University of Alberta

THE POTENTIAL OF KURA CLOVER *(Trifolium ambiguum)* AS A PASTURE LEGUME FOR CENTRAL ALBERTA

JENNIFER ANNE. WALKER ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science
in
Plant Science

Department of Agricultural. Food and Nutritional Sciences

Edmonton. Alberta
Spring 2002

0-612-69773-8

University of Alberta

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Name of Author: Jennifer Anne Walker

Title of Thesis: The potential of kura clover (*Trifolium ambiguum*) as a pasture legume for Central Alberta.

Degree: Master of Science

Year this Degree Granted: 2002

Jennifer Walker

R. R. #1
Wetaskiwin, Alberta
Canada
T9A 1W8

Date submitted: April 16, 2002

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled the potential for kura clover as a pasture legume for central Alberta, submitted by Jennifer A. Walker in partial fulfillment of the requirements for the degree of Master of Science.

Dr. J. R. King

Dr. E. Bork

Dr. J. Hoddinott

April 16, 2002

ABSTRACT

Kura clover (*Trifolium ambiguum*) is a persistent pasture legume that shows great potential for inclusion in pasture mixtures in Alberta. Two experiments measured kura growth under five harvest frequencies, and kura compatibility with four grass species at two seeding dates. Biomass and stand composition were measured for kura grown alone or in mixtures with Kentucky bluegrass (*Poa pratensis*), meadow brome (*Bromus biebersteini*), orchard grass (*Dactylis glomerata*) or timothy (*Phleum* pratense). Early seeding increased establishment success. Production year yields were maximized in kura-meadow brome mixtures. Clover percentage was highest when sown with Kentucky bluegrass. Forage quality of Kentucky bluegrass plots was significantly improved with the inclusion of kura. Yield and uniformity of distribution of kura in monoculture was optimized under a 4-cut harvest frequency. Etiolated regrowth of kura plants varied with planting date. Morphology of kura plants differed between plants sown in monoculture and kura plants sown with a companion grass species.

ACKNOWLEDGMENTS

I would like to thank my supervisor, Dr. Jane King, for seeing the potential of kura and for her patience, advise and encouragement throughout the time it has taken me to complete this project.

To Dr. Edward Bork and Dr. John Hoddinott, thank you for your participation in this project through your thoughtful suggestions and comments.

I would also like to express appreciation for technical support and lots of laughter to Shirley, Lynn, Carla, Gilbert, Jen, Su, Jing and Sue as well as the staff at the Edmonton Research Center.

Finally, to my husband Curtis and to my family, thank you for realizing that I could accomplish this and for telling me so.

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List of Abbreviations

ADF	acid detergent fiber
ADL	acid detergent lignin
Ca	calcium
cm	centimeter
CP	crude protein
Cu	copper
°C	degrees Celcius
d	day
estab.	establishment
Fe	iron
g	gram
ha	hectar
IVDMD	in vitro dry matter digestibility
K	kura clover
KBG	Kentucky bluegrass
kg	kilogram
lb	pound
m	meter
MB	meadow brome
Mg	magnesium
mm	millimeter
Mn	manganese
Mo	molybdenum
NDF	neutral detergent fiber
OG	orchard grass
T	timothy
t	tonne
yr	year
Zn	zinc

CHAPTER ONE

AN INTRODUCTION TO KURA CLOVER

Trifolium ambiguum. commonly known as kura. honey. pellet or Caucasian clover. is a perennial clover originating in the Caucasian region of Russia. In 1808. Marshal L. B. F. von Bieberstein made the original description of this species with a more precise definition given by Hossain in 1961 and again in 1970 by Zohary (Bryant. 1974). In its native habitat. *T. ambiguum* occurs over a range of ecological niches from poorly drained lowlands to meadows 3200 m into the Caucasus Mountains (Speer and Allinson. 1985). Although introduced into the United States in 1911. kura clover use in North America has been limited to apiaries (Sheaffer et al.. 1992).

Research on kura clover has recently been expanded due to interest in its potential as a pasture legume. Forage specialists have long sought a legume that would be persistent in grass swards under grazing. Legumes most often used. alfalfa (*Medicago* sativa). white clover (*Trifolium repens*). red clover (*Trifolium pratense*). are non-persistent under grazing and especially under environmental stresses such as heat or drought (Taylor and Smith. 1998). Alfalfa does not tolerate continuous grazing. white clover is not productive during hot summer months. and red clover does not maintain productive stands past the third season following establishment (Speer and Allinson. 1985). In native habitats kura clover has maintained a strong presence under a century of intensive grazing (Lucas et al.. 1997). Kura is adapted to mountain slopes. valleys and screes. and dry grassy steppes. It is held in high regard because of early season production and persistence (Bryant. 1974). The extensive rhizome and root development of kura clover suggests high potential for long-term productivity however. more research is needed to assess its suitability for use in grazing systems in Western Canada.

MORPHOLOGY

Roots and Rhizomes

Similar to other perennial clovers, kura has a crown system with a large taproot. When grown in Connecticut and Minnesota, kura seedlings showed that rhizomes begin to develop as early as three months after germination and within two years the rooting zone can reach over 1m in diameter and 60cm in depth (Kim, 1996; Speer and Allinson, 1985). During the first 2-3 years following seeding, kura plants develop a root system at the expense of aboveground biomass. Root: shoot ratios in kura clover at this time, are 1.5 – 3.0:1 while in white clover are 0.3 – 0.7:1 (Spencer et al., 1975). Strachan et al., (1994) determined that a 13 year old stand of kura clover in New Zealand, had over 20t/ha of root biomass. Such prolific and massive root systems provide resistance to drought, frost and other environmental extremes and contribute to the long-term productivity.

Stem and Leaves

Leaves of kura clover are trifoliate, with leaflets ranging from 1-8cm in length and from 0.5-5.0cm in width (Taylor and Smith, 1998). Leaflets are glaucus, and petioles are glabrous or slightly pubescent. V-shaped watermarks may or may not be present on leaflets (Taylor and Smith, 1998). Young plants have a single meristem at the crown with additional meristems developing as rhizomes become part of the primary crown (Genrich et al., 1998). Initial stems are procumbent and become ascending as development proceeds (Speer and Allinson, 1985).

Flowers

Kura clover exhibits an indeterminate growth pattern (Speer and Allinson, 1985). Flowering occurs in response to a long-day photoperiod. Flower heads are both lateral and terminal, with stems carrying several heads at varying heights (Speer and Allinson, 1985). Flowers are initially white turning to pink as they mature. Lower flowers deflex after seed is set (Taylor and Smith, 1998). Inflorescences of kura clover are capitate; heads up to 3.5cm long are borne on peduncles up to 10cm in length. The number of florets per head ranges from 33 – 172, varying from 9 – 16 mm in length (Speer and Allinson, 1985). Corollas are twice the length of the calyx, which encloses ovid to oblong pods (Taylor and Smith, 1985). Pods are enclosed in the calyx and contain 2 seeds. Seeds are oblong to reniform and vary in color from yellow to brown, with weight per hundred seeds ranging from 0.1218 – 0.2896 g

(Speer and Allinson. 1985). Kura plants are self – incompatible necessitating cross-pollination (Taylor. 1998).

Ploidy

Kura is one of the few species of clovers. that exhibits natural polyploidy (2x. 4x. and 6x). Evidence shows probable existence of north-Caucasian (Euro-Siberic) and south-Caucasian (Iranian-Turanian) forms of kura clover (Bryant. 1974). Bobrov (1950) described two separate habitats and two separate forms of kura in its native environments. Vacek and Ded (1956) also referred to upland and lowland varieties of kura clover. Hossain (1961) separated the ecotypes into two species – *Trifolium ambiguum* and *Trifolium majus* based on leaflet shape and size as well as the presence or absence of glabrescence.

Kannenberg and Elliot (1962) set out to determine the morphological and physiological differences between the ploidys for the purpose of identification and selection of desirable characteristics by comparing several different traits in both greenhouse and field conditions. Mean leaf size differed significantly between the environments with the 6x plants' leaves in the greenhouse only 60% the size of those observed in the field. and 4x plants 70% of the size recorded in the field (Kannenberg and Elliot. 1962). Pistil length of the 2x plants was consistently shorter than the 4x or the 6x plants. measuring < 4.5 mm. Because the environment does not affect this characteristic it is useful for identifying this group. Additionally. the 2x plants flowered earlier and more profusely that the 4x and 6x ploidies. Irregular meioses of the plants. different ploidy levels as well as incompatibility affected the fertility of the various groups. Significant differences were noted however. when comparing the average weight/100 seeds. Seed weight was greater at higher levels of ploidy. 2x < 4x <6x (Kannenberg and Elliot. 1962). In general. the 6x plants demonstrated a higher degree of vigor and were ranked the best of the three for desirable agronomic traits. Kannenberg and Elliot (1962) noted that kura is unique in that induced polyploids that are generally reported to have fewer flowers and rhizomes but in kura these characteristics increased directly with ploidy.

Variations between ploidies formed the basis for initial plant breeding studies on kura in Australia. Early reconnaissance testing was carried out at 1590 m in the Snowy Mountains. where a diploid and a tetraploid form performed best out of twenty legumes tested. These two forms were subsequently selected over several years for nodulation. flowering and seed production. Ten introductions were then field tested against white and strawberry clovers (*Trifolium fragiferum*). The best performing survivors were polycrossed and developed to form the basis of the cultivars Summit and Treeline (Bryant. 1974).

ADAPTATION

Kura clover is indigenous to Caucasian Russia (Georgia. Armenia and Azerbayd). the Crimea. the Ukraine. eastern Turkey and northern Iran (Speer and Allinson. 1985). Kura has been introduced for agricultural use into Czechoslovakia. New Zealand. Australia. the United States and Canada. This clover has been found naturally over a wide gradient of habitats. It survives well in dry regions such as the montane. subalpine and alpine zones of Australia (Taylor and Smith. 1998).

Sketchy reports exist about the natural habitat of kura clover. although. they imply that it shows preference for non-calcarous. clay and clay-loam soils of the steppes and meadow soils of the mid-latitude mountains. It appears to escape the extremes of low moisture and temperature by becoming dormant (Bryant. 1974). Specimens have been collected from both dry and moist sites but well-drained dry slopes of volcanic origin are most frequently mentioned as collection sites throughout its origin in Turkey and Iran (Bryant. 1974). In the Snowy Mountains of Australia. introduced lines persist in alpine humus soils at 1580 m. eroded mineral alpine humus soil over weathered granodiorite at 2020m. and weathered phyelites and schists at 2140m (Bryant. 1974).

Kura clover demonstrates greater productivity in cool rather than warm climates. During drought. it becomes dormant and remains so until adequate moisture is available (Taylor and Smith. 1998). Variations between cultivars appear in the ability of kura clover to withstand water-logging. Field survival of 80% has been observed after inundation in early spring for 40 days of water up to 15 cm deep (Bryant. 1974). The ability of kura clover to survive on poorly drained soils surpasses that of red or white clover (Speer and Allinson. 1985). Kura clover has been found to be productive on soils of low pH or with phosphorous deficiencies (Taylor and Smith. 1998). Studies examining productivity of kura clover on a soil testing P=16 lb./acre and pH 5.7. showed that kura out yielded white clover by 2.6 t dry matter/ha/year (Daly and Mason. 1987).

While kura clover will grow and persist on acidic soils. dry matter yields respond positively to additions of lime. Poor growth on low pH soils could be due to inadequate uptake of Ca and Mo (Taylor and Smith. 1998). Because of the extensive root and rhizome system. kura clover may be more productive and persistent than other legumes on infertile soils.

Kura has been collected from areas as high in elevation as 3170 m in eastern Turkey where 4-5 months of snow follows late autumn drought. In Armenia. kura naturally occurs in alpine and sub-alpine areas with frost-free periods of 60-90 days (i.e. 4 month effective growing season)(Bryant. 1974). Bergersen et al (1963) found that nodular tissue of kura successfully over-wintered at 2140 m in the Snowy mountains. This enabled new nitrogen fixing material to be formed at least 2 weeks earlier than new roots were developed. Such a mechanism allows for more efficient use of a short-growing season (Bryant. 1974).

ESTABLISHMENT

Perhaps the greatest drawback to the adoption of kura clover for use in pasture. is its slow rate of establishment. High percentages of hard seed. up to 75%. necessitate mechanical or chemical scarification of seed prior to seeding (Speer and Allinson. 1985). Additionally. successful establishment is highly dependent on method of sowing. Moorhead et al.. (1994) compared establishment of kura clover in New Zealand. on a desiccated sod using strip seeding. sod seeding and broadcasting techniques. Strip seeding exhibited the highest percentage of established plants closely followed by sod seeding methods on soils with either high or low fertility. Broadcasting onto desiccated sod showed very poor establishment (9%) and subsequent plants were small. yellowed and without nodulation (Moorhead et al.. 1994). By using the strip seeding method. rhizome development was earlier. root production was greater and faster vegetative spread was encouraged (Moorhead et al.. 1994). Broadcasting is thought to have poor results due to desiccation of germinating seed and rhizobium resulting from remaining exposed on the surface of the sod (Moorhead et al.. 1994).

In Minnesota. a field experiment was designed to evaluate the effects of sod suppression. planting method and legume species on establishment of legumes into existing grass pastures (Cuomo et al.. 2001). Treatments consisted of whole plot suppression using glyphosate or no suppression. no-till drilling. broadcast seed. broadcast seed and harrow or broadcast seed and light disking. Legume species studied included alfalfa. red clover. kura clover and birdsfoot trefoil (*Lotus corniculatas*) Cuomo et al.. (2001) found that suppression of the existing sod was intrinsic to the ability of legumes to establish. If plants emerged and grew in the seedling year the resulting stand in the year following planting was satisfactory for all species regardless of planting method. Kura clover demonstrated poorer establishment than the other legume species due to the limited top growth and greater susceptibility to competition. However, the kura plants that did establish themselves spread and produced secondary crowns. Cuomo et al., (2001) concluded that if kura has the ability to spread following establishment, the initial poor quality of stands is not as debilitating a factor as it would be for other legume species.

The effect of temperature on germination of kura clover was studied by Bryant (1974), who found that significant germination occurred at constant temperatures of 4°C. Germination exceeded 50% after 10 days at 7°C and reached a maximum germination percentage at 15°C (Bryant, 1974). Pre-germination cold treatments did not enhance germination of the clover but rather delayed the germination process. This is perhaps indicative of an endogenous mechanism to protect plants from false breaks of dormancy in the spring (Bryant, 1974).

It has been demonstrated that seedling establishment is directly related to nodulation (Patrick and Lowther, 1995). To ensure effective nodulation, Patrick and Lowther (1995) suggested increasing the inoculation rate well above that currently recommended to significantly improve the establishment of kura clover. Early work with kura in the United States showed that nodules were formed sparingly and that a large percentage of those formed were ineffective at fixing nitrogen (Erdman and Means, 1956). Erdman and Means (1956) isolated nodules from *T. ochroleucon*, *T. spadiceum* and *T. ambiguum* from a region in Turkey where kura clover is known to have originated. In addition to removing nodules from these plants, soil samples were taken and rhizobia isolates from both the soil and nodules were cultivated. Eight known strains of rhizobia were identified from the established plants and 19 new strains were found both in the soil and on the plants. Kura seeds were inoculated with each and sown in a greenhouse (Erdman and Means, 1956). Of the 27 rhizobia strains, 21 produced effective nodules and gave significantly higher yields of vegetative biomass when compared to the uninoculated control and the ineffective strains. Erdman and Means (1956) concluded that kura clover is not dependent on one specific strain of rhizobia and that successful inoculation causes significant dry matter weight increases. Evans and Jones (1966) also documented the benefits of nodulation. They found that the number of nodules and weight of kura plants were significantly increased by inoculation especially in 4x and 6x varieties. Yield of grass:clover mixtures was not affected at first harvest but increased at the subsequent harvests when compared with un-innoculated swards. Evans and Jones (1966) submit that the biomass of grass was increased when sown with kura clover even when the clover exhibited poor germination. This trend was due to the release of nitrogen from the legume roots following harvests.

Parker and Allen (1952) tested the effectiveness of nodulation using 87 rhizobial strains isolated from clover plants as well as from soils. Their findings contradict those of Evans and Jones (1966) as well as Erdman and Means (1956). Evidence of nodules on kura clover was sparse and no evidence was found to support the premise that kura benefited from successful inoculation (Parker and Allen, 1952). Parker and Allen (1952) suggest that nodulation is dependent primarily on the plant rather than the rhizobial strain. The plant, as the dominant agent in the relationship either lacks or contains the factors that preclude a plant-rhizobial complex.

Hely (1957) examined several rhizobial strains and host lines to determine which effective nodulated combinations occurred. The number of nodules formed appeared to be predominantly a function of host without any effect from bacterial strain. Furthermore, a larger number of infections was found to be associated with increases in ploidy (Hely, 1957). Turkish strains of bacteria nodulated earlier, more frequently and effectively when compared with Australian and New Zealand strains, as measured by the weight of vegetative material. Plants thought to be resistant to nodulation belong to a sector of the host population that becomes susceptible to infection at a later stage of development (Hely, 1957). Hely (1957) proposed that kura may be similar to red clover in that infection does not occur randomly but is restricted to discrete foci, each of which is available to infection over a limited period. Kura clover may have foci fewer in number and open to bacterial infection for a short period of time than other clover species (Hely, 1957). In further studies, Hely (1963) found considerable variation within the host lines. Plants with highly effective nodulation were identified only with early nodulation while late nodulating plants demonstrated nitrogen fixation but not effective levels of symbiosis in addition to a short season of fixation (Hely, 1963). Crossing of early nodulating varieties with late nodulating varieties revealed that inheritance of time of nodulation is quantitative and polygenic having a non-additive tendency toward earliness (Hely, 1972).

Hill and Luck (1991) compared the effect that temperature had on 10 legume species, grouping the species by base germination temperature. Alfalfa was found to have the lowest base temperature at <4°C, clovers and birdsfoot trefoil germinated at a minimum between 4-6°C and cicer milkvetch, crownvetch and big trefoil had a base germination temperature of >6°C (Hill and Luck, 1991). However, none of the species germinated well when the average daily temperature fell between 5 - 12°C. Cicer milkvetch (*Astragalus cicer*) and crownvetch

(*Coronilla varia*) demonstrated the greatest rate of cotyledon expansion followed by kura clover. birdsfoot trefoil. white clover and lespedeza (*Sericia lespedeza*) (Hill and Luck. 1991). The clovers (white. red. strawberry and kura) had higher root:shoot ratios and total dry matter yield at day/night temperatures of 15/10°C than the other 6 legumes. When grown at 24/20°C or 20/15°C day/night temperature regimes all 10 legumes had similar dry matter yield (Hill and Luck. 1991). Hill and Luck (1991) concluded that seedlings with cotyledons adapted for photosynthesis. developed true leaves more slowly than those with less well-adapted cotyledons. This strategy of rapid expansion of cotyledons but delay in leaf production may be disadvantageous for seedlings competing in mixed stands or against weeds.

