growth (Thornley et al., 1995). Sheaffer et al., (2008) noted that greater Kura clover establishment and spread occurred when rhizomes with 2 nodes were planted. In our study, very few daughter plants were identified by the end of the second season. Almost all daughter plants were found at site one.

Previous studies in Alberta found that Kura clover had higher contributions to biomass when sown with Kentucky bluegrass than meadow bromegrass (Walker, 2002). Kura clover sown in July in mixture with meadow bromegrass did not achieve a large enough size to successfully over winter (Walker, 2002). Similar to Walker (2002), the results of this study indicate that Kura clover seedlings are unable to successfully over-winter when planted into a live meadow bromegrass stand potentially due to the inability to achieve sufficient size to enable overwintering.

In this study, the herbicide treatment was intended to lessen the competitive effect of the grass on the seedling Kura clover. Individual Kura clover plants did not benefit from the removal of the grass component when planted into a Kentucky bluegrass or smooth brome/mixed grass pasture, potentially due to xerification of the microenvironment compounded by a hot, dry summer. Survival, leaf production and yield were greater when the grass stand was not killed. Higher survival in NH treatments may have resulted from shading, which slowed the loss of soil moisture. Furthermore, chemical suppression of the existing sward resulted in an influx of invader species which may have increased competitive pressure on the clover seedlings.

Seguin et al. (2001) in Quebec, Canada found that herbicidal suppression of sod improved Kura clover establishment. This is similar to our findings in Kura clover – meadow bromegrass mixtures. Kura clover seedlings did not successfully overwinter in live stands of meadow bromegrass due to high levels of competition. In plots where herbicide was used to suppress the meadow bromegrass, Kura clover plants did establish and survive, however, plants merely maintained themselves, with little increase in size recorded. In contrast to Seguin et al., (2001), for Kura clover planted into Kentucky bluegrass or smooth bromegrass pastures, frequent harvests sufficiently reduced the competition from the existing grasses to allow the establishment of seedling Kura clover. At these two sites, herbicidal sod suppression was unnecessary.

Conclusions

Successful establishment of Kura clover seedlings planted into existing pasture stands was influenced by the competitive nature of the dominant grass species. Kura demonstrated the ability to grow and spread in an established stand dominated by the less competitive Kentucky bluegrass. Chemical suppression of the sward had a negative effect on Kura clover survival in Kentucky bluegrass or smooth brome/mixed grass pastures. The removal of the grass component however, allowed colonization by early successional forb species, rapidly growing opportunists which are by nature highly effective competitors. In contrast, introducing Kura clover seedlings into an established stand of highly competitive meadow bromegrass was only successful when glyphosate was used to suppress the grass.

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Table 6-1. Kura clover survival in the first and second seasons following transplanting into an existing pasture sward with (H) or without herbicide (NH) application and harvested at 4 week, 6 week intervals or at the end of the season. Plots were located near Wetaskiwin, Alberta and at the Edmonton Research Station, University of Alberta, Edmonton, Alberta in the years 2004 – 2006.

Site 1		Kura Clover Survival (percentage)					
Kentucky bluegrass (KBG)	**Harvest Frequency**	**Herbicide (H) Season 1**	**Herbicide (H) Season 2**	**No Herbicide (NH) Season 1**	**No Herbicide (NH) Season 2**	**Average of Herbicide Treatments Season 1**	**Average of Herbicide Treatments Season 2**
	4 week	94	85	96	88	95 a	86 *b*
	6 week	92	98	96	100	94 b	99 *a*
	Deferred	90	98	98	98	94 b	98 *a*
	Average	92 b	93 B	97 a	95 A		
				ANOVA			
	F-test (HFxH)						
	SE (HFXH)					-	-
Site 2				**Kura Clover Survival (percentage)**			
Smooth bromegrass/ mixed grass (SB)	4 week	97	94	99	90	98	92
	6 week	97	92	100	89	99	90
	Deferred	94	90	100	87	97	89
	Average	96	92	100	89		
				ANOVA			
	F-Test (HFxH)						
	SE (HFxH)						
Site 3				**Kura Clover Survival (percentage)**			
Meadow bromegrass (MB)	4 week	98	94	94	-	96	
	6 week	99	96	96	-	97	
	Deferred	99	95	97	-	98	
	Average	99	97	96	-		
				ANOVA			
		***	***				

a –b indicate significant differences in the paired comparison of herbicide in season one; *A-B* indicate significant differences in the paired comparison of herbicide treatment in season two; means followed by the same letter are not significantly different p<0.05.

*, **, *** Significant at the 0.05, 0.01, 0.001 probability levels, respectively.

† NS, nonsignificant at the 0.05 probability level.

‡ Standard error of the difference of two least-squares means. Standard errors have been backtransformed where necessary

Table 6-2. Average leaf number per plant of Kura clover seedlings at planting, the end of the first season, at the start and end of the second production year. Clover seedlings were transplanted into an existing pasture sward with (H) or without herbicide (NH) application and harvested at 4 week, 6 week intervals or at the end of the season. Plots were located near Wetaskiwin, Alberta and at the Edmonton Research Station, University of Alberta, Edmonton, Alberta in the years 2004 – 2006.