Early in the establishment period. kura plants are susceptible to damage by frost. Caradus (1994) studied the frost tolerance of leaves and stems of 13 clover species. Plants were subjected to the frost treatments at 5 weeks following seeding. Soil temperature was held constant at 5°C and vegetative growth was held at -4. -8. -12 or -16 °C for 6 hours and then returned to 12°C. Kura had the lowest green leaf dry weight following the -4°C frost treatment. All of the clovers were negatively affected by the lower temperature treatments. Caradus (1994) concluded that the small plant size and poor vigor negatively affected the frost tolerance of kura clover.

Seguin et al.. (1999) compared the effects of an oat companion crop harvested for forage. birdsfoot trefoil companion crop seeded with or without pre-plant herbicide and solo seeding of kura clover with or without pre-plant herbicide on the forage and seed yields of kura clover. Kura yields in the establishment year ranged from 0 – 370 lb/acre for the various treatments. The use of pre-plant herbicides increased yields in only one of three years of the trial. however. as no herbicide is registered for use on kura clover the lower yields may be attributed to herbicidal injury to the plants (Seguin et al.. 1999). The greatest forage yield as well as the most weed-free yield. was obtained when kura is seeded with an oat companion crop. In the year following seeding. forage yield of kura ranged from 250 – 4470 lb/acre. maximized in the solo seeded plots. The kura stands developed rapidly in the year following seeding and the plant densities observed in the spring were not related to forage yields (Seguin et al.. 1999). Seed yield for the year following seeding was greatest for the solo seeded plots that were treated with a pre-plant herbicide. Under these conditions the kura seed yield was 510 lb/acre. The results of the study suggest that both forage and seed yield of kura clover can be maximized by using a pre-plant herbicide and seeding kura without a companion crop (Seguin et al., 1999).

As previously stated, the response of kura to inoculation is variable and nitrogen fixation is generally low. Seguin et al., (2001) examined the effects of nitrogen fertilization on the nodulation and growth of kura clover in the establishment year. The application of nitrogen fertilizer positively affected herbage yields and increased root mass in the year of seeding. Rhizome development occurred more slowly than in other studies, initial observation of rhizomes occurred 100 days after planting versus 65 days after planting as reported by Genrich et al., (1998). Harvested yields in the year following seeding were increased by inoculation in the previous year, thus Seguin et al., (2001) concluded that inoculating kura clover benefits the stand beginning in the year following seeding.

Companion grasses in swards with kura also significantly affect the establishment of the clover. Greenhouse trials show that kura clover is sensitive to sowing density of companion grasses (Taylor and Smith, 1998). Hill and Hoveland (1993) studied how the presence of tall fescue affected legume performance when grown in pots at 2:1 and 1:2 densities. In addition to the competition study, Hill and Hoveland (1993) included observations of response to drought and cutting frequency of kura clover, birdsfoot trefoil, and white clover. Performance of the legumes was negatively affected by the presence of tall fescue. All of the legumes exhibited lower leaf water potentials than did the tall fescue when placed under moisture stress. Kura roots did not branch and spread when seeded with the fescue as compared to pure stands. However, the dry matter yield of the trefoil and the white clover decreased more than the dry matter of the kura in response to moisture stress or cutting frequency (Hill and Hoveland, 1993). Because this trial was performed in pots, restricted rooting depth and volume likely accentuated the differences and limited the flexibility of the plant responses to the imposed treatments, thus impeding accurate interpretation an application of the results.

Hill and Mulcahy (1995) examined the effect of various grass:legume ratios on the ability of kura clover to establish and grow. Kura was sown with *Festuca arundinacea* and *Phalaris aquatica* at grass: legume densities of 1:1, 2:1, 4:1 and 8:1. In addition, plants were harvested at 15 weeks and 31 weeks following sowing or beginning 15 weeks after sowing and following every 4 weeks until the 31-week after sowing. Hill and Mulcahy (1995) also examined the effect that drought had on the establishment of kura clover grown in mixtures with grasses. The number of leaves and shoots expressed by kura clover was greater at low grass densities. Rhizome number was also lower under frequent defoliation in the establishment period and under high levels of fertility when the grass vigor was high. Drought had no permanent effect on the growth rate of the clover causing only temporary reductions in vegetative biomass (Hill and Mulcahy. 1995). Hill and Mulcahy (1995) concluded that root and rhizome development of kura clover is crucial during the establishment period and enhanced when the density of companion grasses is low. The combination of root competition limiting root growth and the high root:shoot ratio of young kura stands results in low competitive ability for capture of light. Hill and Mulcahy (1995) hypothesized that initially establishing pure stands of kura and then direct drilling the desired grass component may improve the ability of kura clover to compete against the aggressive nature of the companion grasses. The competition to establishing grasses would remain minimal however. because the clover is preferentially partitioning carbohydrates towards root development The sensitivity of kura to competition is consistent with studies in Minnesota which demonstrated that when seeded with grasses kura comprised only 30% of the sward. Timothy (*Phleum pratemse*) and Kentucky bluegrass (*Poa* pratensis) were the two exceptions to this. Seeded with kura. these grasses were less than 50% of the mixture when managed for a single end of season harvest (Jeranyama et al.. 2001). When kura was grown with Kentucky bluegrass. more total biomass was harvested. and a higher proportion of clover was recorded than when kura was grown in mixtures with either smooth brome (*Bromus inermis*) or orchard grass (*Dactylis glomerata*) (Kim. 1996).

NITROGEN FIXATION

It is important to quantify the amount of nitrogen fixation by legumes for the purpose of nitrogen budgeting. Seguin et al.. (2000) studied the nitrogen fixation of birdsfoot trefoil and kura clover. evaluating forage yields. atmospheric derived nitrogen and fixed nitrogen at four harvests at two sites in Minnesota. For each of the species. three year old stands had higher forage yields than two year old stands. Variation between location of the tests resulted in different trends for phytomass accumulation over the growing season. The percentage of nitrogen from the atmosphere was nearly constant over the growing season for kura clover. averaging 57%. Nitrogen fixation for kura varied with site and showed a decreasing trend from 37. 32. 20 and 11 % of the total season nitrogen fixed at the first. second. third and fourth harvest respectively (Seguin et al., 2000). Kura clover showed higher levels of nitrogen fixation than birdsfoot trefoil at one site, and similar levels of fixation were found at the other site. Seguin et al., (2000) concluded that while both species showed low levels of nitrogen fixation, kura clover fixed greater annual total nitrogen and showed a more stable nitrogen fixation when compared to birdsfoot trefoil.

Zemenchik et al., (2001) noted the value of legumes in forage mixtures for improving yield via biological fixation of nitrogen. Kura clover and birdsfoot trefoil were compared when grown in mixtures with Kentucky bluegrass, orchard grass or smooth brome to determine the fertilizer replacement value of the legume. In the year following seeding, nitrogen fertilizer was split applied to pure grass stands sown at the full recommended seeding rate, at six rates ranging from 0 – 336 kg/ha nitrogen. The three grass species had also been established at half the recommended seeding rate, in mixtures with either kura or birdsfoot trefoil and did not receive any chemical fertilization (Zemenchik et al., 2001). In the first production year kura accounted for 29% of the dry matter yield when sown with smooth brome grass. Over the second and third production years however, kura increased to 62% of the dry matter yield of the mixture. In contrast, the percentage of birdsfoot trefoil decreased over the three year trial period (Zemenchik et al., 2001). The fertilizer replacement value of the kura was positively correlated with its yield. As the yield of kura in the mixtures increased so did the fertilizer nitrogen value realized by the grass (Zeminchik et al., 2001).

VEGETATIVE PRODUCTIVITY

Kura clover grown in pure stands, when compared with pure stands of 6 other legume species, demonstrated greatest persistence under 2-cut, 3-cut or 4-cut harvest frequencies per season (Sheaffer and Marten, 1991). Yield was determined by harvesting 1 x 6m, ground cover and crude protein content was also assessed. Kura clover stands showed only 65% ground cover in the year following seeding but improved over the next two years surpassing the other legume stands (Sheaffer and Marten, 1991). Kura yields over a growing season were similar whether harvested two, three or four times per season averaging 60% of the biomass obtained from the pure alfalfa stands (Sheaffer and Marten, 1991). Under the four cuts harvest frequency, kura, alfalfa and cicer milkvetch were the only stands with sufficient biomass to harvest (Sheaffer and Marten, 1991).

Although kura clover has yields less than alfalfa, cicer milkvetch and birdsfoot trefoil grown in pure stands in Minnesota, its far superior persistence in pure stands suggests high potential value as a forage legume (Sheaffer and Marten, 1991).

In Minnesota, grazing trials were established to compare the persistence of kura clover with birdsfoot trefoil in monoculture and in mixture with each other, using the put-and-take method of stocking (Sheaffer et al., 1992). Birdsfoot trefoil demonstrated poor persistence whereas pure stands of kura clover showed excellent persistence when grazed by sheep. In mixture with birdsfoot trefoil, kura clover increased from the seeded proportion of 10% to over 95% ground cover over the duration of the study (Sheaffer et al., 1992). Animal gains/ha initially did not differ between the legume species however, four years following seeding, lamb gains were 105% and 86% greater for kura-trefoil mixture and the kura monoculture respectively, compared with pure stands of birdsfoot trefoil (Sheaffer et al., 1992).

Peterson et al., (1994) conducted a two part study in the United States (Minnesota), examining the response of a 5-year-old stand of kura clover to 4 harvest frequencies and 4 grazing treatments. Pure stands of kura clover were subject to one of 3, 4, 5 or 6 cuts per year over 3 growing seasons. Forage yield was measured on a 1 x 6.1 m area. In the first two treatment years, total forage yield was not affected by cutting frequency. However by the third treatment year, 5 and 6-cut plots yielded 80 and 70 % of plots harvested 3 and 4 times over the growing season (Peterson et al., 1994). The second portion of the study involved grazing sheep using the put-and-take method of stocking. Pure swards of kura clover were either continuously stocked, rotationally grazed with 14d of rest, rotationally grazed with 28d rest until September, or rotationally grazed with 28d rest until October. Grazing periods lasted 3 to 6d, and sheep remained in a paddock until all available forage was defoliated to 1 cm in height (Peterson et al., 1994). Accumulated biomass was measured at the beginning of every grazing period. In the second and third treatment years, 14d rest rotational system produced 72 and 84% of the biomass produced in the rotational systems with 28d rest periods (Peterson et al., 1994). Rotational grazing with 28 d rest ending in September, exhibited the highest yields of all treatments and provided the most uniform forage distribution over the season (Peterson et al., 1994).

Peterson et al., (1994-b) also examined the response of belowground biomass to clipping and grazing. Clipping frequency had little effect on root carbohydrate levels. Changes in root mass under clipping treatments were due to time of year and age of stand rather than harvest frequency. In contrast, grazing treatments visibly altered below ground morphology (Peterson et al.. 1994-b). Primary crown and taproot mass and number decreased over the duration of the test. Secondary crown number and mass varied with grazing treatment. Continuous and 28d rest rotation had the greatest secondary crown number and mass (Peterson et al.. 1994-b). In all treatments. secondary crown number and mass were similar at the end of the study suggesting that kura clover is capable of maintaining a secondary root system under a range of defoliation treatments (Peterson et al.. 1994-b). The number of rhizome initials (buds) has been used as a measure of overall plant vigor because they are indicative of active belowground growth. Both fall grazed plots and continuous stocking reduced the number of rhizome initials and decreased spring regrowth (Peterson et al.. 1994-b). Storage of TNC was highest in taproots in spring and summer shifting to rhizomes during the late summer and fall. The 14d rest and 28d rest rotations showed the highest TNC concentrations. Peterson et al.. (1994-b) concluded that under frequent close defoliation. Kura clover reduces above ground growth to maintain stored TNC's.

Contrary to Peterson et al.. (1994). results of grazing treatments with set stocking and rotational treatments showed significant reductions in biomass and rhizome mass of kura clover (Lucas et al.. 1997). Kura clover was compared to white clover under four grazing regimes. set stocked or rotational grazed under high or low intensities using sheep as the grazing animal. In the first treatment year. kura clover showed superior percentage cover than did white clover. In the second treatment year. for the set stocked treatment under high grazing pressure kura was reduced to half of the percentage cover found in the white clover plots. Under the intensive. continuously stocked treatment. the root and rhizome mass of kura clover was severely depleted (Lucas et al.. 1997). This is contradictory to previous studies which showed that the below ground mass of kura was not affected by defoliation (Peterson et al.. 1994 b). In the rotationally grazed plots however. where the perennial ryegrass was extremely vigorous. kura clover survived the smothering effect of high grass cover better than the white clover.

In Wisconsin. a study was developed to evaluate defoliation management on three different kura – grass mixtures using orchard grass. smooth brome or Kentucky bluegrass. under 3. 4. or 5 cut harvest frequencies at two cutting heights (Kim. 1996). Over the three year trial period. kura - grass mixtures had higher forage production on average than solo seeded kura. Kentucky bluegrass – kura mixtures showed the best total forage production and had the most consistent kura – grass ratio over the three years. The proportion of kura in the mixtures increased between the first and second trial years and remained constant between years two and three. Kura yield in the mixtures was highest when sown with Kentucky bluegrass. lowest when sown with orchard grass and intermediate when sown with smooth brome (Kim. 1996). Cutting frequency did not affect the yield of kura. however. less frequent harvests increased the total forage production and prevented weed appearance. Over the three years. kura phytomass in the mixtures increased while the grass phytomass decreased. Quality analysis of the kura clover showed consistent nutritive value at each defoliation (Kim. 1996).

Kim (1996) also examined the spreading ability of kura when sown into three different types of swards: grass. grass fertilized with nitrogen or killed grass using 1 year old Kentucky bluegrass or smooth brome swards. Kura plants were started either from seed or from rhizome cuttings and then transplanted into the plots. Individual plants spread up to 1 m over two growing seasons when grown without competition from the grass but spread only 29 cm when sown into a fertilized grass sward. The kura plants demonstrated a greater degree of spread in the fertilized smooth brome plots as compared to the fertilized Kentucky bluegrass plots but the spread was similar between the species for unfertilized plots (Kim. 1996). Defoliation of young kura plants over a 12 week period at 2. 4. or 6 week intervals under partial or complete defoliation treatments was compared for kura plants started from seed or clones from rhizome cuttings to determine changes to root and rhizome mass. The leaf dry matter produced was higher for kura plants that were started from seed as compared to cloned plants (Kim. 1996). Rhizome development from cloned plants occurred more quickly than plants started from seed although. root growth was slower for the cloned plants. Kim (1996) suggested that root development is not directly related to rhizome development but does have an association with leaf development in kura clover.

FORAGE QUALITY

Nutritional characteristics of kura clover have been evaluated by measuring in vitro dry matter digestibility (IVDMD). crude protein (CP) content. analysis of Ca. Mg. Fe. Mn. Cu and Zn. K and P. acid-detergent fiber (ADF) and acid-detergent lignin (ADL). Allinson et al.. (1985) compared this analysis of kura clover with alfalfa. birdsfoot trefoil. white clover. crownvetch and cicer milkvetch. Kura clover had the highest IVDMD values and Ca concentrations. Other minerals and CP content were similar to those of other legumes. Kura clover had lower concentrations of ADF and ADL than alfalfa (Allinson et al.. 1985). Results of this analysis suggest that kura has a higher digestibility than many commonly utilized legume species. A small decrease in quality of kura clover has been observed in association with flowering (Peterson et al.. 1994).

Forage quality of kura clover increased with increasing cutting frequency as measured by CP content and IVDMD values (Sheaffer and Marten. 1991). CP content of kura clover exceeds the requirements of growing lambs. Lamb gains obtained on stands of kura clover were similar to those achieved by grazing alfalfa (Marten et al.. 1990).

When seeded in mixtures with grasses. crude protein concentration was found to be negatively correlated with increasing percentage of grasses and positively correlated with the presence of kura clover (Jerenyama et al.. 2001).

Kura clover possesses non-lethal amounts of hydrocyanic acid. Levels are much less than those observed in white clover and do not affect quality (Taylor and Smith. 1998). The greatest detriment to grazing kura clover is the potential for bloat. Grazing pure stands of kura clover caused 6% of lambs to bloat. 2% of which died as a result (Sheaffer et al.. 1992). Frequency of bloating from kura clover is similar to that of alfalfa and is most likely due to the high leaf : shoot ratio of kura. Sheaffer et al. (1992) recommended that incidence of bloat could be reduced by using grass-kura mixtures instead of pure stands. Evaluation of the compatibility of various pasture grasses with kura clover is necessary as little information currently exists.

SEED PRODUCTION

Seed yields of kura have been found to be closely associated to the availability of bees. On a breeders seed plot in Cooma. Australia. 4-5 hives per acre of kura was found to be the optimum during the dense spring flowering period (Bryant. 1974).

Removing a hay crop prior to flowering is a frequently used practice in forage/legume seed production systems in Oregon. the purpose of which is to improve seed yield and to control insects and diseases that would otherwise damage the crop. By removing a hay crop prior to harvesting seed. the temperature is more conducive to pollinator activity due to the delay in flowering. in addition the vegetative regrowth is less susceptible to early season diseases and insect infestations. Steiner (1992) examined the effects of haying date and establishment times on the seed yield of kura clover. Kura clover seed yield was adversely affected by haying at any time. Later haying removed more flowers than earlier harvests and further decreased yield. Yield reductions as high as 90% were experienced when compared to the seed yield of un-hayed treatments (Steiner. 1992). Seeding date did not affect the seed yield. however. the later planting dates reduced the amount of ground cover in the plots (Steiner. 1992). Steiner (1992) concluded that to re-establish flowers following cutting. new reproductive stems must be initiated. This concurs with Pellet's (1956) observations. which stated that kura flowers only once in a season.

Steiner and Snelling (1994) examined the viability of intercropping an alternative crop during the establishment year of kura clover to heighten the economic incentive for kura seed production. Intercropping may give sufficient advantage to seed growers during the establishment period when monocultures of kura clover produce low seed yields. Kura was found to be sensitive to competition during establishment (Steiner and Snelling. 1994). The greatest economic return was realized when kura clover was relay-intercropped with wheat that had been cross-seeded using a wide row spacing (Steiner and Snelling. 1994).

BREEDING

Seedling vigor of kura clover is lower than other legume species due to allocation of carbohydrates toward root development. DeHaan et al.. (2001) postulated that by selecting kura clover for increased carbohydrate allocation toward shoot development. seedling vigor would be improved. The breeding program focused on phenotypic selection over three cycles. for kura plants with reduced root : shoot ratios. Selection resulted in an increase of shoot weight by 39% from the parent population by the third cycle (DeHaan et al.. 2001). Variation in shoot height and weight between the parent and F3 populations were more pronounced under high fertility conditions. In addition. DeHaan et al.. (2001) found that seed size in kura clover was correlated to shoot and root growth in the field and concluded that selection for larger seeds may be beneficial.

Taylor and Cornelius (1994) investigated the effectiveness of selection programs to enhance forage and seed yield in kura clover. Cultivars developed in Australia include Summit (2x). Alpine (2x). Forest (2x). Treeline (2x). Monero (6x). and Prarie. the cultivar Rhizo (6x) was developed in the United States. Hexaploid forms of kura typically include the most vigorous plants. Plots were established in Kentucky. Plants flowering in their second season were left to open pollinate. collections of the half-sib seeds taken from the heads with the highest number of seeds were then used for the next generation (Taylor and Cornelius. 1994). This process was repeated over 6 generations. First season selection plants were chosen over 3 years for 2nd season blooming followed by 6 years of selection for 1st season blooming. These selections were then seeded into field plots. Plants were mowed once annually following seed harvest. Dry matter yield was measured at 5 harvests over a 3 year period. Taylor and Cornelius (1994) concluded that first season selection significantly increased blooming while second season selection gave a greater number of heads per plant and corresponded to more grams of seed/ plant.

Several studies have been initiated with the intent of utilizing desirable characteristics exhibited by kura to improve various qualities of commonly used clovers. Marshall et al. (2001) examined the potential of creating a hybrid between kura clover and white clover to improve the drought resistance of white clover. Kura and the hybrids successfully maintained higher leaf relative water content and leaf water potential than did white clover. Over a 4 week period without watering to simulate drought conditions. kura sustained a leaf relative water content at more than 80% whereas white clover declined over the study period reaching 50% by the end (Marshall et al., 2001).

Kura clover is closely related taxonomically to white clover but has several characteristics such as cold tolerance, drought tolerance, disease resistance, to which white clover is susceptible. In previous tests in which the two species were crossed, fertilization occurred but viable seed was not produced. Williams (1978) utilized kura as the female parent and white clover as the male parent to more closely examine the potential for developing a cross. From 100 pollinated flowers, 30 embryos were large enough to use for testing, of these 6 grew in culture with 2 producing roots and 4 producing roots and shoots. Of the 4 that produced shoots only 2 were predicted to flower (Williams, 1978). The hybrid plant resembled white clover exhibiting stoloniferious growth and small leaves that are more oval in shape (resembling kura) and have more marginal veins than white clover. The hybrid demonstrates the presence of leghaemoglobin but shows no rhizome development and is highly sterile (Williams, 1978). Further study involving culture nurse endosperm *in vitro* has yielded a partially fertile hybrid (2n = 32) between kura and white clover. Through hand pollination, F2, F3 and backcross progeny have been developed. Although these plants are only partially fertile, they demonstrate vigorous growth and show potential for genetic exchange between the two species (Williams and Verry, 1981).

Williams (1980) also crossed kura clover with alsike clover to improve disease resistance, rhizome development and winter-hardiness as well as increasing establishment and nodulation potential. Crosses produced hybrid plants with some embryo development but no viable seed. Embryos from the cross were dissected and cultured *in vitro* using nurse endosperm from normally developing kura. Williams (1980) found that there was greater chance of seed set in crosses where the female parent had a higher chromosome number than the male parent. Of the hybrids, 2 survived to flowering showing growth habits similar to that of alsike clover. The plants expressed some rhizome development and had leaves and stipules more closely resembling the kura parent (Williams, 1980).

STATEMENT OF PURPOSE

Our objective was to determine the suitability of kura clover as a pasture legume for use in Central Alberta. We examined best-suited companion grasses through measuring dry matter production and stand composition of four kura-grass mixtures. In addition, dry matter production of pure stands of kura clover under five harvest frequencies assessed to provide insight into optimal management strategies.

It was hoped that through analysis of the treatments imposed on kura clover (*T. ambiguum*), that we would gain increased understanding of its productiondynamics and regrowth ability. More specifically, we aimed to determine management strategies that maximize seasonal biomass production both through identification of grass species most compatible as well as grazing frequency to optimize regrowth. Theoretically there is much support for kura as a potential component in the pasture swards of Central Alberta. The purpose of these trials was to gain a practical understanding of the ability of kura clover to adapt to Alberta conditions and to develop guidelines for its use.

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CHAPTER TWO

THE COMPATIBILITY OF KURA CLOVER WITH FOUR GRASS SPECIES

HYPOTHESIS TESTED

Total phytomass production over a growing season of kura clover (K) grown alone or in mixture with either Kentucky bluegrass (KBG). meadow brome (MB). orchard grass (OG) or timothy (T) or kura clover grown alone will be the same.

Phytomass production at each of 4 harvests will be the same for kura clover grown alone or in mixture with either KBG. OG. MB or T.

Percentage of kura clover in the sward. when grown with either KBG. MB. OG or T will be the same.

Percentage of kura clover in the sward. when grown with either KBG. MB. OG. or T will be the same at each of 4 harvests.

INTRODUCTION

Kura clover *(Trifolium ambiguum)* is a perennial clover originating in the Caucasian region of Russia. Kura is naturally found over a wide range of habitats and demonstrates a greater range of tolerances to drought, soil type, pH, elevational changes and poorly drained soils than legumes currently used in North American pasture swards (Taylor and Smith, 1998). During the first two to three seasons following sowing, kura develops its root system at the expense of vegetative structures, demonstrating root:shoot ratios of 1.5 - 3.0:1 whereas white clover of the same age shows root:shoot ratios of 0.3 - 0.7:1(Spencer et al., 1975). Rhizomes are initiated as early as three months following germination and within two years a plant's root system can span 1m in diameter and at least 60cm in depth (Speer and Allinson, 1985). The focus on development of the root system results in a large rooting zone and the extensive nature of the root matrix suggests high potential for long-term productivity.

As a result of the preferential partitioning of carbohydrates towards root development, establishment is slow. Kura is a poor competitor with weeds and can be negatively affected by the presence of aggressive companion grasses at the early stages of its development (Taylor and Smith, 1998). Hill and Mulcahy (1995) examined the effects of grass:legume ratios on the ability of kura to establish and grow. Kura was sown with *Festuca arundinacea* or *Phalaris aquatica* at grass : legume ratios of 1:1, 2:1, 4:1, or 8:1. The number of leaves and shoots expressed by kura increased as the density of the companion grass decreased. An inverse relationship was also shown between grass density and kura rhizome number, as the density of the grass increased the number of rhizomes decreased (Hill and Mulcahy, 1995). They concluded that root and rhizome development is crucial during the establishment period of kura and this ability is enhanced when the density of the companion grass is low.

Kura demonstrates persistence in pure stands under 2, 3, or 4-cut harvest frequencies. Yields were similar whether harvested two, three or four times per season (Sheaffer and Marten, 1991). Grazing trials were imposed using sheep with either continuous stocking, rotational grazing with 14d rest periods, rotational grazing with 28d rest periods through September or rotational grazing with 28d rest periods through October (Peterson et al., 1994). Grazing intervals lasted 3 to 6d based on the time required to take all available forage to a height of 1cm. Accumulated phytomass was measured at the beginning of each grazing period. In the second and third treatment years the 14d rest rotational system produced only 72% and 84% of the phytomass accumulated in the rotational systems that had 28d rest periods. Rotational grazing with 28d of rest ending in September exhibited the highest yields of all treatments and provided the most uniform forage distribution (Peterson et al.. 1994).

In Wisconsin a study was developed to evaluate defoliation management on three grass – kura mixtures (Kentucky bluegrass. orchard grass or smooth brome) under three harvest frequencies (3. 4. or 5 cut) at two cutting heights (Kim. 1996). Kura – grass mixtures were found to have higher forage production over three years than did solo seeded kura. Less frequent harvests increased total forage yield and prevented weed appearance. The proportion of kura in the mixtures increased between the first and second production years and remained constant between the second and third production years. Over the duration of the trial. the clover biomass of the sward increased while the grass biomass decreased. Kura clover – Kentucky bluegrass mixtures demonstrated the greatest total forage production and had the most consistent legume to grass ratio over the three years (Kim. 1996).

Sleugh et al.. (2000) compared the effects of alfalfa. birdsfoot trefoil. and kura clover grown in mixtures with orchard grass. smooth brome grass or intermediate wheatgrass for forage yield and quality. Inclusion of legumes improved the seasonal yield distribution and increased the quality of the pasture sward. The alfalfa – intermediate wheatgrass mixture produced the most biomass over the season. Alfalfa in monoculture showed the least decline in yield over the four harvests taken in the first production year followed by kura clover monocultures (Sleugh et al.. 2000). Kura in monoculture had the highest in vitro dry matter digestibility and crude protein levels of the six species studied and when sown with grass. kura clover improved the quality of the sward (Sleugh et al.. 2000).

The nutritional forage quality of kura clover was compared to alfalfa. birdsfoot trefoil. white clover. crownvetch and cicer milkvetch (Allinson et al.. 1985). Kura had the highest in vitro dry matter digestibility and Ca concentrations of the legumes studied. Kura demonstrated lower ADF and ADL concentrations than alfalfa. Results of the comparison suggest that kura is of high forage quality having higher digestibility than several commonly utilized legume species.

Several investigations have addressed various aspects of kura growth parameters. establishment and quality however information regarding binary mixtures using kura is very limited particularly in western Canada. The objective of this study was to determine the best-suited companion grass to be sown with kura clover for use in pasture swards. in

Central Alberta, utilizing dry matter production, stand composition and forage quality as measures of kura:grass compatibility.

DESIGN & METHODOLOGY

Mixture tests were of a split-plot design with main treatments of seeding date, and sub-plots of grass-kura clover mixtures. The trial ran from May 1999 through the 2001 growing season and consisted of four replications with a total of eight blocks. Seeding occurred at the end of May (SD1) or end of July (SD2), 1999 and 2000. Seeding dates were selected to demonstrate the ability of plants to establish themselves at different stages in the growing season. Plots were established on a black chernozemic soil with a pH = 5.6, salinity of 0.52 dS/m^2, 200 kg/ha available nitrogen, 68 kg/ha of available phosphorous, 750 kg/ha available potassium and 81 kg/ha of available sulfates. Individual plots were 6 x 2.4m and consisted of 8 seeded rows. Grasses were seeded with a double disc press drill 1.25 cm deep with a 30cm row spacing at recommended seeding rates (Table 2-1). Grass species were selected for their range of competitive abilities and varying growth habits. Kura clover was broadcast by hand over the plot area, raked by hand and packed.

TABLE 2-1. Seeding rate of 4 grass species and kura clover for kura-grass mixture plots seeded at the end of May and July in the years 1999 and 2000.

Kura Clover (T. ambiguum) cv. NF201	10 kg/ha
Meadow Brome (Bromus beiberstienii) cv. Fleet	8 kg/ha ***
Orchard Grass (Dactylis glomerata) cv. Kay	6 kg/ha
Timothy (Phleum pratense) cv. Champ	4 kg/ha
Kentucky Bluegrass (Poa pratensis) cv. Troy	4 kg/ha

*** Coated MB was seeded at 15 kg/ha as adjusted for weight of coating and poor germination (85%)

Plots were hand weeded as needed to prevent excessive competition from weeds. Due to the unexpectedly fast growth rate of the four grass species seeded at the end of May 1999, a harvest 8wk after seeding was added to the design of the experiment. All plots (SD1 and SD2) were harvested at the end of the season in the year of their establishment (Table 2-2).

Yield was measured from a sample 0.6 x 5.4 m collected with a flail mower, harvested material was placed in cloth bags. Prior to collecting the harvest sample, a 30 cm swath was removed from each end of the plot to remove any "edge effect" that might skew data. Harvests included grass rows 3 and 4 and clover in the intervening spaces. Species composition was determined using a 50 x 50cm quadrat subsample taken from grass rows 6 and 7 and clover in the intervening spaces. Plants were harvested by hand, separated into grass or legume and species composition determined from the dried weights of each. Material collected from both the machine harvest and the subsample was placed in a forced air dryer at 65°C for 48 hours. Following each harvest, plots were mowed with a sickle mower and the trash was removed. In the production years, harvests occurred at monthly intervals beginning approximately in the first week of June of each season (Table 2-2). Because of the minimal amount of vegetative growth on the late seeded plots in 2000, these plots were not machine harvested at the end of season but hand harvested samples of 1m^2 were taken.

TABLE 2-2. Harvest schedule of kura-grass mixture plots for 1999 through 2001 growing seasons.

[redacted]			
May 25, 1999	July 28, 1999	May 30, 2000	June 11, 2001
		July 7	July 9
	October 4	August 23	August 13
		September 29	September 23
July 27, 1999	October 4, 1999	May 30, 2000	June 11, 2001
		July 7	July 9
		August 8	August 13
		September 29	September 23
May 26, 2000	July 31, 2000	June 11, 2001	
	September 20	July 10	
		August 13	
		September 23	
July 28, 2000	September 20	June 11, 2001	
		July 10	
		August 13	
		September 23	

Both the machine harvested material as well as the subsample collections were weighed. Dry matter weights were statistically analyzed to determine production of individual mixtures at each harvest and for total yield over the growing season. Separations of the grass and clover were analyzed for percentage clover in each mixture at each harvest. In addition, all samples were analyzed for crude protein percentage, ADF, and NDF at Norwest Labs, Edmonton, Alberta.

Data were analyzed using the general linear model to test the analysis of variance for the effects of seeding date, forage mixture and the interactions of seeding date by mixture and mixture by harvest (SAS Institute, 1998), means were separated using an adjusted Tukey test. Significance was tested at the 95% confidence interval ($p<0.05$). Results for analysis of variance tests are presented in tabular form located in Appendix II (Table II-1 through Table II-14).

RESULTS AND DISCUSSION

Forage Production

Establishment Year

There were statistically significant differences between the two establishment years (1999 and 2000) for total phytomass produced in the establishment year of the mixtures. The yield data were therefore analyzed separately by year.

The general trend for vegetative productivity in the year of establishment was that greater forage yield was produced when plots were seeded early in the spring as opposed to mid-summer (Figure 2-1). In both 1999 and 2000 the early seeded orchard grass-kura clover mixture yielded the greatest total phytomass in the establishment year (Appendix I, Table I-1).

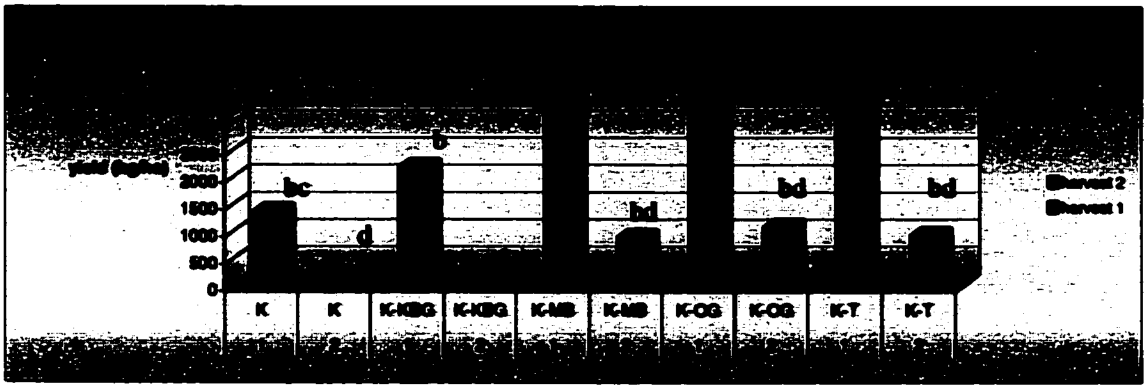


FIGURE 2-1. Total annual dry matter yield (kg/ha) of mixtures of kura clover with either Kentucky bluegrass(K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or kura clover grown alone (K) in the establishment year (1999) at two seeding dates. (1 = early seeding; 2 = late seeding)
*Interaction of seeding date by mixture was significant at (p=0.0041).
**Columns with the same alphabetical letter are not significantly different when p<0.05.

Replicate was significant for the 1999 establishment data and remained significant when the first replicate, which exhibited very poor germination and emergence, was removed. Therefore data are presented with the first replicate included.

In 1999, for the total annual yield in the establishment year the interaction of seeding date by mixture was significant. Kura clover – orchard grass mixtures (K-OG) gave the highest total annual yield at both the early and late seeding dates but was not significantly different than the kura – meadow brome (K-MB) and kura – timothy (K-T) mixtures (Figure 2-1). At the time of the first harvest, the MB, OG and T were in a reproductive stage of growth. At this point the density of the grasses was not high, however, this is an indication of the vigorous nature of these plants (Plate 2-1). The vigorous growth demonstrated by the grass species might have arisen as a result of the high levels of available nitrogen (200 kg/ha) at the time of seeding.

Phytomass produced from the late seeded plots was significantly less (p=0.0041) than the early seeded plots. Pure stands of late seeded kura did germinate and emerge, however, the young kura plants grew low to the ground, appearing almost rosette like, and therefore plants were below the cutting height at the end of the season. Speer and Allinson (1985) noted that the stand formation of kura clover is variable, typically falling into one of three trends. The first demonstrates mother plant development to appreciable size, the second vigorous rhizome and daughter plant development resulting in a compact stand and the third type demonstrating colonization of a wide area by few daughter plants (Speer and Allinson, 1985). For the solo seeded kura plots, plant development followed the final pattern, showing wide spread growth over the plot area.

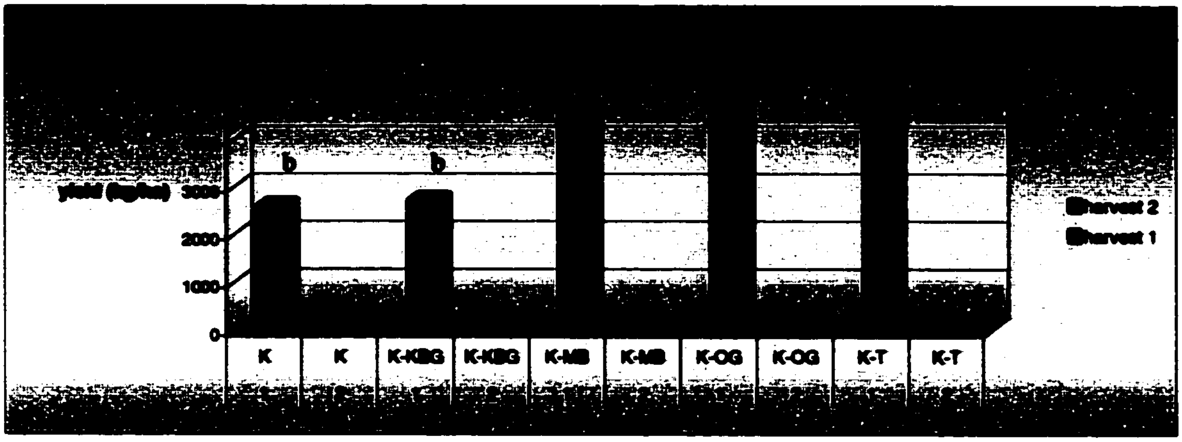


FIGURE 2-2. Total annual dry matter yield (kg/ha) of kura clover grown in mixture with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or grown alone (K) in the establishment year (2000) at two seeding dates (1 = early seeding; 2 = late seeding).
*Interaction of seeding date by mixture was significant at ($p < 0.0001$).
** Columns with the same alphabetical letter are not significantly different when $p<0.05$.