		Kura Clover Leaf Number (mean no/plant)				
	Harvest	Season 1		Season 2		Total Daughter
	Frequency	At Planting	Year End	Spring	Year End	Plants
Site 1 - Kentucky bluegrass (KBG)						
Herbicide (H)	4 week	9.7 a	11.7 b	15.7 b	7.9 b	14
	6 week	10.7 a	14.0 b	15.9 b	13.3 b	5
	Deferred	10.3 a	8.9 b	18.7 b	16.2 b	1
No Herbicide (NH)	4 week	10.8 a	33.1 a	37.6 a	14.9 b	15
	6 week	11.2 a	31.3 a	35.7 a	28.7 a	22
	Deferred	13.4 a	29.0 a	36.4 a	27.6 a	3
			ANOVA			
	F-Test (HFxH)	NS	***	***	**	
	SE (HFxH)	-	1.7	2.0	2.3	
Site 2-Smooth bromegrass/ mixed grass (SB)						
Herbicide (H)	4 week	10.3 a	9.0 b	15.0 b	2.6 a	0
	6 week	10.5 a	10.3 b	18.6 b	2.7 a	0
	Deferred	11.2 a	5.1 b	13.1 b	0.3 b	0
No Herbicide (NH)	4 week	16.1 a	33.1 a	41.1 a	1.7 a	0
	6 week	12.7 a	50.5 a	37.9 a	1.4 a	0
	Deferred	10.3 a	31.3 a	45.7 a	3.1 a	3
			ANOVA			
	F-Test (HFxH)	NS	***	***	**	
	SE (HFxH)	-	0.45	0.22	0.39	
Site 3-Meadow bromegrass (MB)						
Herbicide (H)	4 week	4.4 a	10.0 a	7.3 b	5.6 ab	0
	6 week	4.4 a	13.1 a	11.2 a	7.7 a	0
	Deferred	4.6 a	14.1 a	9.1 ab	4.4 b	0
No Herbicide (NH)	4 week	4.0 a	2.1 b	-	-	-
	6 week	3.4 a	2.4 b	-	-	-
	Deferred	4.5 a	3.1 b	--	-	-
			ANOVA			
	F-Test (HFxH)	NS	***			
	SE (HFxH)	-	0.42			

a–b means followed by the same letter are not significantly different $p<0.05$.
*, **, *** Significant at the 0.05, 0.01, 0.001 probability levels, respectively.
† NS, nonsignificant at the 0.05 probability level.
‡ Standard error of the difference of two least-squares means. Standard errors have been backtransformed where necessary.

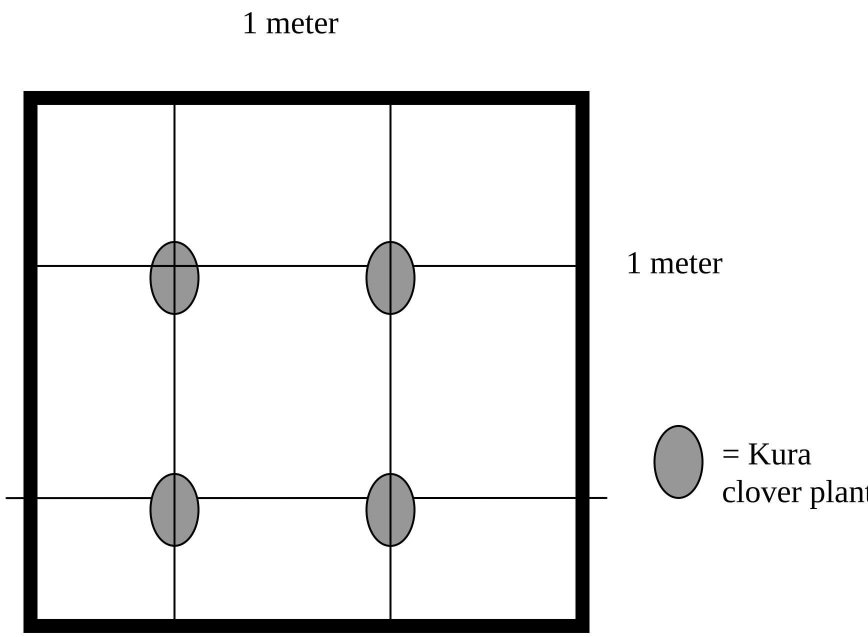
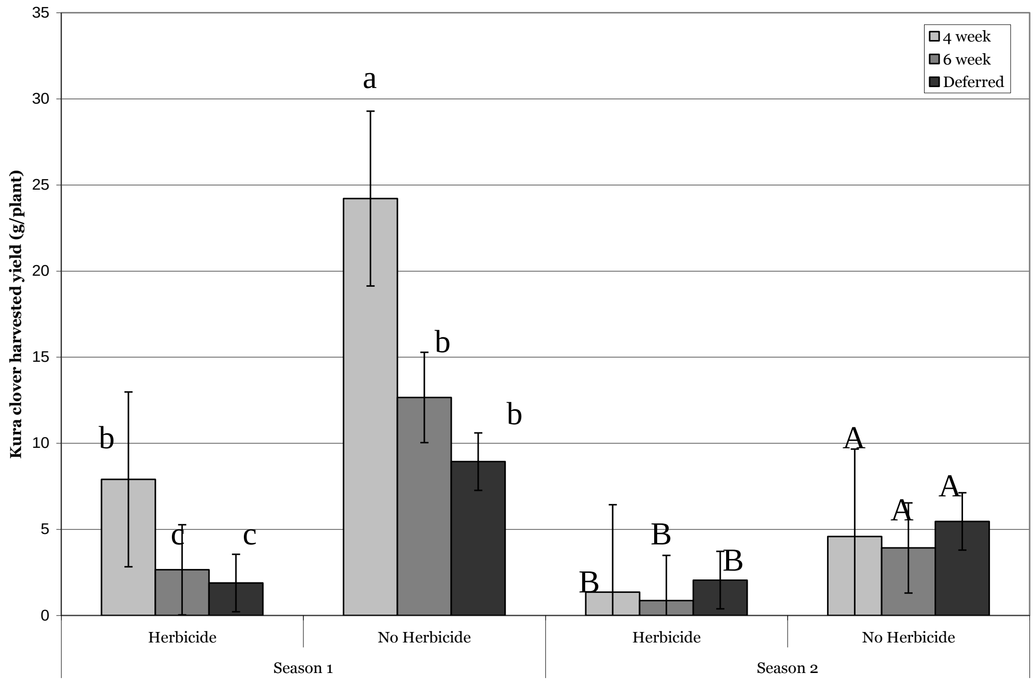