In 2000, as in the 1999 establishment year data, the general trend was for the earlier seeded mixtures to produce greater total phytomass than the late seeded mixtures (Figure 2-2). Very little precipitation fell in the year following the late seeding date, consequently, there was no emergence observed in the late seeded plots two weeks following seeding. Following irrigation (1.25 cm 3 weeks after planting), germination was still slow and for this reason very little development of grass or clover was observed at the year end harvest. Kura plants sown at the end of July 2000, had approximately 2-3 true leaves and grass plants sown at the end of July 2000, had approximately 3-5 true leaves at the end of season harvest date. This is consistent with previous observations that kura seedlings are highly susceptible to stresses such as frost (Caradus, 1994), drought (Taylor and Smith, 1998) or competition (Hill and Hoveland, 1993).

Mixtures of K–OG at the early seeding date again produced the greatest total phytomass compared to the other kura – grass mixtures (Appendix I,Table I-2). There was no significant difference in the forage yield between the K-OG, K-MB and K-T mixtures. At the year end harvest. the early solo seeded kura plots demonstrated rhizome growth and daughter plant formation. Kura growth and development was similar to that observed in other studies. Rhizome development has been known to occur as soon as 3 months after seeding with daughter plant formation within 45 days after planting single node rhizome segments (Speer and Allinson. 1985).

Yields in the seeding year of kura clover grown in monoculture in Minnesota ranged from 0 – 350 lb/acre for spring seeded plots (Seguine et al.. 1999). The total annual yield in the establishment year for both 1999 and 2000 seeded plots greatly exceeded the forage yield of the above trial. In 1999. solo seeded kura plots at the early seeding date yielded 1.373 kg/ha and in 2000 yields of 2.456 kg/ha were realized at the early seeding date. High levels of plant available nitrogen in the soil potentially influenced production in the establishment year.

For both 1999 and 2000. harvested material was comprised primarily of grass. For the early seeded plots. the percentage of kura in each mixture was consistently higher at the second harvest than for the first harvest of the season. The increase in percent clover results from the reduction in competition for light with the grass species. which grew rapidly in the spring and had slower rates of regrowth in the latter half of the season. Average percentage kura was greater in the year 2000 than in 1999 for the early seeded plots. this is potentially due to conditions at the time of seeding. In 2000. early plots were seeded following a period of rain whereas in 1999 the spring conditions were dry and consequently germination was slower.

First Production Year:

There were significant differences between the years when analyzing the phytomass production in the first production year of the mixture plots. As a result, the data for the plots established in 1999 are presented separately from the plots established in the year 2000. The significant difference in values is probably due to the extremely poor establishment of the late seeded plots in the year 2000, which in turn, affected the yield in subsequent growing seasons.

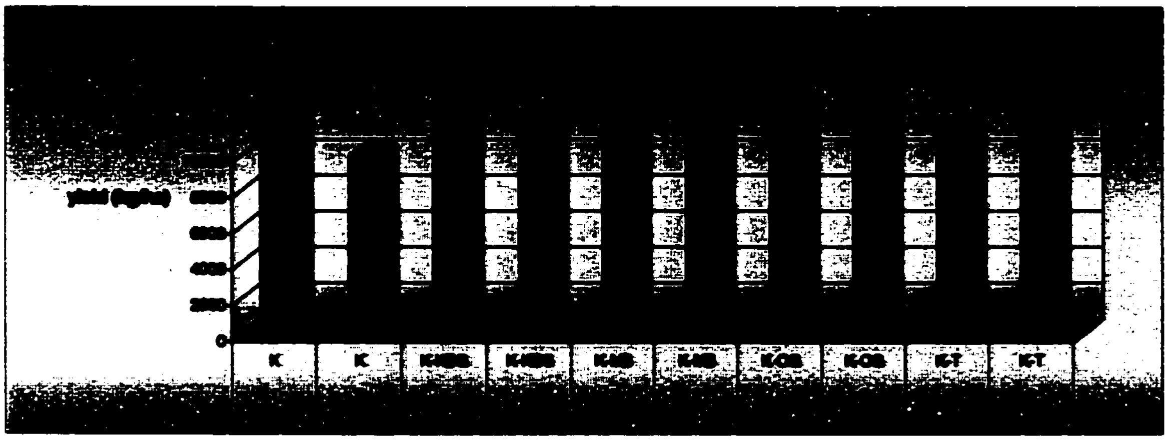


FIGURE 2-3. Total annual dry matter yield (kg/ha) of mixtures of kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG), or timothy (K-T) or kura in monoculture (K) in the first production year (2000) established at either early (1) or late (2) seeding dates (1999).
*Interaction of seeding date by mixture was significant at $p< 0.0001$.
**Columns with the same letter are not significantly different at $p<0.05$.

Seeded 1999

For the harvested yield in the first production year there were significant interactions between seeding date and mixture as well as between mixture and harvest. Total annual dry matter yield ranged from 10,008 kg/ha for late seeded kura to 15,772 kg/ha for late seeded K-MB (Figure 2-3).

When comparing results for the seeding date by mixture interaction a general trend emerges. Kura clover in mixtures with meadow brome produced the greatest amount of phytomass at both early and late seeding dates (Appendix I, Table I-3). Total annual yield of early seeded K-MB was significantly higher than early seeded K-T mixtures and did not differ from the early seeded K alone, K-OG or K-KBG mixtures in the first production year. For each mixture the general trend was for greater forage production by the late seeded plots. The opposite trend is shown by the K-KBG and kura alone plots, where phytomass of late seeded plots was lower, reflecting their poor establishment at the late seeding date. Early seeded K-KBG accumulated 12% more phytomass when compared to the late seeded plots. The negative effect of late seeding is even stronger in the solo plots of kura where the early seeded plots yielded 26% more than the late seeded plots. The slow development of the late seeded kura can be explained by the rhizomatous nature of the kura plants. In the establishment year energy is allocated toward the development of roots therefore little vegetative growth occurs, a delay in seeding perhaps accentuates the lack of production of above ground structures into the first production year.

FIGURE 2-4. Yield (kg/ha) of mixtures of kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG), or timothy (K-T) or kura grown alone (K) at each harvest in the first production year (2000) for plots established in the year 1999.
*Interaction of mixture by harvest was significant $p<0.0001$.
**Columns with the same letter are not significantly different at $p<0.05$.

When examining the accumulation of above ground phytomass over the growing season for each of the various kura – grass mixtures, it becomes apparent that the period of greatest growth occurred between the first and second harvests with the exception of the K-MB plots (Figure 2-4). This is probably due to the earlier initiation of growth of MB in the spring resulting in a more advanced maturity at the time of the first harvest. Following the removal of a large percentage of reproductive grass tillers at the time of MB first harvest, a lag in the regrowth was experienced. For all of the mixtures. the period of lowest regrowth occurred between harvest three and harvest four at the end of the growing season. The slow rate of growth at this time may be explained by the lack of precipitation throughout this period. and to the decreasing day length and cooler temperatures. Consequently. very little growth was observed as the plants initiated winter dormancy. The growth pattern of the mixtures did not follow patterns observed in previous studies where forage production was greatest at the first harvest of the season (Kim. 1996). The low yield at the last harvest did follow earlier reports when the least amount of forage production occurred at the end of season harvest (Kim. 1996).

At the first harvest in the first production year. K-MB mixtures produced significantly more forage than the K-KBG. K-OG and K-T mixtures (Figure 2-4). All grass mixtures yielded significantly more than the kura grown alone. At harvest two the highest yield was obtained from the kura alone. K-OG or K-T mixtures followed by K-KBG and K-MB. The rapid regrowth of the kura plants may arise from the high number of growing points available to initiate growth. Because at the first harvest several of the grasses showed development of reproductive heads the active growing points were above the cutting height whereas the meristems of kura plants remained low to the ground. Yields were similar for all mixtures at harvest three. K-MB produced the greatest amount of harvested forage followed by K-OG. K. K-KBG and K-T. K-MB mixtures produced significantly more phytomass at harvest four than all other kura – grass mixtures.

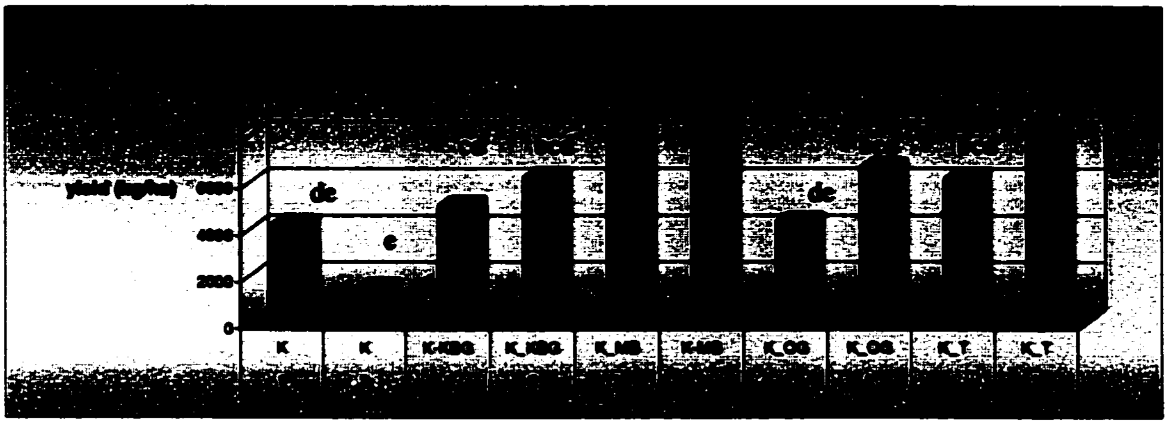


FIGURE 2-5. Total annual dry matter yield (kg/ha) of mixtures of kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or kura in monoculture (K) in the first production year (2001) following establishment at early (1) or late (2) seeding dates (2000).
*Interaction of seeding date by mixture was significant at $p=0.0012$.
**Columns with the same letter are not significantly different at $p<0.05$.

Seeded 2000

The plots that were seeded in 2000 experienced severe drought following the late seeding date and as a result, the kura plants that germinated were not able to grow and develop the structures necessary to allow survival through winter. Consequently the data that is presented for the late seeded plots established in the year 2000 is comprised primarily of grass. Some kura plants did establish but contributed very little to the harvested yield. It is interesting to note however, that many of the kura plants present in the first production year from the late seeding date, appeared to arise from seeds that did not germinate in the year of planting but rather in spring 2001. Furthermore, by the end of the first production year these plants developed rhizomes and were beginning to spread throughout the plots.

In the first production year (2001) there was a significant interaction between seeding date and mixture when considering total annual yield (Table I-4). The data shows a general trend of higher phytomass production for the late seeded plots compared to the early seeded plots in each mixture (Figure 2-5). This is an indication of the ability of the four grass species to perform in monoculture, as there was essentially no clover component in the late seeded mixtures, and reveals that the presence of kura does have an affect on the performance of the grass species. Similar to the plots established in 1999, the K-MB plots produced more forage yield than the other mixtures.

Early seeded K-MB mixtures produced 1.3x, 1.3x, 1.9x, and 1.9x more harvested phytomass than early seeded K-T, K-KBG, K-OG and K alone respectively (Figure 2-5). Due to the environmental conditions throughout the previous winter when no snow cover was present and temperatures fluctuated greatly the orchard grass plots did not break dormancy until after the first harvest of the season occurred. Additionally, some of the orchard grass plants were damaged by the winter and did not re-grow. Death loss was visually observed to be approximately 10%. The vulnerability of orchard grass to winter temperatures is widely accepted. It will not survive northern climatic conditions if snow cover is lacking (Christie and McElroy, 1995).

FIGURE 2-6. Dry matter yield (kg/ha) of mixtures with kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or kura clover grown alone (K) at four harvests in the first production year (2001) for plots established in the year 2000.
*Interaction of mixture by harvest was significant at $p<0.0001$.
**Columns with the same letter are not significantly different at $p<0.05$.

Growth cycle of the kura – grass plots in the year following seeding differed between the plots established in 1999 and plots established in 2000. For the 2000 seeded plots the third harvest yielded the greatest amount of phytomass (Figure 2-6) as opposed to the second harvest, in the first production year of the plots established in 1999 (Figure 2-4). This could mainly be attributed to the environmental conditions in 2001, as no precipitation events occurred during the re-growth period between the first and second harvest in 2001, resulting in a delayed pattern of growth for all mixtures. Additionally, the total annual yield for mixtures established in 2000 ranged from 1.512 kg/ha for late seeded kura to 10.309 kg/ha, significantly ($p<0.0001$) less than the harvested yield for plots established in 1999.

At the first harvest, the greatest yield was from the K-MB mixtures but this was not significantly greater than the K-KBG or K-T mixtures (Figure 2-6). Kura grown alone and K-OG mixtures produced significantly less phytomass at the first harvest than the other mixtures. The mixtures did not significantly differ at the second harvest. K-MB yielded the highest followed by K-T, K-KBG, K-OG and K alone. At harvest three, K-MB yielded significantly more than kura alone but did not differ from the other mixtures. There were no significant differences between the mixtures at the fourth harvest in the first production year.

Second Production Year:

In the second production year (2001) of the kura – grass mixture plots, data analysis revealed that seeding date had a significant effect on the total annual yield of the 1999 seeded plots. Early seeded plots produced more harvested forage than did late seeded plots($p<0.0001$). This trend was true for all mixtures. Also, the main effect of mixture significantly impacted the total annual yield of the kura – grass mixture plots. Total annual yield ranged from 4.290 kg/ha for K-T to 10.174 kg/ha for K-MB (Figure 2-7).

FIGURE 2-7. Total annual dry matter yield of kura clover grown alone (K) or in mixture with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the second production year (2001) for plots established in 1999.
*Main effect of mixture was significant at $p<0.0001$.
**Columns with the same letter are not significantly different at $p<0.05$.

In the second production year, K-MB mixtures produced significantly more phytomass than the other kura – grass mixtures (Appendix I, Table I-5). The total harvested yield of kura alone did not differ from K-KBG and both were significantly higher than K-OG and K-T. K-T produced significantly less biomass than all other mixtures. Productivity of the K-KBG plots was similar to mixtures of white clover and Kentucky bluegrass in West Virginia where between 4,270 kg/ha and 9,760 kg/ha of forage was produced, depending on moisture received during the growing season (Bryan et al., 2000).

It is important to note that the yield from the plots containing orchard grass was accumulated over the last portion of the growing season. Due to the lack of spring moisture and the adverse conditions the previous winter, the orchard grass plots remained dormant for an extended period of time (Plate 2-3). At the end of season harvest the K-OG plots demonstrated 85% recovery of the plants with 15% loss due to winter kill as noted through visual observations.

The seeding date treatment effect on the yield of the various mixtures is essentially removed by the completion of the second production year. Additionally, due to the extremely dry conditions throughout the growing season, biomass production of the mixtures in 2001 is somewhat indicative of the species drought tolerance.

FIGURE 2-8. Dry matter yield (kg/ha) of mixtures with kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or kura grown alone (K) at the first harvest in the second production year (2001) following establishment at early (1) or late (2) seeding dates (1999).
*Interaction of seeding date by mixture was significant at $p<0.0001$.
**Columns with the same letter are not significantly different at $p<0.05$.

For the initial harvest in the second production year there was a significant seeding date by mixture interaction. The general trend is for the early seeded plots to show greater levels of spring growth when compared to the late seeded plots (Figure 2-8). Comparison within mixture however, showed no significant differences. The interaction arose from the difference between the yield of late and early seeded K-T, which showed late seeded mixtures producing greater amounts of forage than early seeded mixtures whereas early seeded mixtures out-yielded late for the other kura – grass mixtures. This is in contrast to the first production year when the late seeded plots tended to out-yield the early seeded plots. K-MB plots demonstrated the greatest amount of early growth significantly higher than the other kura –grass mixtures. This is indicative of the growth habit of the meadow brome grass, which is more tolerant to drought than the other species in the study.

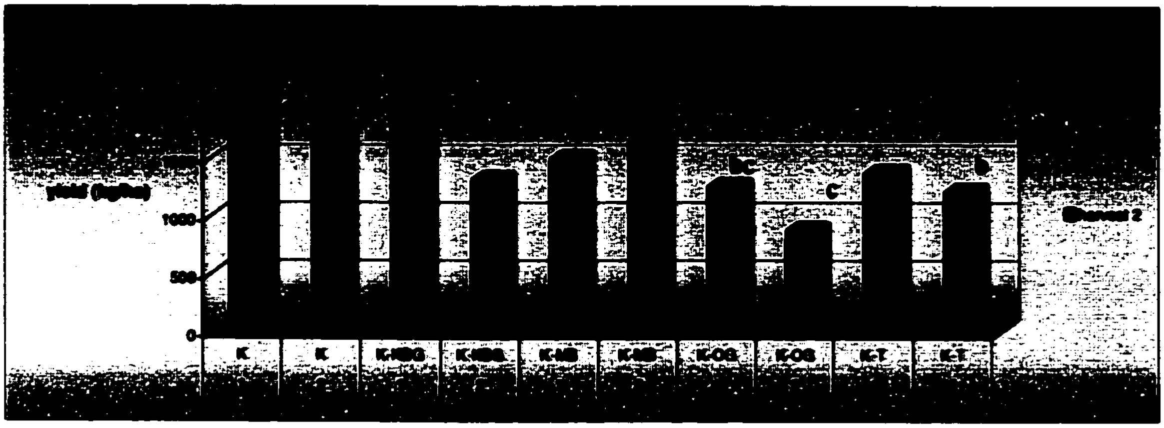


FIGURE 2-9. Dry matter yield (kg/ha) of mixtures with kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or kura clover grown alone (K) at the second harvest in the second production year (2001) following establishment at early (1) or late (2) seeding dates (1999).
*Interaction of seeding date by mixture was significant at $p < 0.0001$.
**Columns with the same letter are not significantly different at $p < 0.05$.

The yield at the second harvest also demonstrated a significant seeding date by mixture interaction. Similar to the data from the first harvest, early seeded plots yielded more than late seeded plots with the exception of K-MB (Figure 2-9). Late seeded K-MB yielded more than early seeded K-MB and thus, caused the interaction of seeding date by mixture for the second harvest. The only significant difference however, was found between the early and late seeded kura monocultures where early seeded plots produced 625 kg/ha more forage than the late seeded kura alone plots.

At the second harvest date, solo seeded kura showed significantly higher amounts of regrowth than any of the kura – grass mixtures. The ability of kura to regrow following the initial harvest may result from the large number of growing points that were below the cutting height. Also, because of the growth habit of kura, more residual leaf area following the harvest may have provided greater opportunity for the plants to photosynthesize. At the time of the second harvest very little precipitation had fallen indicating that perhaps once established, kura is better adapted to extracting moisture from the soil during periods of drought than the other species.

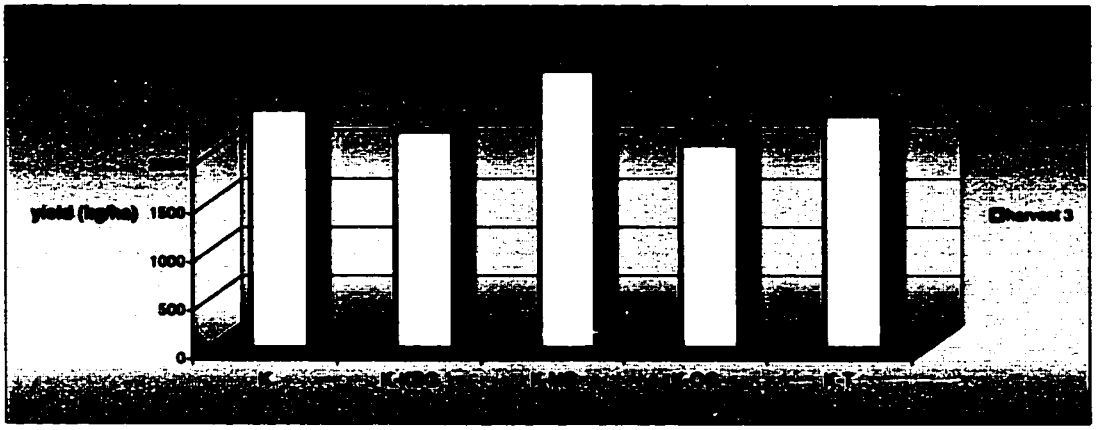


FIGURE 2-10. Dry matter yield (kg/ha) of mixtures with kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or kura clover grown alone (K) at the third harvest in the second production year (2001) of plots established in 1999.
*Main effect of mixture was significant at $p<0.0001$.
**Columns with the same letter are not significantly different at $p<0.05$.

By the time of the third harvest in the second production year the effects of seeding date were no longer evident and the only significant difference in yield occurred due to the mixture combinations (Figure 2-10). The greatest regrowth potential was shown by the K-MB plots, which produced 2,882 kg/ha of harvested material, significantly more than K-KBG and K-OG. By the third harvest the K-OG plots had recovered substantially but at this point still yielded significantly less than K-MB.

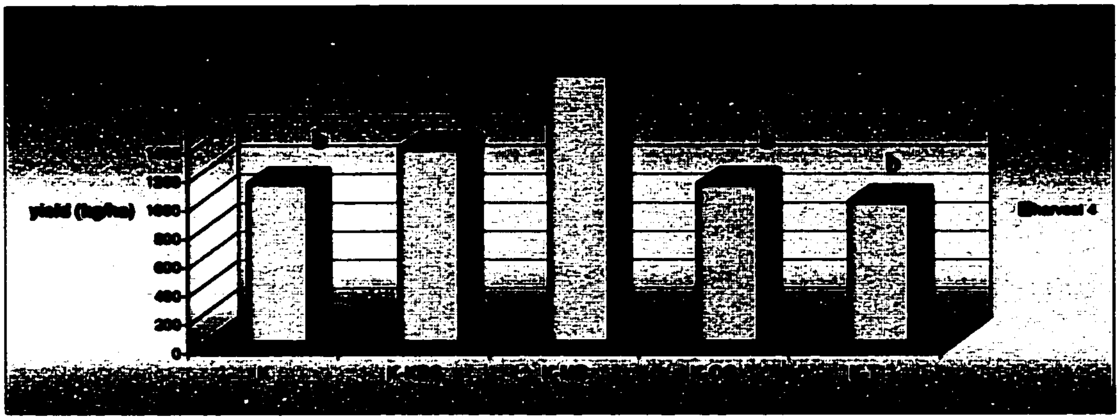
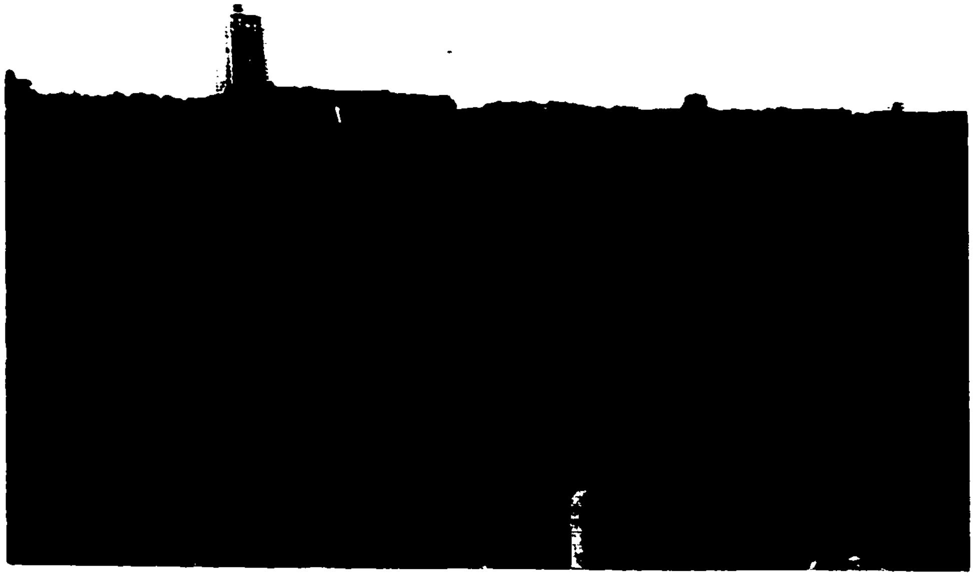


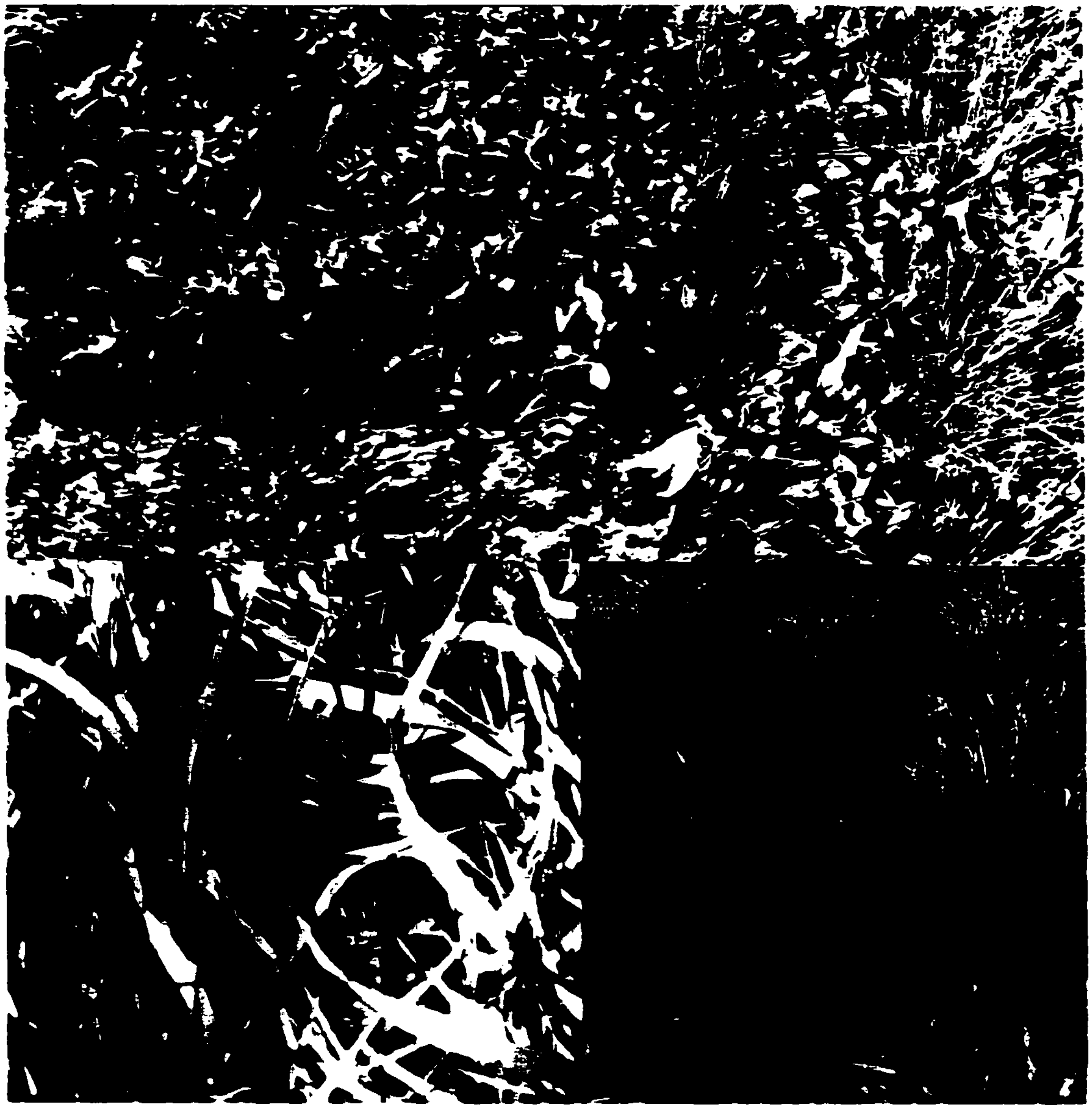
FIGURE 2-11. Dry matter yield (kg/ha) of mixtures with kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or kura clover grown alone (K) at the fourth harvest in the second production year (2001) of plots established in 1999.
*Main effect of mixture was significant at $p<0.0001$.
**Columns with the letter are not significantly different at $p<0.05$.

In the second production year, K-MB demonstrated the greatest amount of regrowth at the time of the last harvest (Figure 2-11). K-MB yielded significantly more than K-OG, K-T and kura alone. Over the final growing period the timothy component of the K-T plots showed very little regrowth. The timothy plots were infested with rust, yellowed and generally appeared to be in an unhealthy state. This potentially is a reflection of the environmental conditions between the last two harvests when no precipitation fell and drought conditions were prevalent.



A. Kura clover:grass mixture plots in the establishment year (July 1999) eight weeks following seeding .

B. Kura clover: grass mixture plots in the first production year (May 2000) of plots established in 1999.

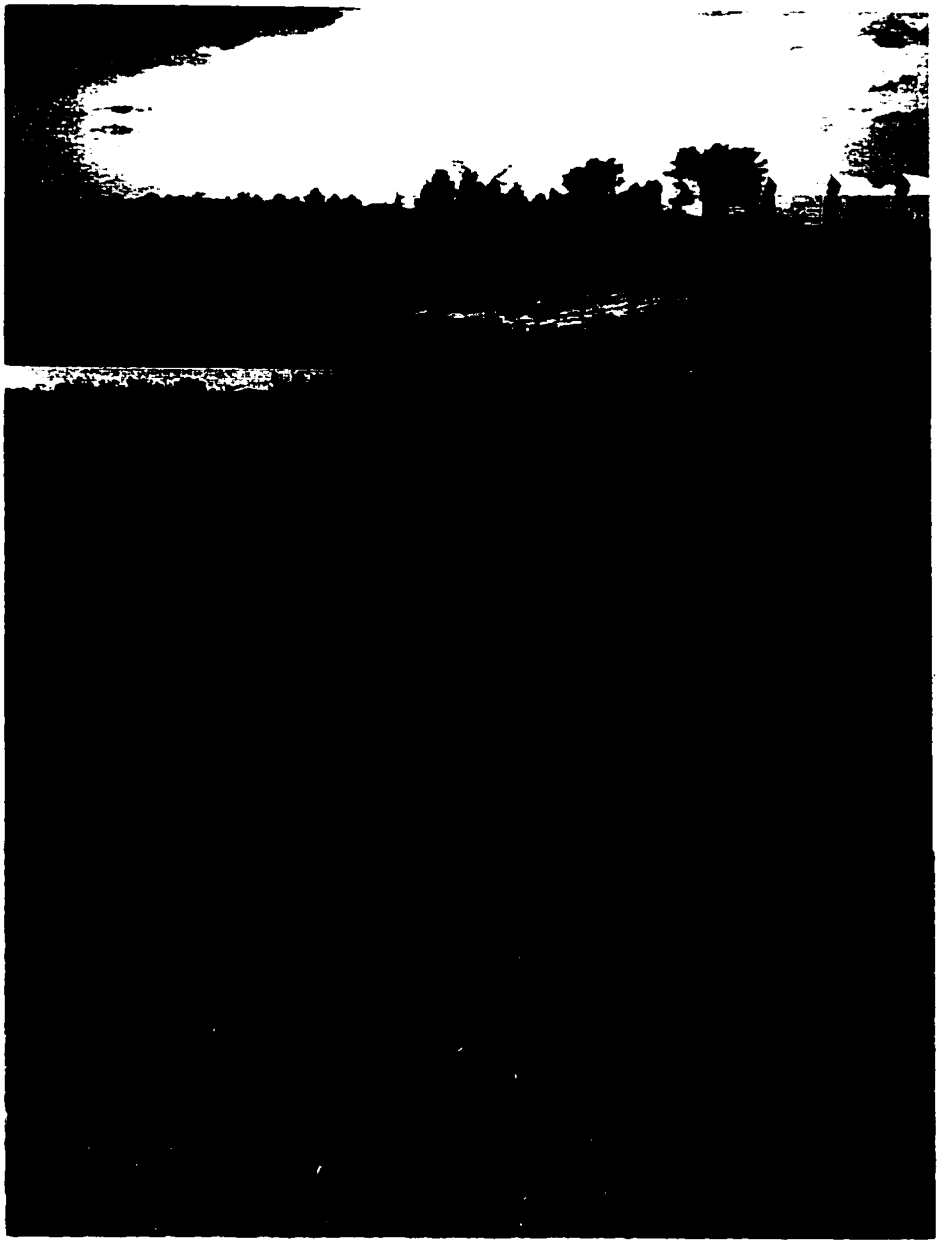


A. Kura clover in monoculture in the first production year (May 2000).

B. K-KBG mixture in the first production year (May 2000).

C. K-MB mixture in the first production year (May 2000).

D. K-T mixture in the first production year (September 2000).



A. Kura clover:grass plots in the second production year (June 2001), from left to right K, K-OG, K-KBG, K-T, K-MB, note dormancy of the OG.

B. Kura clover:grass mixtures in the second production year (August 2001), from left to right K-T, K-MB, K-OG, K-KBG, K.

C. K-OG and K-KBG plots at the final harvest of the second production year (September 2001).

D. Species Composition

First Production Year

Composition of the plots was determined using the hand separated ¼ m^2 samples to calculate clover and grass proportions in the sward. There was a significant interaction between seeding date and mixture in the first production year for percent kura in the mixtures ($p<0.0001$).

TABLE 2-3. Average percentage of kura clover in mixtures of kura – Kentucky bluegrass, kura – meadow brome, kura – orchard grass or kura – timothy over four harvests in the first production year (2000) for plots established in 1999 at early or late seeding dates

Early	kura – Kentucky bluegrass	45.0 ± 0.15	a
Late	kura – Kentucky bluegrass	32.9 ± 0.17	b
Early	kura – meadow brome	7.0 ± 0.08	c,d
Late	kura – meadow brome	5.0 ± 0.05	d
Early	kura – orchard grass	5.2 ± 0.08	c,d
Late	kura – orchard grass	2.7 ± 0.03	d
Early	kura – timothy	12.2 ± 0.07	c
Late	kura – timothy	8.1 ±0.04	c,d

* Interaction of seeding date by mixture was significant $p=0.0031$.
**Means in rows with the same alphabetical letter are not significantly different at

The general trend shows earlier seeding results in a higher proportion of kura clover in the total harvested yield (Table 2-3). The only significant difference however is found between early and late sown K-KBG mixtures. For all other kura – grass mixtures the proportion of kura remained consistent between the seeding dates. Early sown K-KBG mixtures have 1.4x more kura than the late seeded K-KBG. To maximize the percentage of kura in a clover–grass sward it is necessary to optimize establishment by sowing early to offset the slow growth rate which can impede the production of the stand in subsequent years (Hill and Mulcahy. 1995). The sensitivity of kura to competition found in this study, is consistent with studies in Minnesota that found that with the exception of timothy and Kentucky bluegrass. kura comprised only 30% of clover:grass swards (Jerenyama et al., 2001).

In this study, kura contributed most to harvested forage when sown with Kentucky bluegrass (Plate 2-2). Early sown K-KBG mixtures had 38.1%, 39.8%, and 32.8% more kura than the early seeded K-MB , K-OG and K-T respectively. Because both kura and Kentucky bluegrass expend energy in the development of rhizomes the overall competition between species is perhaps reduced. Kentucky bluegrass also has a more creeping growth habit that is not aggressive in the early stages of development whereas meadow brome grass, orchard grass and timothy show rapid growth and establish vigorous bunches soon after establishment. Hill and Mulcahy (1995) concluded that the combination of root competition and high root:shoot ratio of kura plants results in low competitive ability to capture light. This is confirmed by visual observations of the kura plants in our plots. When growing with Kentucky bluegrass, height of the kura plants were similar or slightly taller than the grass plants, and leaves of the kura were large and dark green in color. In contrast, when sown with meadow brome, kura plants demonstrate petiole extension, and leaves were small and pale.

TABLE 2-4. Percentage of kura clover in mixtures of kura clover and Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the first production year (2000) for plots established in 1999.

Mixture	Harvest 1	Harvest 2	Harvest 3	Harvest 4
K-KBG	24.5 ± 0.11 b	53.1 ± 0.13 a	48.8 ± 0.15 a	28.9 ± 0.10 b
K-MB	5.7 ± 0.06 c	12.5 ± 0.08 c	3.9 ± 0.04 c	1.4 ± 0.01 c
K-OG	4.6 ± 0.04 c	5.9 ± 0.07 c	3.9 ± 0.08 c	1.6 ± 0.02 c
K-T	5.8 ± 0.04 c	10.6 ± 0.05 c	12.9 ± 0.08 c	11.4 ± 0.07 c

*Interaction of mixture by harvest was significant at $p<0.0001$.
**Means with the same letter are not significantly different at $p<0.05$.