Figure 6-1. Diagram of the frame and transecting lines used as a pattern to plant Kura clover seedlings.

a)



b)

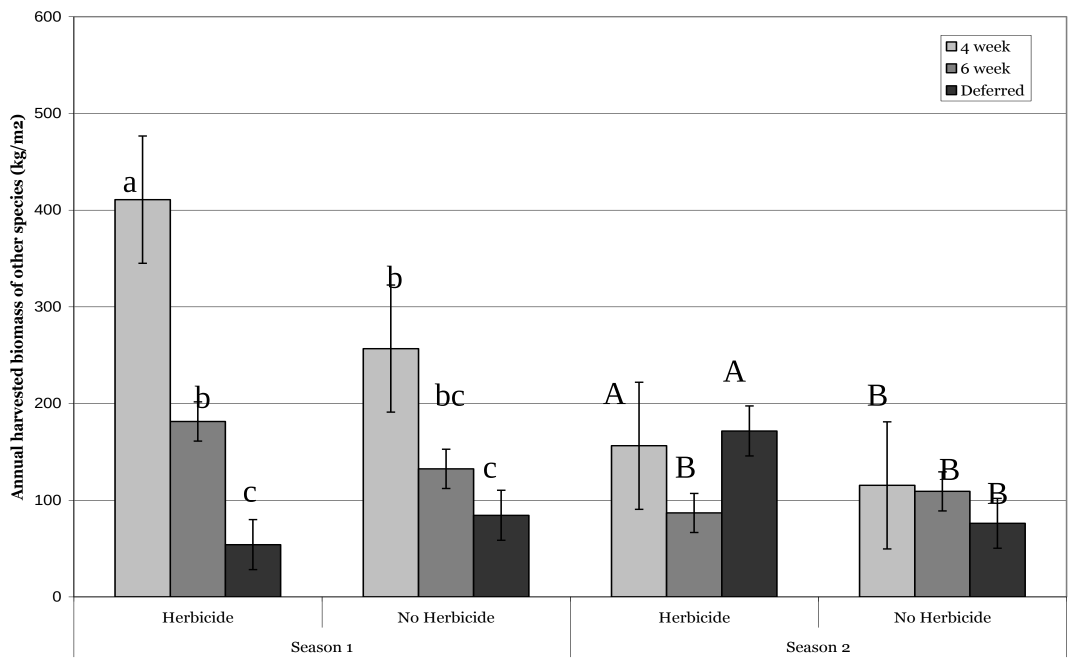


Figure 6-2. Total seasonal dry matter yield (g/plant) of a) Kura clover plants and b) total plot yield (g/m^2) in the first and second season when Kura clover plants were introduced into an existing Kentucky bluegrass pasture sward with (H) or without herbicide (NH) application, at three harvest frequencies. Plots were located near Wetaskiwin, Alberta, Canada. Data were collected in the year 2004-5.

Paired comparisons between herbicide and no herbicide treatments; Season 1: *a* – *b* indicate significant differences $p<0.0001$; Season 2: A-B indicates significant differences $p< 0.001$.

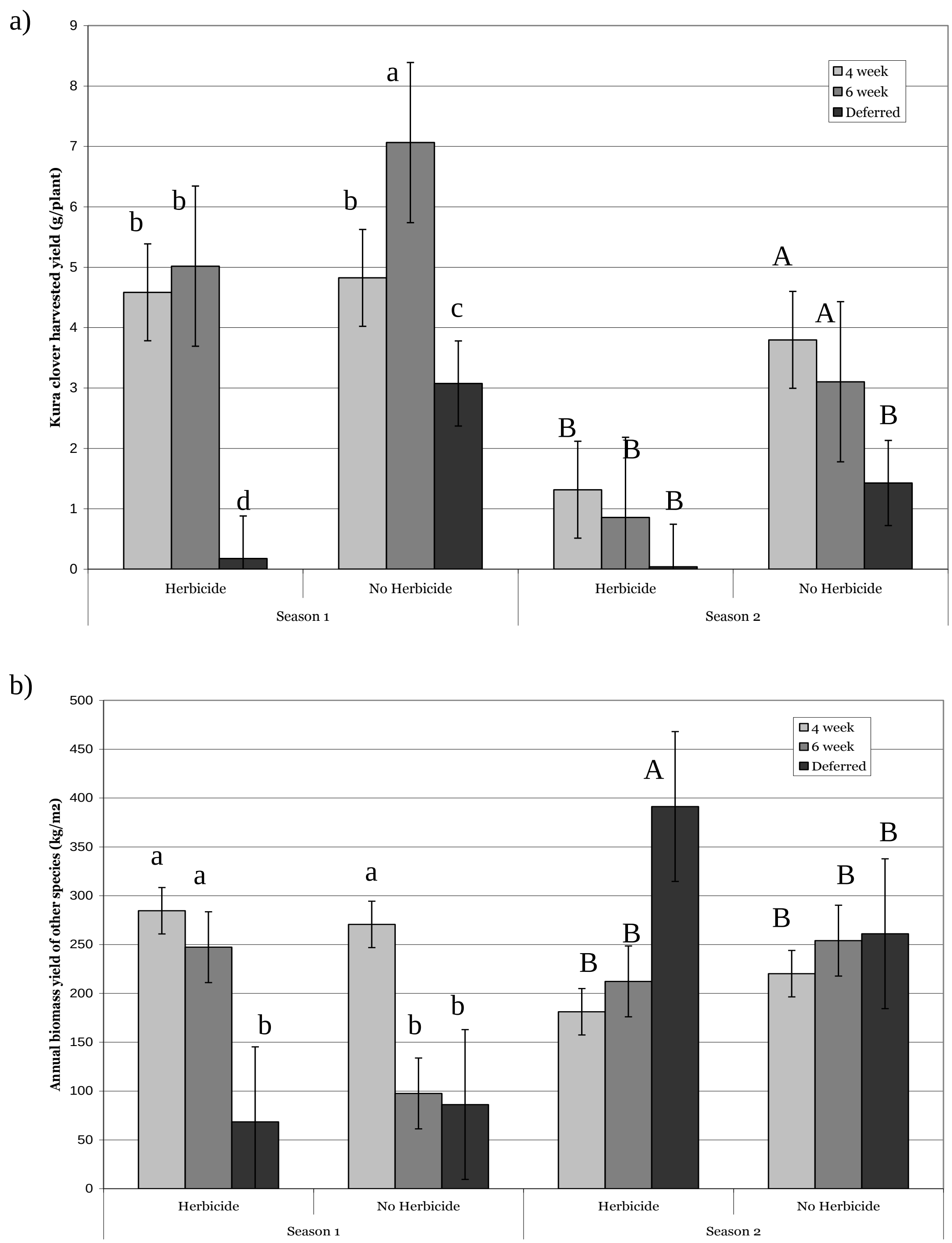


Figure 6-3. Total seasonal dry matter yield (g/plant) of a) Kura clover plants and b) total plot yield (g/m^2) in the first and second season when Kura clover plants were introduced into an existing mixed grass pasture sward with or without herbicide application, at three harvest frequencies. Plots were located near Wetaskiwin, Alberta, Canada. Data were collected in the year 2004-5.
Paired comparisons between herbicide and no herbicide treatments; Season one: *a – b* indicate significant differences p<0.0001; Season two: *A – B* indicate significant differences p<0.0001.

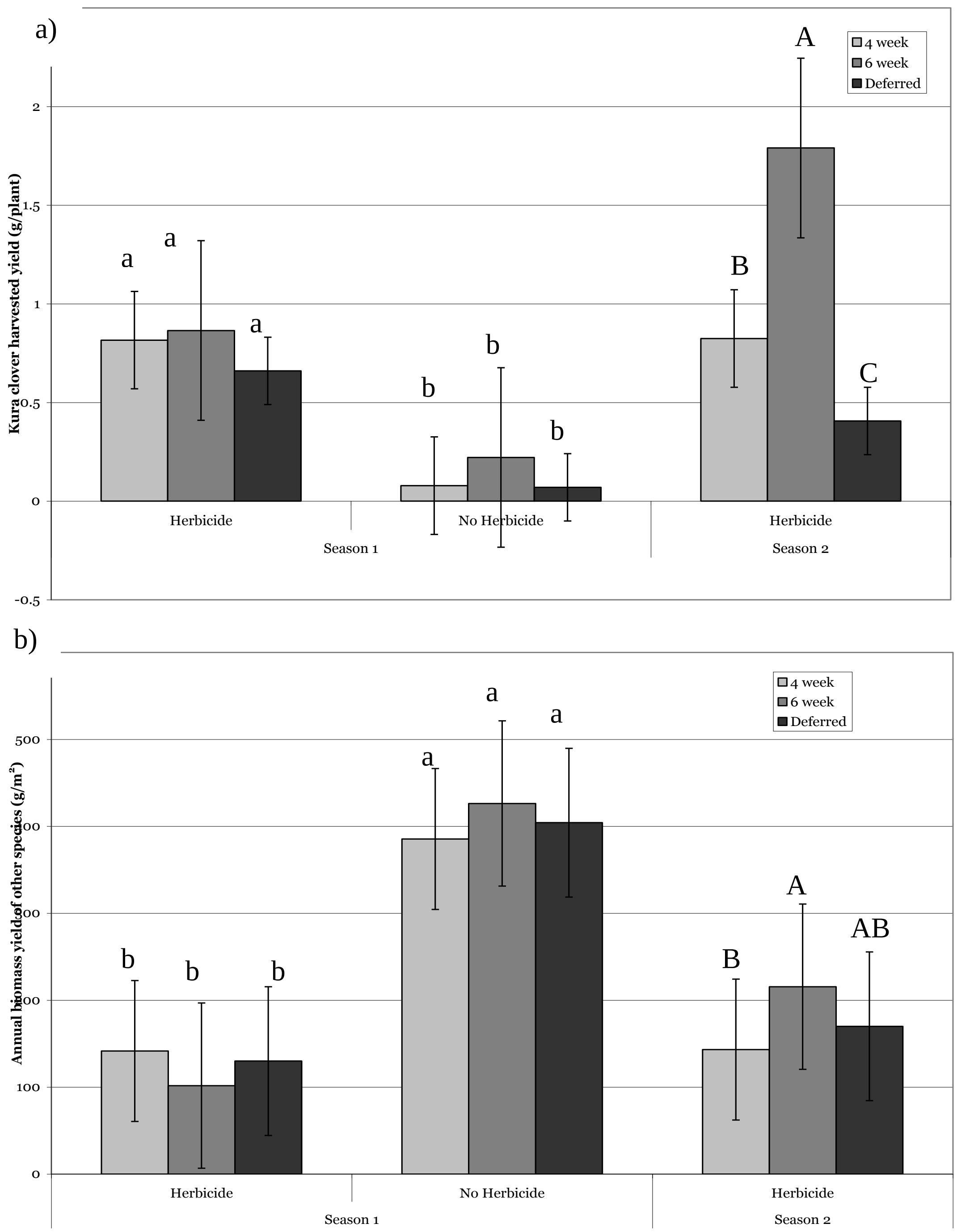
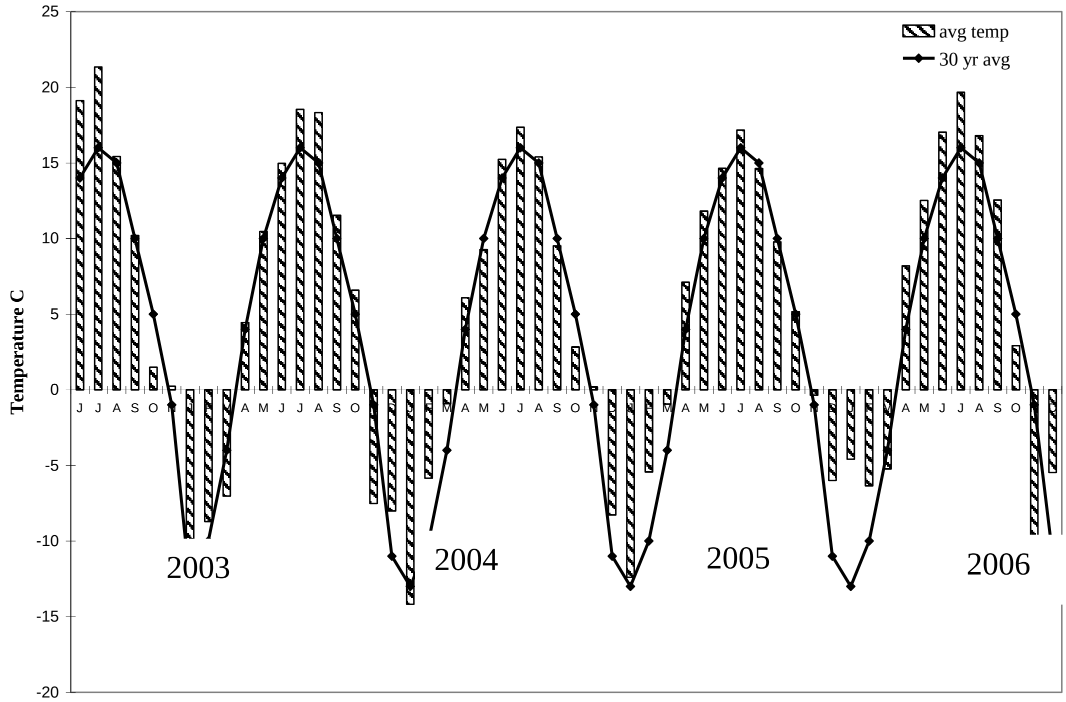