The greatest percentage of kura clover for each mixture occurred at the second or third harvest of the growing season (Table 2-4). This indicates that the grass component of the mixture appears to have been more vigorous early in the season, taking advantage of the early moisture. The dry conditions early in the spring of 2000 may also have influenced the composition of the sward in that the kura will remain dormant until moisture is present in sufficient amounts (Taylor and Smith, 1998) – this may be reflected in the increase in proportion of kura found at later harvests after more precipitation fell.

In the first production year for 1999 established plots, the greatest percentage of kura was found when sown with Kentucky bluegrass (Table 2-3). Significantly more kura was present in the sward at the second and third harvests than in the first and last harvests of the season for the K-KBG mixture (Table 2-4). It is important to note however that at one year of age, kura is still preferentially partitioning carbohydrates toward root development and the true growth potential has not yet been realized (Taylor and Smith, 1998). Thus, the proportion of kura clover in the sward during the first production year may not be an accurate indication of the ability of kura to coexist with the more aggressive grass species.

The data presented for species composition for the plots established in the year 2000, was significantly different than the data presented for the composition of plots established in 1999. The effects of seeding date on percentage kura in the sward was not tested as the late seeded plots were not included in the analysis. The extremely dry conditions in 2000 following late sowing did not allow the kura plants sufficient time to establish themselves and many did not even germinate. As a result, the contribution to the biomass was minimal and therefore the late seeded plots were not considered in the following analysis.

TABLE 2-5. Average percentage of kura clover in mixtures of kura – Kentucky bluegrass, kura – meadow brome, kura – orchard grass or kura – timothy over four harvests in the first production year (2001) for plots established in 2000 at early seeding dates.

kura – Kentucky bluegrass	47.0 ± 0.18	a
kura – meadow brome	14.0 ± 0.12	b
kura – orchard grass	24.1 ± 0.27	a
kura – timothy	33.0 ± 0.15	a

*Main effect of mixture was significant at $p<0.0001$.
**Means with the same letter are not significantly different at $p<0.05$.

The percentage of kura clover found in the various kura – grass mixtures was significantly affected by the grass component of the sward (Table 2-5). This differed from the plots established in 1999 in that for the first production year of the 1999 seeded plots the interaction of mixture and harvest was significant. Similarity occurs between the years however with the highest percentage of kura contribution to the biomass occurring when seeded with KBG. The delay in growth of the orchard grass that occurred due to winter damage, may have allowed the kura opportunity to better establish its presence in the K-OG sward in 2001, becoming a more dominant contributor to the harvested forage biomass. This gives an indication of the negative effect that is placed upon the kura by the vigorous and competitive nature of orchard grass. When comparing the amount of kura present in the 1999 plots with the kura present in the 2000 orchard grass plots we see evidence of the repressive effects of the competition. In 1999 seeded plots the average percentage of kura in early seeded K-OG mixtures was 5.2% as opposed to 2000 early seeded K-OG plots which contained 42.1% kura.

K-KBG, K-OG and K-T all had significantly higher proportions of kura in the sward than was found in the K-MB plots (Table 2-5). K-KBG had 33% more kura in the harvested forage when compared to the K-MB plots and only 14% and 5% more kura than the K-T and K-OG plots respectively.

<u>Second Production Year:</u>

TABLE 2-6. Average percentage of kura clover in mixtures of kura – Kentucky bluegrass, kura – meadow brome, kura – orchard grass or kura – timothy over four harvests in the second production year (2001) for plots established in 1999 at early or late seeding dates

Early	kura – Kentucky bluegrass	45.1 ± 15.0	a
Late	kura – Kentucky bluegrass	27.9 ± 6.0	b
Early	kura – meadow brome	2.9 ± 6.0	d
Late	kura – meadow brome	1.8 ± 0.3	d
Early	kura – orchard grass	12.7 ± 19.0	c,d
Late	kura – orchard grass	10.2 ± 16.0	d
Early	kura – timothy	24.2 ± 17.0	b,c
Late	kura – timothy	12.9 ± 9.0	c,d

*Interaction of seeding date by mixture was significant at $p=0.0391$.
**Means with the same letter are not significantly different at $p<0.05$.

Similar to the first production year, early seeded kura–grass plots show higher percentages of kura clover than the late seeded plots (Table 2-6). The only significant difference however, is found when comparing early and late seeded K-KBG, where early seeded mixtures have 13% more kura than late seeded plots. The proportion of kura clover has increased in both K-OG and K-T mixtures from the first to the second production years though not significantly. For mixtures of K-OG this may be due to the delay in spring growth of the orchard grass allowing the kura plants to initiate growth earlier, reducing competition. The increase in proportion of kura in mixtures of K-T may be due to the poor response of timothy to multiple harvests, which would serve to reduce the competition between the legume and grass. The high proportion of kura in K-KBG mixtures is consistent with observations from previous studies. In Wisconsin, a mix of kura–Kentucky bluegrass produced more total forage and had the most consistent clover:grass ratio over a three year trial period when compared to mixtures of kura–smooth brome and kura–orchard grass (Kim, 1996)

TABLE 2-7. Percentage of kura clover in mixtures of kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the second production year (2001) for plots established in 1999.

Mixture	Harvest 1	Harvest 2	Harvest 3	Harvest 4
K-KBG	32.6 ± 0.14 a,b,c	47.3 ± 0.20 a	35.3 ± 0.06 a	30.1 ± 0.08 a,b,c
K-MB	0.0 ± 0.02 f	3.4 ± 0.09 f	1.0 ± 0.02 f	1.9 ± 0.03 f
K-OG	12.0± 0.15 c,d,e,f	20.4 ± 0.30 b,c,d,e,f	3.7 ± 0.04 e,f	9.9 ± 0.08 d,e,f
K-T	8.1 ± 0.07 e,f	29.4 ± 0.22 a,b,c,d	12.7 ± 0.08 c,d,e,f	24.2 ±0.08 b,c,d,e

*Interaction of mixture by harvest was significant at $p=0.0017$.

**Means with the same letter are not significantly different at $p<0.05$.

A significant interaction between mixture and harvest was observed in the percentage of kura clover in the mixtures in the second production year. The proportion of kura varied greatly both between and within mixtures. The highest proportion of kura was found in association with mixtures of K-KBG (see Table 2-7). As in the first production year, the percentages of kura increased at harvest two and harvest three for K-KBG mixtures. With the exception of K-KBG and K-OG, the lowest amount of kura occurred at the first harvest of the season. The variation in amounts of kura may be due to the changes in the environmental conditions between the harvest dates. Following the first harvest that was primarily comprised of grass, the kura that had been below the cutting height was allowed to grow with reduced competition for light. With the exception of KBG, the grasses at the time of the first harvest were at a reproductive stage of growth, new tiller production was therefore delayed because of the advanced stage of maturity. Also, due to the lack of moisture the regrowth of the grass was slowed further reducing the amount of competition to the clover. As a result, kura contributed more to the harvested yield at the second harvest for each of the kura – grass mixtures. Prior to the third harvest, in mid July, a few days of rain enabled the grass species to again dominate the mixtures decreasing the amount of kura. At the final harvest the percentage of kura increased for all mixtures except K-KBG. This is consistent with the findings of Sleugh et al., (2000) who concluded that the inclusion of legumes in a pasture setting evens out the seasonal distribution of the available forage.

The ability of the kura plants to survive is reflected through changes in species competition between the first and second production years. Changes in kura contribution to harvested yield could result from an increase in the kura, a decrease in the grass component or a combination of both. An increase in the proportion of kura in the mixtures occurred at both early and late seeding dates for K-KBG. K-OG and K-T (Table 2-4. Table 2-6). Kim (1996) found similar results in Wisconsin where the proportion of kura in binary mixtures increased between the first and second production years and remained constant between the second and third production years. In a similar trial. the dry matter yield of kura increased over a three year period when sown in binary mixtures with either Kentucky bluegrass. orchard grass or smooth brome (Zemenchik. 2001). In this study the proportion of kura in the K-MB plots decreased between the first and second production years. It has been found that because the grass and clover compete for light. moisture and soil nutrients. and because of the aggressive nature of the grass. the growth of clovers is often suppressed. The effect of shading when the grass has grown to relatively advanced stages of development reduces the population of the legume component (Donald. 1963).

Forage Quality:

TABLE 2-8. Percentage of crude protein per dry weight of Kentucky bluegrass, meadow brome, orchard grass, timothy, or kura, or mixtures of kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) at four harvests in the first production year (2000) for plots established in 1999.

		Grass Component	Kura Clover	Grass – Kura Mixture
Kura alone	-1		25.38 *a*	
	-2		20.78 *a,b,c,d*	
	-3		21.13 *a,b,c*	
	-4		22.63 *a,b,c*	
K-KBG	-1	13.39 *b*	23.80 *a,b*	20.53 *a,b,c*
	-2		19.17 *b,c,d*	19.25 *b,c,d*
	-3		20.53 *a,b,c,d*	18.48 *b,c,d,e*
	-4		23.43 *a,b*	20.35 *a,b,c*
K-MB	-1	19.81 *a*	N/A	23.63 *a*
	-2		17.78 *c,d*	19.35 *b,c,d*
	-3		15.2 *d*	19.80 *a,b,c*
	-4		N/A	20.43 *a,b,c*
K-OG	-1	17.36 *b*	N/A	21.35 *a,b*
	-2		18.60 *b,c,d*	15.70 *d,e,f*
	-3		17.50 *b,c,d*	14.35 *f*
	-4		N/A	19.08 *b,c,d*
K-T	-1	16.38 *b*	N/A	20.23 *a,b,c*
	-2		19.50 *b,c,d*	14.87 *e,f*
	-3		18.35 *b,c,d*	16.95 *c,d,e,f*
	-4		21.38 *a,b,c*	18.80 *b,c,d,e*

*N/A = too small sample size to perform quality analysis
**For the grass component – main effect of species was significant at $p<0.0001$; for the kura component the interaction of mixture by harvest was significant at $p<0.0001$; for the mixtures the interaction of mixture by harvest was significant at $p=0.0021$
***Means within columns with the same letter are not significantly different at $p<0.05$.

In the first production year there were significant differences between the average percentage of crude protein of the four grass species (Table 2-8). Meadow brome grass had significantly more protein then OG, T or KBG. The high level of protein in MB is due mainly to the large amount of leaf material produced by each plant. Thus, despite having some reproductive stems at the time of harvest, the number of flowering stems was small relative to the number of tillers. In contrast, OG and T also had reproductive stems at the time of harvest but these were in much higher proportion to the total number of tillers and quality may have been lower due to the stage of maturity of the plants. Crude protein content of grasses varies greatly depending on the timing of harvest. For orchard grass. the percentage of crude protein ranged from 23.2% at an early vegetative stage. to 6.6% at early seed set for plants grown in Guelph. Canada (Christie and McElroy. 1995). At the same location the crude protein content of timothy ranged from 23.0% to 5.7%.

The percentage of crude protein in the kura plants varied according to which grass species they were planted with. When sown with MB or OG the kura plants had lower levels of crude protein than when sown with KBG. T or alone (Table 2-8). The decrease in quality may have arisen from the shading effect of the MB and OG. In mixtures with MB or OG. the individual kura plants were observed to have extended petioles and much smaller leaflets than when sown with KBG. T or alone. When comparing the percentage of crude protein in each of the mixtures there is a significant mixture by harvest interaction. The quality was highest. for each species at the first harvest of the season. Because of the low proportion of kura in the MB. OG and T mixtures (<15%). the effects of including kura in the sward on the percentage of crude protein. were not obvious. For the K-KBG mixtures however. kura was a dominant contributor to harvested forage. When comparing the percentage of crude protein of the KBG with the percentage crude protein in the K-KBG mixture it is evident that the inclusion of kura enhanced the quality of the plant material. The presence of kura increased the amount of crude protein of the harvested biomass in K-KBG mixtures. from 5.09 – 7.14% over the growing season. Jerenyama et al.. (2001) had similar findings. concluding that in kura : grass mixtures. crude protein content was negatively correlated to the increasing percentage of grass and positively correlated to the increasing percentage of kura in the mixture.

TABLE 2-9. Percentage of neutral detergent fiber (NDF) per dry weight of Kentucky bluegrass, meadow brome, orchard grass timothy, kura, or mixtures of kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG), or timothy (K-T) at four harvests in the first production year (2000) for plots established in 1999.

		Grass Component	Kura Clover	Grass – Kura Mixture
Kura alone	-1		26.70 *a*	
	-2		35.70 *a*	
	-3		38.35 *a*	
	-4		37.00 *a*	
K-KBG	-1	55.67 *a,b*	30.47 *a*	46.60 *b,c*
	-2	64.93 *a*	33.50 *a*	45.50 *b,c*
	-3	58.95 *a,b*	32.20 *a*	52.88 *a,b,c*
	-4	59.58 *a,b*	24.75 *a*	49.98 *a,b,c*
K-MB	-1	53.20 *a,b*	N/A	49.58 *a,b,c*
	-2	48.33 *a,b*	36.95 *a*	55.08 *a,b*
	-3	49.33 *a,b*	43.27 *a*	57.30 *a,b*
	-4	44.93 *a,b*	N/A	53.78 *a,b,c*
K-OG	-1	40.53 *b*	N/A	46.90 *b,c*
	-2	61.8 *a*	31.85 *a*	60.58 *a*
	-3	58.48 *a,b*	33.05 *a*	61.33 *a*
	-4	50.83 *a,b*	N/A	50.95 *a,b,c*
K-T	-1	48.88 *a,b*	N/A	43.15 *c*
	-2	61.20 *a*	30.38 *a*	54.47 *a,b,c*
	-3	45.73 *a,b*	38.20 *a*	45.88 *b,c*
	-4	50.90 *a,b*	38.03 *a*	45.85 *b,c*

*N/A = too small sample size to perform quality analysis

** For the grass, kura and mixtures the interaction of mixture by harvest was significant at $p=0.0376$, $p<0.0001$ and $p=0.0090$ respectively.

***Means within columns with the same letter are not significantly different at $p<0.05$.

A significant interaction between mixture and harvest occurred for each of the components of the quality study. For the grass component of the mixtures NDF was highest at the second harvest of the season with the exception of meadow brome grass (Table 2-9). This pattern was not apparent in the kura component of the sward or in the grass kura –

mixture where the third harvest generally showed the highest NDF values with the exception of the K-KBG and K-T mixtures. In all cases. the kura component had a lower percentage of NDF than the grass component of the mixtures. and ranged from 24.8% (K-KBG. harvest four) to 43.3% (K-MB. harvest three). When kura was sown with Kentucky bluegrass. NDF in the mixture ranged from 45.5 to 52.9% as compared to the Kentucky bluegrass component alone. which ranged from 55.7 to 64.9% over the growing season. The comparison between grass and mixture demonstrates the effect of adding kura to the sward. In the case of Kentucky bluegrass. the overall quality is enhanced due to a reduction in the NDF. As NDF is inversely proportional to animal intake. including kura in the sward would increase the intake of the grazing animal. For K-MB and K-OG mixtures. little change in NDF occurs between the grass component and the mixture (Table 2-9). The kura component of these mixtures was less than 10% and therefore was not enough to affect the quality parameters. When sown with timothy. kura contributed between 6 and 13% to the biomass. Despite a limited contribution to yield. comparison of the NDF values for timothy and kura- timothy mixtures shows that the inclusion of kura reduced the NDF of the mixture up to 6.73% over the growing season. Similar results were found in Wisconsin. where inclusion of kura in the mixtures increased crude protein and reduced NDF. Kim (1996) reported NDF values of 289 g/kg for solo seeded kura. 410 g/kg for K-KBG. and 435 g/kg for K-OG mixtures.

TABLE 2-10. Percentage of acid detergent fiber (ADF) per dry weight of Kentucky bluegrass, meadow brome, orchard grass or timothy, kura, or mixtures of kura clover with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) at four harvests in the first production (2000) year for plots established in 1999.

		Grass Component ($p<0.0001$)	Kura Clover ($p<0.0071$)	Grass – Kura Mixture ($p<0.0001$)
Kura alone	-1		18.30 *a*	
	-2		27.83 *a*	
	-3		28.78 *a*	
	-4		24.55 *a*	
K-KBG	-1	33.79 *a*	19.47 *a*	29.00 *c,d,e*
	-2		24.23 *a*	28.38 *d,e*
	-3		22.75 *a*	35.83 *a,b,c*
	-4		16.65 *a*	30.10 *b,c,d,e*
K-MB	-1	29.02 *a*	N/A	30.70 *b,c,d,e*
	-2		26.35 *a*	33.48 *a,b,c,d*
	-3		32.43 *a*	36.08 *a,b,c*
	-4		N/A	32.20 *a,b,c,d,e*
K-OG	-1	29.82 *a*	N/A	26.55 *d,e*
	-2		24.75 *a*	37.18 *a,b*
	-3		26.65 *a*	39.20 *a*
	-4		N/A	28.33 *d,e*
K-T	-1	28.98 *a*	N/A	25.40 *e*
	-2		23.38 *a*	33.30 *a,b,c,d,e*
	-3		33.20 *a*	28.88 *d,e*
	-4		20.17 *a*	26.13 *e*

*N/A= too small sample to perform quality analysis

** For the grass component the main effect of species was significant at $p<0.0001$; for the kura and the mixtures the interaction of mixture by harvest was significant at $p=0.0071$ and $p<0.0001$ respectively.

***Means within columns with the same letter are not significantly different at $p<0.05$.

The inclusion of kura showed similar effects on the ADF value of the mixtures as it did on the NDF values (Table 2-10). Kentucky bluegrass had an ADF value of 33.8% on average over the growing season. In contrast the ADF of the K-KBG mixture was reduced to 28.4% at the second harvest. As with NDF. the kura component of the sward had lower ADF values than did the grass component although not significantly so. Thus. if kura is present in the sward and contributes to biomass. the ADF component of the mixture will be less than the grass alone resulting in higher digestibility and greater nutritional value of the fodder.

CONCLUSIONS

Similar to the findings of previous studies. we can conclude that the slow rate of establishment of kura clover leaves it highly susceptible to the effects of interspecific competition. Hill and Mulcahy (1995) described the negative effects that high grass density had on establishing kura swards. Zemenchik et al.. (2001) found that percentage of kura clover in mixture with orchard grass was less than when sown with smooth brome or Kentucky bluegrass due to the productivity of orchard grass over the summer months and the summer dormancy of the other species. Kura was also found to be more competitive in mixtures with short versus tall grasses (Zemenchik et al.. 2001). The seeding rates used for the four grass species in this test followed the recommendations given for central Alberta. These rates however. refer to sowing mixtures with alfalfa. We submit that by reducing the seeding rate of the grass component. the kura will establish itself more prominently in the sward. It has also been suggested that even if the initial plant density of the kura population is low. because of the spreading growth habit. it will increase its contribution to forage biomass over time (Cuomo et al.. 2001).