Figure 6-4. Total seasonal dry matter yield (g/plant) of a) Kura clover plants and b) total plot yield (g/m^2) in the first and second season when Kura clover plants were introduced into an existing meadow bromegrass sward with or without herbicide application, at three harvest frequencies. Plots were located near at the Edmonton Research Station, University of Alberta, Edmonton, Alberta. Data were collected in the year 2005-6.

Paired comparisons between herbicide and no herbicide treatments; Season 1: *a* – *b* indicate significant differences p<0.0001; Season 2: A-B indicates significant differences p< 0.001.



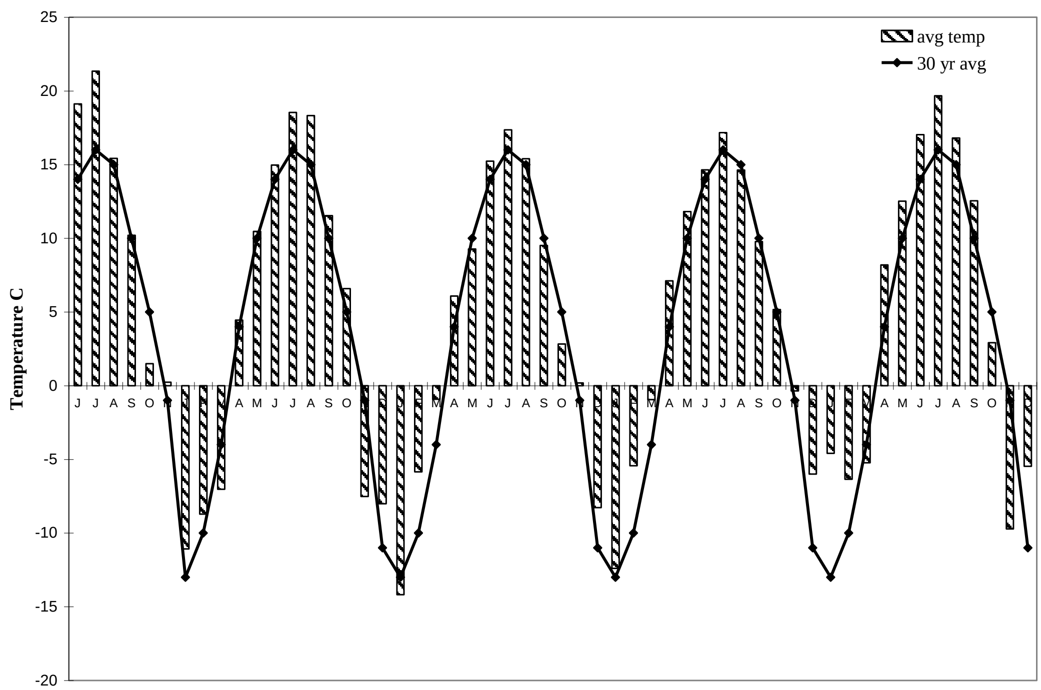


Figure 6-5. Average monthly temperature (°C), indicated by the columns, at University of Alberta, Edmonton Research Station, Edmonton Alberta, from January 2003 through December 2006, and the 30 year average monthly temperature (°C), indicated by the line, for Edmonton, Alberta.

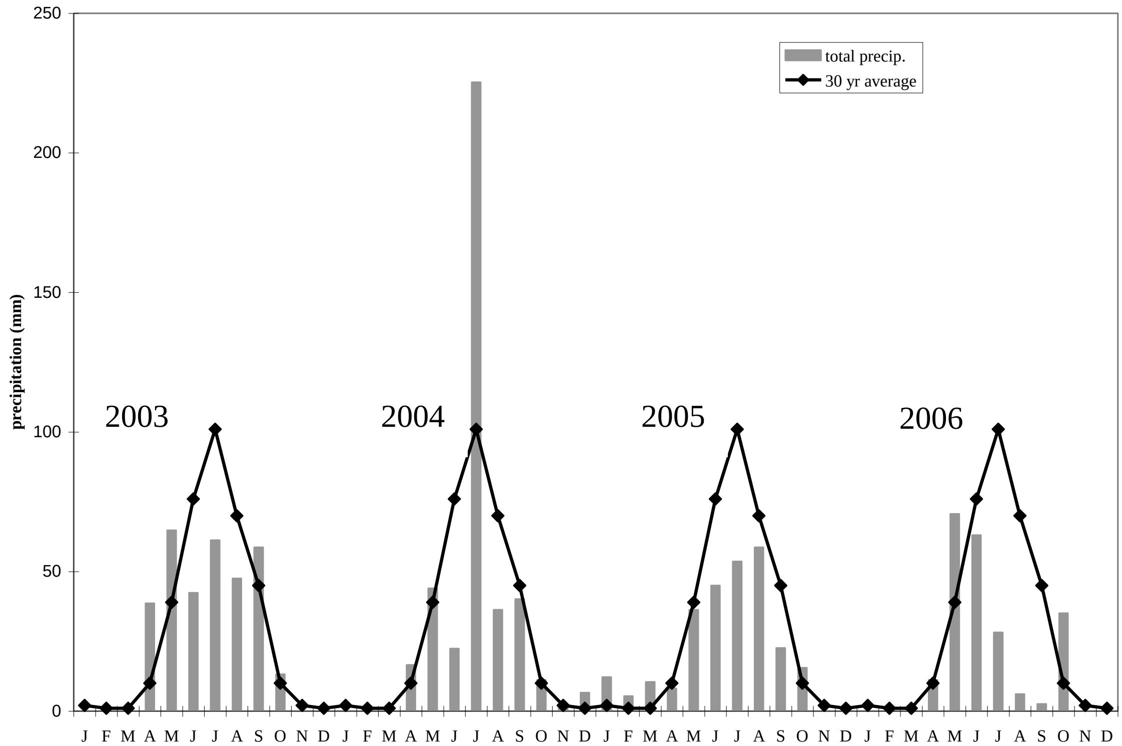


Figure 6-6. Average monthly precipitation (mm), indicated by the columns, at University of Alberta, Edmonton Research Station, Edmonton Alberta, from January 2003 through December 2006, and the 30 year average monthly precipitation (mm), indicated by the line, for Edmonton, Alberta.

Plate 6-1. a) Quadrat used for placing Kura clover seedlings in individual plots; b) plot at time of planting, chemical sod suppression c) transplanted Kura clover seedling.

Chapter 7

Synthesis

Overall Purpose:

To determine the potential for Kura clover seed production in Alberta and to develop Kura seed production guidelines.

To investigate methods of improving Kura clover establishment in newly planted mixtures with grasses. To identify the relative importance of above versus below ground competition in Kura clover – grass mixtures, and to examine Kura clover survival and spread in existing swards.

Summary of Major Findings:

Seed production

The potential of Kura clover as a pasture legume is well accepted in Australia, New Zealand and the United States. Kura clover has excellent persistence, yield and quality under a variety of conditions. However, a local source of seed is needed in order for this species to be adopted for wider use in Canada. Seed is currently imported from the United States at considerable cost. To our knowledge, there are no forage seed producers growing Kura clover in Alberta. The first two chapters of this thesis addressed management strategies to optimize seed yield and quality in an Alberta growing environment.

- Kura clover flowered and produced seed in central Alberta.
- Of the two cultivars tested, 'Endura' was found to have higher seed yields than 'Cossack'.
- Row spacing did not affect harvested seed yield.
- Seeding rate did significantly affect the amount of harvested seed. Low seeding rates (3 and 6 kg/ha) resulted in greater yields than higher seeding rates (9 and 12 kg/ha).

- Harvested seed yields reached as high as 700 kg/ha, much higher than recorded seed yields in the United States and New Zealand.
- Seed yield was greatest in the first production year.
- Although harvestable seed was produced in the second and third production years, yield was greatly reduced.
- Desiccation is recommended to aid harvest ease of Kura clover for seed.
- Additional processing of seed is required following combining to provide a marketable product.
- Establishing Kura clover under a cover crop is not recommended, especially if intended for seed production.
- Of the cover crops tested, corn and faba bean were the least detrimental to Kura clover growth and seed production. Seed yield under these cover crops was similar to those in the United States but lower than yields in Alberta.
- Barley and triticale provided the highest degree of competition to the Kura clover. Kura clover plants grown under barley or triticale had the lowest percentage ground cover and the lowest seed yield of all treatments.