The high nutritional value of kura clover indicates that when included in pasture swards. the quality of the forage will increase. Kura has a high crude protein content. also the NDF and ADF values are much lower for kura than for the grasses. The use of binary kura – grass mixtures improved the crude protein value of the harvested forage as well as reducing the NDF and ADF values improving the potential digestibility of the forage and should result in increased animal intake. This is especially relevant for producers who have a limited land base. By including kura in pastures, not only will the soil fertility improve due to the nitrogen contribution from the legume, but also the amount of forage intake required by the grazing animal will decrease due to the superior forage quality, increasing the production per land unit.

Based on the results of this study, which is similar to previous studies, kura clover is most compatible when grown with Kentucky bluegrass in Central Alberta. In association with KBG, kura contributed half of the harvested forage yield over the two production years and improved the overall quality of the sward. Due to the aggressive seedling growth of meadow brome and orchard grass, the kura experienced a high degree of competition for light when sown in mixtures with these grass species, which prevented it from becoming a major component of the sward. When the growth of these grasses is slowed, as was experienced in the 2001 growing season by the extended period of dormancy in the orchard grass, the kura was less restricted by competition and greatly increased its presence in the sward. Based on our observations we see potential for the inclusion of kura clover as a tool to increase the time that a legume species remains in pastures, as well as to provide a superior quality of fodder for the grazing animal. Further investigations are required however, to improve our understanding of the best method for integrating kura into the sward.

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CHAPTER THREE

THE EFFECTS OF HARVEST FREQUENCY ON KURA CLOVER YIELD AND FORAGE QUALITY

HYPOTHESIS TESTED

Pure stands of kura clover harvested 1, 2, 3, 4, or 5 times over a growing season will yield the same total phytomass.

Forage quality of kura clover harvested 1, 2, 3, 4 or 5 times over a growing season will be the same at each harvest.

Forage quality at each harvest of kura clover will be the same whether cut 1, 2, 3, 4, or 5 times over the growing season.

INTRODUCTION

Kura clover (*Trifolium ambiguum*) is a perennial clover that is native to the Caucasian region of Russia. Introduced into the United States in 1911, it was used almost exclusively in apiaries (Sheaffer et al., 1992). Kura clover demonstrates development of a massive root system as a result of the preferential partitioning of carbohydrates toward root development in the first two years following seeding (Taylor and Smith, 1998). Kura shows a greater range of tolerances to drought, soil type, pH, elevation, and poorly drained soils than the commonly used legumes in North America (Taylor and Smith, 1998).

Kura grown in pure stands demonstrated persistence under 2, 3, and 4 cut annual harvest frequencies (Sheaffer and Marten, 1991). In the first year following seeding kura showed only 65% ground cover but improved over the next two years surpassing the ground cover of 6 other legume species including alfalfa (Sheaffer and Marten, 1991). The dry matter yield of kura was similar between the 2, 3, and 4 cut treatments and averaged 60% of the biomass obtained from alfalfa.

Peterson et al., (1994) examined the response of 5 year old stands of kura to 3, 4, 5 or 6 cut harvest frequencies per year over three growing seasons. In the first two treatment years the total annual yield was unaffected by cutting frequency. By the final treatment year, the 5 and 6 cut treatments yielded 80% and 70% of the 3 and 4 cut plots respectively (Peterson et al., 1994). For the same treatments, Peterson et al., (1994 b) found that clipping frequency had little effect on the root carbohydrate level of kura plants. Below ground morphology was altered by sheep grazing the plots, with primary crown and taproot mass having decreased under more frequent grazing episodes. For all treatments the secondary crown number and mass were the same at the end of the study. Peterson et al., (1994 b) concluded that kura clover has the ability to maintain a viable root system under a range of defoliation treatments.

In Wisconsin, a study was developed to evaluate defoliation management on kura – grass mixtures using Kentucky bluegrass, orchard grass or smooth brome and solo seeded kura (Kim, 1996). Less frequent harvests increased the total forage production of the plots and prevented weed growth. For the solo seeded kura plots, harvest frequency did not affect the total forage production similar to the findings of Peterson et al., (1994). The forage quality of the kura clover was most uniform over the season under more frequent harvesting regimes (Kim, 1996). The solo seeded kura plots under all harvest frequencies demonstrated consistent yield at each defoliation (Kim, 1996).

The objective of this study was to develop an understanding of the response of kura clover to various harvest frequencies under central Alberta growing conditions via comparison of dry matter accumulation over the growing season. In addition, the nutritive value of the kura was assessed to determine which harvest frequency best suits the nutritive management goals of different producers.

DESIGN AND METHODOLOGY

This study used a complete randomized block design with five treatments and four replications for each treatment. Harvest frequencies ranged from a single, end of season harvest to five cuts taken at three week intervals. The two cut treatment represented a typical hay management strategy and the four cut treatment simulated a rotational grazing system. The trial took place from May 1999 through the 2001 growing season. Pure stands of kura clover seeded at 10 kg/ha were established in the last week of May 1999 and 2000 on a black chernozemic soil with pH = 5.9, salinity = 0.52 dS/m^2, 200 kg/ha available nitrogen, 68 kg/ha available phosphorous, 750 kg/ha available potassium and 81 kg/ha of available sulphates. Individual plots measured 2.4 x 6m, and consisted of 8 seeded rows. In 1999, one half of the kura (5 kg/ha) was seeded with a double disc press drill, 1.25 cm deep with 30 cm row spacing. The remaining clover was broadcast by hand, raked in and packed. Normally the legume would be broadcasted, however, due to the logistics of harvesting, half of the seed was planted using the drill to increase the ease of harvest. In 2000, all kura in plots were seeded with a double disc press drill at 30 cm row spacing. Plots were hand weeded to prevent excessive competition from weeds.

TABLE 3-1. Harvest schedule for kura clover plots established in 1999 and 2000 under 1, 2, 3, 4 or 5-cut harvesting frequency treatments.

1 cut	End of season	September 29, 2000	September 31, 2001	September 31, 2001
2 cut	8 weeks	July 14, 2000 September 29	July 24, 2001 September 31	July 24, 2001 September 31
3 cut	6 weeks	May 30, 2000 July 14 September 29	June 12, 2001 July 24 September 31	June 12, 2001 July 24 September 31
4 cut	4 weeks	May 30, 2000 June 27 July 24 September 29	June 12, 2001 July 11 August 15 September 31	June 12, 2001 July 11 August 15 September 31
5 cut	3 weeks	May 30, 2000 June 20 July 14 August 3 September 29	June 12, 2001 July 3 July 24 August 20 September 31	June 12, 2001 July 3 July 24 August 20 September 31

Plots were harvested for yield using a flail mower and removed a 0.6 x 5.4 m area of the plot. Harvested material was transferred into cloth bags and placed in a forced air drier at 65°C for 48 hours. After each harvest, plots were mowed down with the flail mower to ensure that all trash was removed from the plot area. Plots with a harvest occurring once in the season were harvested by hand, removing a 1m x 1m area. Quality analysis was performed at Norwest Labs in Edmonton, Alberta, samples were analyzed for crude protein, NDF and ADF content.

Statistical analysis of dry matter production at each harvest and over the growing season was compared to determine under which harvesting frequency the greatest biomass production was achieved. The general linear model procedure of SAS (1999) was used for analysis of variance to test for the main effect. Treatment means were separated using a Tukey test and significance was determined at $p<0.05$ (SAS Institute, 1999). Results from the analysis of variance tests are presented in tabular form located in Appendix III (Table III-1 through Table III-6).

RESULTS AND DISCUSSION

Forage Yield

For the total annual yield in the first production year there were statistically significant differences between the plots seeded in 1999 and 2000. Thus, each year was analyzed separately for the total annual yield.

FIGURE 3-1. Total annual dry matter yield (kg/ha) of kura clover grown in monoculture at five harvest frequencies in the first production year (2000) for plots established in 1999.
*Main effect of harvest frequency was significant at $p < 0.0001$.
** Columns with the same letter are not significantly different at $p<0.05$.

In the first production year for plots established in 1999 the main effect of harvest frequency was significant ($p<0.0001$). The greatest total annual yield of kura clover was harvested under 4 cut system which yielded 12986 kg/ha dry matter (Figure 3-1). The four cut system yielded significantly more phytomass than the 2, 3, and 5 cut systems, which did not differ from each other but yielded significantly more than the single harvest treatment. These results differ from previous studies in Minnesota, which found no statistical difference between 2, 3, or 4 cut harvest frequencies (Peterson et al., 1994).

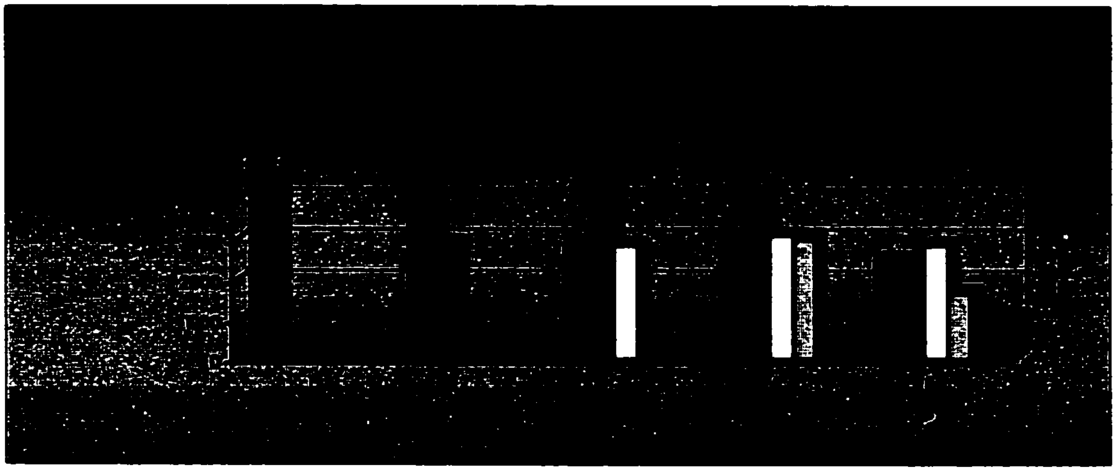


FIGURE 3-2. Dry matter phytomass accumulation (kg/ha) of kura clover grown in monoculture at five harvest frequencies over the first production year (2000) for plots established in 1999.

The regrowth potential of kura clover under the various harvest frequency treatments is shown in the forage yield at each harvest. The most uniform pattern of growth was associated with the 4 cut treatment, which yielded 3001, 3936, 3232, and 3025 kg/ha dry matter at harvest one to four respectively (Figure 3-2). The low yield of the single harvest plots may be related to the stage of maturity the plots reached. Yield under a single harvest system did not maximize growth potential of kura plants which demonstrated a greater amount of growth when kept vegetative through multiple harvests throughout the season. Under the 3, 4, and 5 cut harvest frequency treatments the kura plants remained vegctative for the duration of the growing season. The 1 and 2 cut stands however flowered, and the 1 cut plots even reached a stage of seed maturation by the end of the season when the harvest was initiated (Plate 3-2). The morphology of the plants appeared to be different between harvest frequency treatments. Under the 4, and 5 cut treatment plants were on average 30 cm high at the time of harvest and under a 3 cut system the plants were 45 cm high at the time of harvest. The kura plants under the three, four and five cut treatments had large leaves and did not show the development of a vertical stem. In contrast, under 1 and 2 cut harvest frequencies the plants demonstrated stem development, and under a single harvest plants showed a high degree of lodging. Under a single harvest the canopy height averaged 50cm while stem length was from 75 cm to 100 cm.

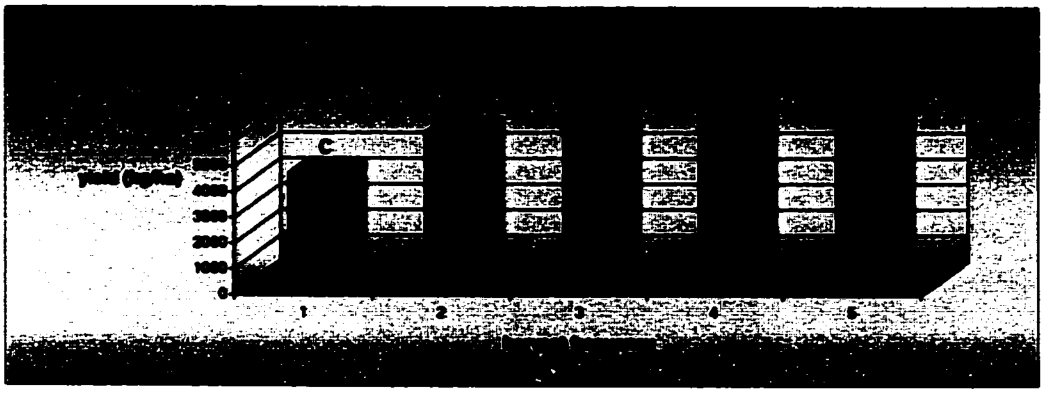


FIGURE 3-3. Total annual dry matter yield (kg/ha) of kura clover grown in monoculture at five harvest frequencies in the first production year (2001) for plots established in 2000.
*Main effect of harvest frequency was significant at $p=0.0002$.
**Columns with the same letter are not significantly different at $p<0.05$.

The plots established in the year 2000 demonstrated a similar pattern of phytomass accumulation as the plots established in 1999 however the total annual yield for plots established in 2000 was up to 4400 kg/ha dry matter lower (2 cut harvest frequency). Because the total yield of plots established in 2000 was overall lower, the differences between treatments became more apparent. As in the 1999 seeded plots, the greatest biomass was obtained from the 4 cut harvest frequency, which yielded 8812 kg/ha dry matter (Figure 3-3).

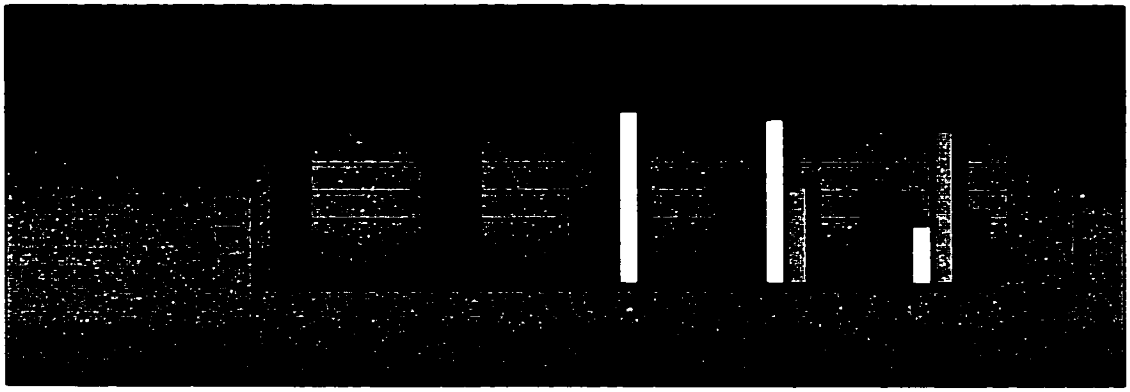


FIGURE 3-4. Dry matter phytomass accumulation (kg/ha) of kura clover grown in monoculture at five harvest frequencies in the first production year (2001) for plots established in 2000.

The 4 cut harvest frequency, once again provided the most uniform distribution of forage yield across the growing season, (Figure 3-4). For plots established in 2000, however, the period of the greatest biomass accumulation was not the same as that seen in plots established the previous year. For the 2 cut plots established in 1999, the first harvest yielded 2.5x more biomass than the second harvest. In contrast, for the plots established in 2000, 2 cut system yields were nearly identical at the first and second harvests. Additionally, the peaks in production were more pronounced for plots established in 2000 as compared to plots established in 1999 in the first year of production. The potential explanation for the more pronounced peaks in production experienced in the 2001 growing season, are the precipitation events that occurred which were later in the season than the previous year. The peaks in production may explain the different pattern of accumulation observed between the two years as well as the performance of each treatment relative to the others. For example, in the first production year of plots established in 1999 the five cut treatment yielded less biomass than the 4 and 3 cut treatments, showing decreasing yields at each subsequent harvest. In contrast, the total annual yield of 5 cut plots established in 2000 was only less than the 4 cut treatments and the difference was not statistically significant. These plots showed a marked increase in the yield following harvest 3, which occurred the second week in July 2001, and after the first rain of the season. The increase in production of harvest four as a result of the rain, under a 5 cut harvest frequency system, was the cause of the increase in total annual yield for the first production year.

The plant height at the time of harvest for the 3 and 4 cut treatments averaged 40 cm, while the 5 cut treatment plots were only 25 cm at the time of each harvest. Similar to the plots established in 1999, the 1 and 2 cut harvest frequency treatments demonstrated the development of a stem however the plants were not as tall as in the previous year and did not show the same degree of lodging. Although flowers were present for both the 1 and 2 cut treatments, seed maturity was not reached most likely because the flowering period occurred later in the 2001 growing season due to the dry conditions (Plate 3-1).

FIGURE 3-5. Total annual dry matter yield (kg/ha) of kura clover grown in monoculture at five harvest frequencies in the second production year (2001) for plots established in 1999
*Main effect of harvest frequency was significant at p= 0.002.
**Columns with the same letter are not significantly different at p<0.05.

Variation in total annual yield between treatments became more apparent in the second production year. With the exception of the single year-end harvest treatment however, the differences between treatments were not statistically significant. Yields in the second production year were also less than the first production year ranging from 3727 kg/ha dry matter for the 1 cut treatment to 9838 kg/ha dry matter for the 4 cut treatment (Figure 3-5). As in the first production year, the 4 cut treatment yielded the greatest biomass in the second production year yielding 9838 kg/ha dry matter. The 4 cut harvest frequency treatment yielded significantly more than the 1 cut treatment but did not differ from the 2, 3 or 5 harvest frequency treatments. Kim (1996) observed that frequency of defoliation did not affect kura clover productivity. Regrowth is largely a function of the photosynthetic capacity of the plant (Kim, 1996). The productivity of kura clover under frequent defoliation treatments may result from the number of leaves that remain below the cutting height (5 cm) allowing the plants to immediately begin regrowth via photosynthates rather than from stored carbohydrates. In contrast, Lucas et al., (1997) found that under frequent defoliation, kura stands lose productivity. The results may be due to the use of sheep, which are able to graze plants to the level of the soil, potentially damaging growing points and removing all vegetative biomass. Current grazing management typically involves leaving a certain percentage of material (30 – 50%) for the purpose of vigorous regrowth and maintenance of stand health. Under such a grazing regime, kura clover remains productive and demonstrates persistence (Kim, 1996; Peterson et al., 1994).