Establishment in Mixtures

Once a local seed supply is available in Alberta, the greatest challenge will be to successfully establish Kura clover in mixtures. Several studies in the United States have addressed the parameters surrounding Kura clover establishment, however, in Alberta, establishment success in mixtures has been limited. Increasing our understanding of Kura clover's competitive ability, complimentary grass species, weed control and harvest management during the slow –growing seedling phase of Kura clover will determine the acceptance of Kura clover as a pasture legume in Alberta. In chapters four – six, we examined (i) the relative importance of above and below-ground competition between Kura clover and meadow bromegrass during seedling growth, (ii) the success of temporal planting separation of Kura clover and meadow bromegrass, or Kura clover and orchard grass mixtures, (iii) the effects of physical and chemical sod suppression and defoliation frequency on seedling growth of Kura clover in existing pastures.

(i)
- In a greenhouse study, Kura clover leaf number and leaf area were maximized when clover roots did not interact with meadow bromegrass roots.
- Vegetative biomass of Kura clover doubled when there was no root competition.
- Shoot competition from meadow bromegrass did not alter Kura clover leaf number, leaf area, or leaf biomass.
- Successful establishment of Kura clover appears to be dependent on reduction of root competition during the seedling phase.

(ii)
- In mixtures with either meadow bromegrass or orchard grass increasing the competitive ability of the slower growing clover was achieved by either reducing the grass seeding rate or by delaying seeding of the grass for up to two months.
- Delaying grass seeding beyond two months resulted in poor grass establishment.
- Sod-seeding grass into an established Kura clover stand is not recommended.

(iii)
- Survival and productivity of Kura clover seedlings was greatest when planted into Kentucky bluegrass pastures.
- Chemical suppression of the grass sward had a negative effect on Kura clover seedling survival in Kentucky bluegrass or smooth bromegrass/mixed grass pastures.
- Introducing Kura clover seedlings into an established stand of meadow bromegrass was only successful when glyphosate was used to suppress the grass.

Benefits to Agriculture in Alberta

Prior to the mid 1900's, grass pastures were successfully maintained by the presence of clovers and the return of manure. With the development of nitrogen fertilizer, agriculture experienced a shift towards more intensive farming systems. The movement toward intensification has created a reliance on heavily fertilized grass pastures. Rochon et al., (2004) note a decline in the use of legumes in European agriculture in favor of more intensive production. As the cost of fertilizer increases however, producers are starting to return to more sustainable, plant-based systems which are dependent on legume species.

Alberta industries, directly or indirectly dependent on forages, have identified ten key issues surrounding forage research and use (Collins, 2008). Included in the list was the lack of seed sources within Alberta, the high cost of nitrogen fertilizer reducing fertilization of grass pastures, range and pasture health and the greater reliance on a smaller forage landbase (Collins, 2008). Collins (2008) indicated that the primary concern was the lack of research, funding and extension programs with forages as the focal point.

<u>Seed Production</u>

Our research determined that seed production is possible in a central Alberta environment and provided valuable practical knowledge that can be used to establish Kura clover for seed. We addressed various methods for processing the harvested Kura clover seed to obtain a saleable product.

The cover crop experiment was designed to compare a range of cover crop species. Although several studies examine the effects of one or two annual cover species very few provide such a wide selection. Our comparison of time to canopy closure, and Kura clover ground cover provided us with a clearer picture of the extent of the competitive interaction between the cover crops and Kura clover. We were able to identify annual species that are a better alternative than the traditional barley cover crop.

Establishing a local seed source will reduce the cost of Kura clover seed in the market (currently at $16.00/kg) and provide a new high value opportunity crop for Alberta forage seed growers. Producing seed within Alberta will positively impact not only the seed grower, but also seed processors and cleaners, companies providing inoculant, and retail companies. Kura clover will also provide an alternative nectar crop for Alberta honey producers

Establishment in Mixtures

Understanding the above and below-ground dynamics between species is crucial to finding grass and legume species that are complimentary in mixtures. This work emphasizes the importance of below-ground interactions between Kura clover and meadow bromegrass.

Once established, the inclusion of Kura clover in mixtures has the potential to increase the sustainability of the sward (Peterson et al., 1994; Speer and Allinson, 1985; Sheaffer and Marten, 1991). Establishing these mixtures in Alberta has been challenging. Walker (2002) reported less than 5% clover in mixtures of Kura clover and meadow bromegrass, or Kura clover and orchard grass. This work shows that through temporal separation of planting, and reduction in the seeding rate of the grass, it is possible to establish a 50:50 grass – clover mixture. It is generally accepted that 30% legume can provide the nitrogen needs of a mixed stand. With the current cost of nitrogen fertilizer at $1.35/kg (34-0-0; Viterra, 2009), and an application rate of 50 – 100 kg/ha (actual N), the addition of legumes into a grass sward could reduce input costs by $200-400/ha/year. A comparison of the cost and potential value of nitrogen benefit of using Kura clover versus other legumes, demonstrates the economic benefits to incorporating this species into pastures (Table 7-1).

Table 7-1. Comparison of initial cost, and estimated value of nitrogen return in pastures of common legume species used in Alberta.

	Cost of seed ($/kg)	**Longevity**	**Cost/ha over time**	**Estimated fertilizer saving ($/ha)**
Alfalfa (AC Grazeland BR)	$8.80	5 years	$14.08	$1,000-2,000
Alsike Clover	$3.30	5 years	$6.60	$1,000-2,000
Kura Clover†	$16.80	25 years	$6.40	$5,000-10,000
Kura clover ‡	$ 8.40	25 years	$3.36	$5,000-10,000
Red ClovEser	$4.40	3 years	$14.70	$600-1,200
Birdsfoot Trefoil	$12.20	5 years	$19.52	$1,000-2,000
Sainfoin	$6.55	5 years	$13.10	$1,000-2,000
Cicer milkvetch	$8.75	10 years	$8.75	$2,000-4,000

* estimated cost/ha based on seeding rates recommended in mixed grass pastures in Alberta (Pickseed Canada, personal communication).

† based on current seed prices (imported from USA and New Zealand)
‡ projected cost, based on seed yields obtained in Alberta (Chapter 2)

Future research needs

Seed Production

Kura clover seed yield varied between growing seasons. It would be valuable to test Kura clover seed yield in southern Alberta, which has a longer growing season.

With the release of the new cultivar Kuratas in 2011, developed in Tasmania for improved seed yields (Hall and Hurst, 2008), determining seed and forage yield potentials in Alberta may provide additional cultivar choices.

Above and Below-Ground Growth

Quantifying the nature of above and below ground competition of Kura clover with other grass species will aid in determining optimal species mixtures.

The above and below ground responses of Kura clover under water, nutrient and defoliation stress would provide additional insight into establishing Kura clover in mixtures with grasses.

Kura Clover in Mixtures with Grasses

We have demonstrated that it is possible to establish Kura clover with high yielding grass species. It would be useful to follow the species composition of these mixtures over a longer period of time.

The positive impact on forage quality of adding Kura clover into meadow bromegrass and orchard grass stands should be measured to indicate potential for increase carrying capacity and the impact on animal gain.

While challenges remain, the major concerns preventing the adoption of Kura clover as a viable legume species for Alberta, were successfully addressed in this research.

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Appendix

Table A-1. Summary of treatment effects for the effects of cultivar (CV), row spacing (RS) and seeding rate (SR) on Kura clover seed yield (kg/ha).

	First Production Year Seed Yield	**Second Production Year** Seed Yield	**Third Production Year** Seed Yield
	Plots sown in 2002		
Cultivar	***		
Row Spacing	ns		
Seeding Rate	ns		
CV*RS	ns		
CV*SR	ns		
CV*SR*RS	ns		
	Plots sown in 2003		
Source			
Cultivar	***	***	***
Row Spacing	ns	ns	ns
Seeding Rate	***	***	**
CV*RS	ns	ns	ns
CV*SR	ns	**	ns
CV*RS*SR	ns	ns	ns
	Plots Sown in 2004		
Source			
Cultivar	***	**	
Row Spacing	ns	ns	
Seeding Rate	***	*	
CV*RS	ns	ns	
CV*SR	**	ns	
CV*RS*SR	ns	ns	

* $p<0.05$; ** $p< 0.001$; ***$p<0.0001$; ns – not significant

Table A-2. Summary of treatment effects for cover crop yield (kg/ha), percentage ground cover of Kura clover, and seed yield of Kura clover (kg/ha) in the first production year.

Source	**Cover crop Yield**	**Percentage Ground Cover**	**Kura Clover Seed Yield**
Cover crop species F test	***	***	***

* p<0.05; ** p< 0.001; ***p<0.0001; ns – not significant

Table A-3. Summary of treatment effects for above and below ground biomass (g) of Kura clover and meadow bromegrass, grown with or without above and/or below ground separation.

Source	**Kura clover above ground biomass**	**Kura clover below ground biomass**	**Meadow bromegrass above ground biomass**	**Meadow bromegrass below ground biomass**
Treatment	*	ns	*	ns

* p<0.05; ** p< 0.001; ***p<0.0001; ns – not significant

Table A-4. Summary of treatment effects for harvested yield (kg/ha) and species composition of Kura clover- meadow bromegrass mixtures

	Harvested yield				**Percentage Kura clover**			
Source	Establishment year	First Production year	Second production year	Third production year	Establishment year	First production year	Second production year	Third production year
Treatment	***	***	***	***	***	***	**	ns
Harvest	ns	ns	ns	ns	ns	ns	ns	ns
Treatment*Harvest	ns	ns	ns	ns	ns	ns	ns	ns

* p<0.05; ** p< 0.001; ***p<0.0001; ns – not significant

Table A-5. Summary of treatment effects for harvested yield (kg/ha) and species composition of Kura clover- orchard grass mixtures

	Harvested yield				Percentage Kura clover			
Source	Establishment year	First Production year	Second production year	Third production year	Establishment year	First production year	Second production year	Third production year
Treatment	***	ns	***	***	***	***	***	***
Harvest	ns	ns	ns	ns	ns	ns	*	*
Treatment*Harvest	ns	ns	ns	ns	ns	ns	***	***

* p<0.05; ** p< 0.001; ***p<0.0001; ns – not significant

Table A-6. Sources of variation for Kura clover leaf number, Kura clover harvested yield (g/plant), and harvested yield of other species (g/m2).

Site One	**Kura clover leaf number**				**Kura clover harvested yield**		**Meadow bromegrass harvested yield**	
Source	At planting	End of season	Spring	End of season	Season 1	Season2	Season1	Season 2
Herbicide (H)	***	***	***	***	***	***	***	***
Harvest Frequency (HF)	***	***	***	***	***	**	***	**
H*HF	**	**	**	**	***	***	***	***
Site Two								
Source	At planting	End of season	Spring	End of season	Season 1	Season2	Season1	Season 2
Herbicide (H)	***	***	**	**	***	***	***	*
Harvest Frequency (HF)	***	***	***	***	**	***	***	***
H*HF	**	**	**	**	**	**	***	**
Site Three								
Source	At planting	End of season	Spring	End of season	Season 1	Season2	Season1	Season 2
Herbicide (H)	***	***			***		***	
Harvest Frequency (HF)	**	**	ns	ns	**	**	ns	*
H*HF	**	**			**		ns	

* p<0.05; ** p< 0.001; ***p<0.0001; ns – not significant