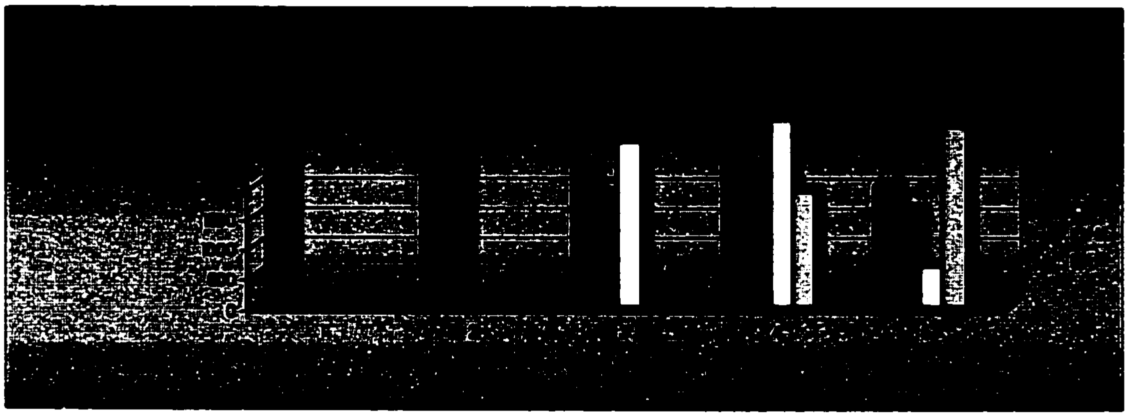


FIGURE 3-6. Dry matter phytomass accumulation (kg/ha) of kura clover grown in monoculture at five harvest frequencies over the second production year (2001) for plots established in 1999.

As in the first production year, the 4 cut harvest frequency treatment demonstrated the most uniform forage distribution across the growing season. When comparing the first and second production years we see similar patterns of biomass accumulation in the 2 cut treatment where more forage was produced at the first harvest of the season (Figure 3-6). For the 3, 4 and 5 cut harvest frequencies the pattern of growth is somewhat altered from the first production year. Whereas in the first production year the second harvest yielded the greatest biomass, the greatest phytomass in the second production year came at a later harvest. Additionally, the spikes in production were more pronounced in the second production year as compared to the first year of production. The potential explanation for the peaks in production experienced in the 2001 growing season are the precipitation events, which were later in the season than the previous year. The peaks in production in the second production year (Figure 3-6) that occurred at the third harvest of the four cut treatment and the fourth harvest of the five cut treatment are also evident in the first production year of the 2000 seeded plots (Figure 3-4). The growth curve of the kura in monoculture was highly dependent on the moisture available for growth, thus the differences in regrowth pattern between the first and second production years.

<u>Forage Quality</u>

The analysis for forage quality of kura clover under multiple harvests was performed on samples obtained from the first production year of plots seeded in 1999. The morphology of the plants in the single harvest treatment, where vertical stem extension and lodging was common, made harvesting difficult, so a quality sample was not taken. Obtaining a representative sample from the collection was not possible as the leaves and flowers had fallen off the stems during the drying process, and the appropriate leaf : stem ratio could not be ascertained.

TABLE 3-2. Average percentage of crude protein, neutral detergent fiber and acid detergent fiber per dry weight of kura clover in the first production year for four harvest frequency treatments.

2 cut	17.58	c	41.53	a	34.80	a
3 cut	19.52	b	33.97	ab	25.67	b
4 cut	22.36	a	33.01	b	24.33	b
5 cut	23.49	a	31.40	b	24.40	b

* Main effect of harvest frequency was significant for crude protein $p=0.0004$; NDF $p=0.0216$; ADF $p=0.0046$.
**Means within columns with the same letter are not significantly different at $p<0.05$.

Harvest frequency was positively correlated to the percentage of crude protein and inversely proportional to the percentage of neutral detergent fiber and acid detergent fiber of the kura (Table 3-2). As the frequency of harvests in the growing season decreased the kura plants had longer to regrow and the leaf:stem ratio appeared to decrease as the plants developed more supportive structures. This is consistent with other forage legumes, as the plants mature the concentrations of NDF and ADF increase due to a decrease in the quality of the stem and an increase in the proportion of stem to the total plant (Albrect and Hall, 1995). The forage quality of hay is graded according to the species composition and nutritive value.

For alfalfa hay to be considered prime it must have a crude protein content greater than 19%, NDF of less than 40% and an ADF of less than 31% of the dry matter (Ball et al., 1991). Kura clover under 3, 4, and 5 cut harvest frequencies exhibited quality characteristics that easily met these requirements and kura harvested twice per season followed closely in category number one.

TABLE 3-3. Percentage of crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) per dry weight for kura clover at each harvest under a two cut harvest system.

[redacted]

			Crude Protein		NDF		ADF	
1	July 24, 2000	7391.98	17.05 ± 0.63	a	47.25 ± 2.33	a	44.85 ± 1.62	a
2	September 31	2932.10	18.00 ± 0.71	a	35.80 ± 0.42	b	24.75 ± 0.63	b

* Main effect of harvest frequency was significant for NDF $p=0.0208$; ADF $p=0.0038$.
**Means within columns with the same letter are not significantly different at $p<0.05$.

Unlike the three and four cut treatments, the two cut treatment showed higher quality at the second harvest than at the first (Table 3-3). The higher level of crude protein and lower levels of the fiber components at the second harvest likely result from the stage of growth the kura plants were in at the time of harvest. As the first cut was not initiated until mid-way through the summer, kura plants were allowed to develop more vertical stems. The volume of forage harvested was larger at the first harvest than the second, however the overall nutritional value of the plants was reduced. The decrease in forage quality associated with an increase in harvested yield is well documented for alfalfa, where the timing of harvest must achieve a balance between yield and quality.

Following the first harvest, due to the decreasing daylength as well as the need to store carbohydrates for winter survival, kura plants did not grow to the stage observed at the first harvest. Therefore, at the second harvest the plants had only leaves and petioles and did not have the need for more supportive structures and the overall quality was then higher than the first harvest of the season.

TABLE 3-4. Percentage of crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) per dry weight for kura clover at each harvest under a three cut harvest system.

1	May 30, 2000	2770.06	24.05 ± 0.49	a	23.35 ± 0.92	a	17.65 ± 3.46	a
2	July 14	5655.86	16.30 ± 0.99	b	43.85 ± 3.61	b	36.10 ± 0.57	b
3	September 29	2862.65	18.20 ± 0.71	b	34.70 ± 5.37	a,b	23.25 ± 0.49	a,b

*Main effect of harvest frequency was significant for crude protein p=0.0041; NDF p=0.0279; ADF p=0.0062).
**Means within columns with the same letter are not significantly different at p<0.05.

When harvested three times per year, kura plants demonstrate changes in quality. The first harvest had significantly more crude protein than the second and third harvests and showed significantly lower levels of NDF and ADF than the second harvest in the season (Table 3-4). The variance in quality between harvests was not found in a Wisconsin study where the quality of kura clover remained consistent between harvests when cut 3, 4, or 5 times per season (Kim, 1996). The increase in forage quality that occurred at the end of the growing season may have arisen due to reduced growth, consistent with lack of moisture and decreasing day length. These factors may have contributed to a concentration of nutrients in the foliage as the plant continued to take up minerals at a fast rate even when growth slowed.

TABLE 3-5. Percentage of crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) per dry weight for kura clover at each harvest under a four cut harvest system.

1	May 30, 2000	3000.96	25.05 ± 0.35	a	31.15 ± 9.40	20.10 ± 1.56
2	June 27	3935.19	24.15 ± 0.64	a	N/A	N/A
3	July 24	3132.72	24.15 ± 0.49	a	36.30 ± 3.39	27.70 ± 1.56
4	September 29	3024.69	18.25 ± 1.20	b	30.10 ± 3.68	24.50 ± 4.38

*Main effect of harvest frequency was significant for crude protein p= 0.0025.
**N/A = too small sample size to perform quality analysis
***Means within columns with the same letter are not significantly different at p<0.05.

Forage quality of kura under a four cut harvest frequency showed a significant decrease in the amount of crude protein at the final harvest of the growing season (Table 3-5). The levels of NDF and ADF did not differ between harvests, and remained well below the threshold qualifications for prime quality alfalfa (Rohweder et al., 1979).

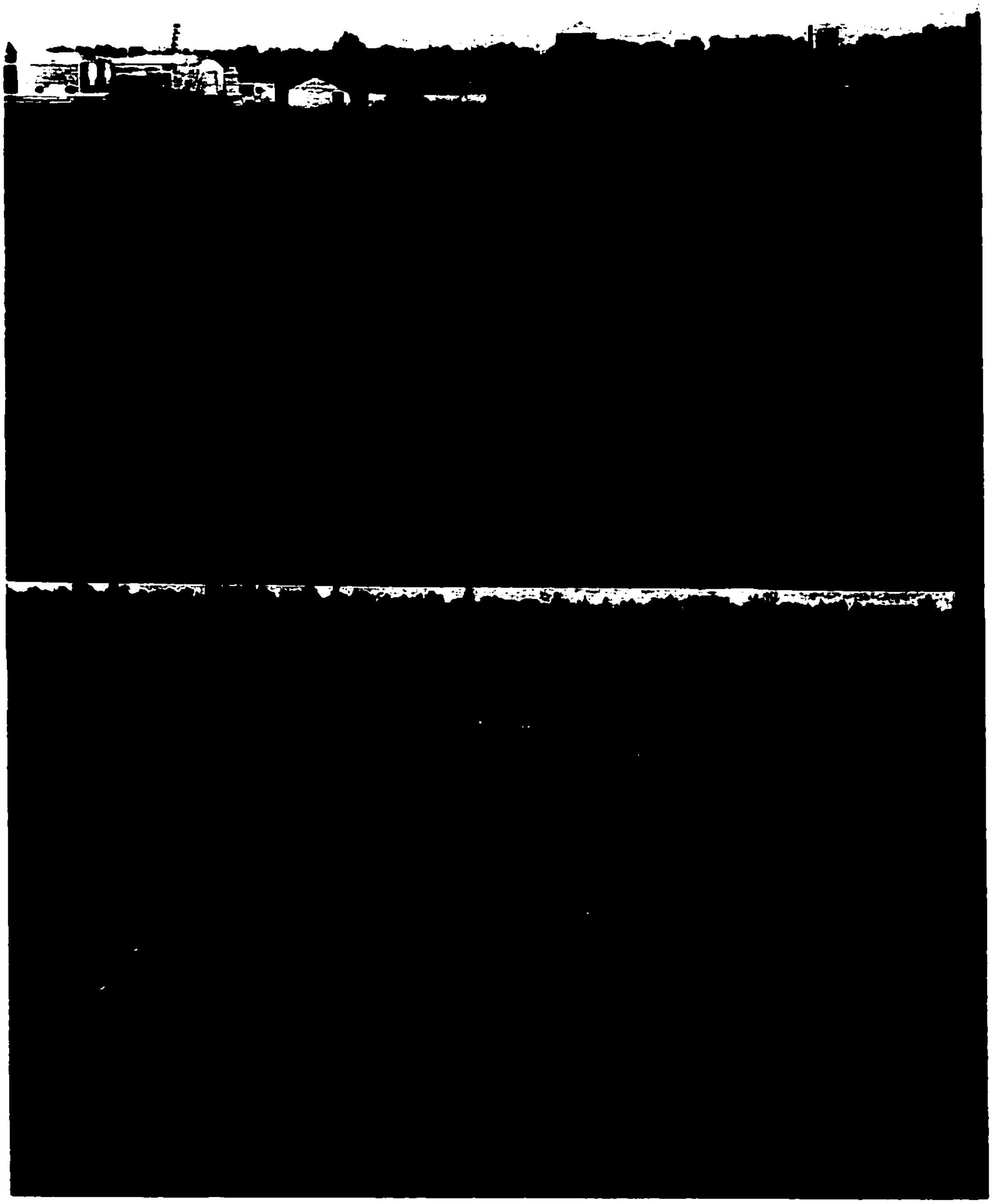
TABLE 3-6. Percentage of crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) per dry weight for kura clover at each harvest under a five cut harvest system.

[redacted]

1	May 30, 2000	2546.30	25.50 ± 1.27	a	26.35 ± 0.49	a	20.80 ± 0.42	a
2	June 20	2430.56	24.15 ± 0.07	a,b	N/A		N/A	
3	July 14	2878.22	22.60 ± 0.71	b	34.10 ± 1.56	b	28.15 ± 5.16	a
4	August 3	1682.10	26.60 ± 0.28	a	34.40 ± 0.71	b	25.80 ± 0.42	a
5	September 29	956.79	20.75 ± 0.64	b	27.65 ± 2.19	a	20.75 ± 0.21	a

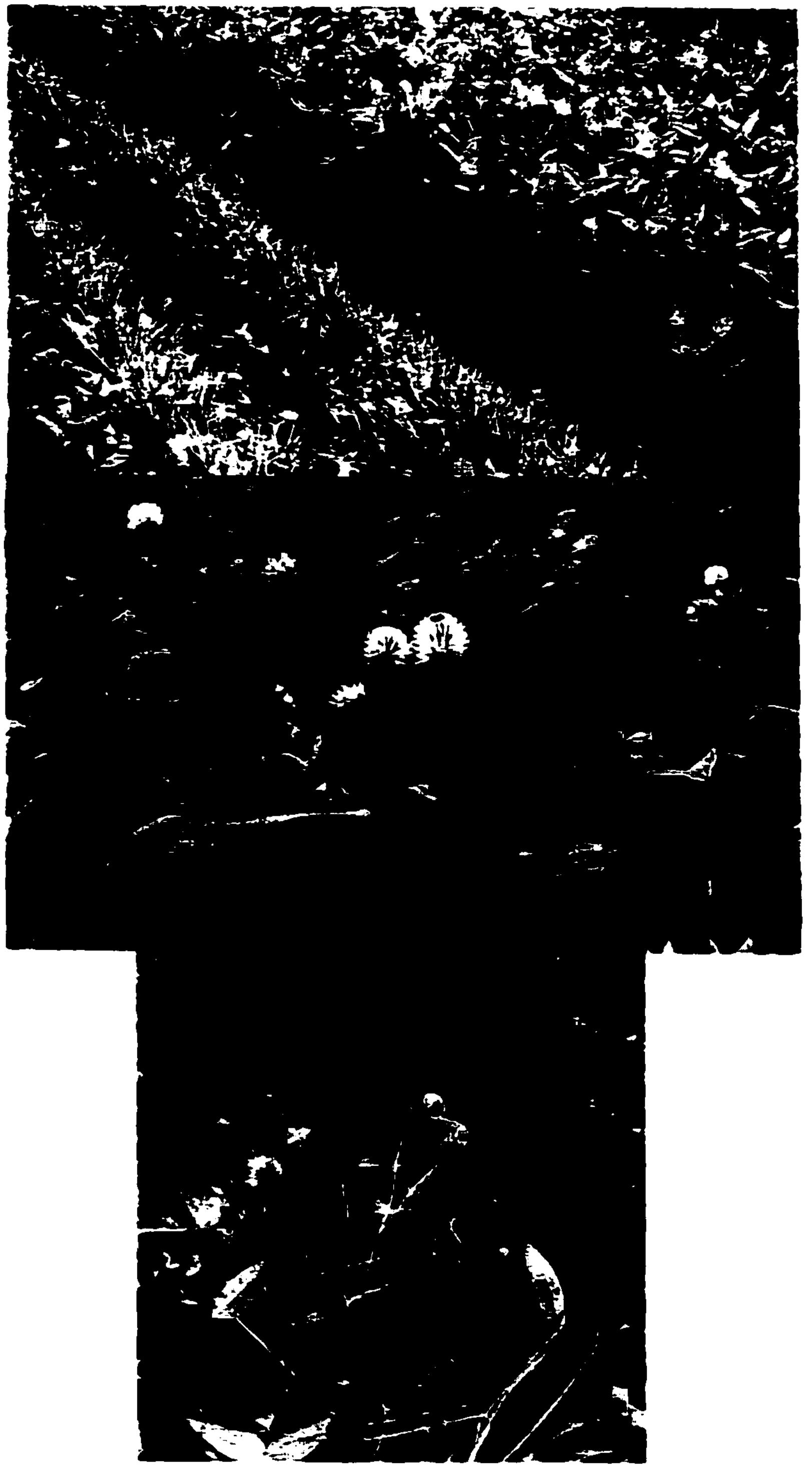
*Main effect of harvest frequency was significant for crude protein $p=0.00026$; NDF $p=0.0039$).
**N/A = too small sample size to perform quality analysis
***Means within columns with the same letter are not significantly different at $p<0.05$.

Variation occurred in the crude protein and NDF percentages when kura was harvested five times per growing season. With the exception of the fourth harvest, crude protein percentages tended to decrease and NDF levels to increase as the season progressed. There was no differences between the ievels of ADF in the kura plants at each of the five harvests. Because the frequency of cutting is high, the kura plants regrew leaves and petioles resulting in high quality forage. This is consistent with the nutritional value of alfalfa, which is higher under a more intense harvest schedule (Albrecht and Hall, 1995).



A. Kura clover harvest frequency test in the first production year (July 2001) for plots established in 2000.

B. Kura clover harvest frequency test in the second production year (July 2001) for plots established in 1999.



A. Kura plots following harvest, showing harvested rows as well as rows that were not harvested (July 2001).

B. Kura plants in flower.

C. Kura flowers at maturity.

CONCLUSIONS

The data from this experiment agrees with that found previously by Peterson et al. (1994). and Kim (1996). Kura was found to be productive under a variety of defoliation treatments. Total phytomass production was found to be similar whether the clover was harvested 2. 3. 4. or 5 times over the growing season. yielding up to 9.8 t/ha in the second production year. The most uniform forage growth was found to occur when kura clover was harvested at 4 week intervals over the season. Under a five cut harvest frequency system. the yield of kura at the end of season is reduced and in the case of drought. there may not be enough regrowth to warrant a harvest. The ability to produce consistent amounts of forage under a variety of harvest frequencies demonstrates the plasticity of kura. Kura therefore has the potential to be utilized in a number of different ways and under several management strategies.

The nutritional value of kura when harvested multiple times in a season was found to be consistently high. Compared with the quality standards that have been determined for alfalfa hay. kura surpasses the "prime" requirements for crude protein content. NDF and ADF when harvested 3. 4. or 5 times per season and remains in the first category under a two cut harvest system (Rohweder et al.. 1979). This is similar to other studies. which found that the quality of kura clover increased with harvest frequency (Sheaffer and Marten. 1991). Previous work comparing the forage quality of kura to several other legume species found that kura had a higher in vitro dry matter digestibility than alfalfa. birdsfoot trefoil. white clover. crown vetch and cicer milk vetch (Allinson et al.. 1985). Kura was also found to have lower ADF and ADL levels than alfalfa under similar harvest treatments (Allinson et al.. 1985). suggesting that the digestibility of kura clover is similar to or potentially greater than alfalfa and other commonly used legume species.

The ability of kura clover to be productive when harvested at high frequencies in a simulated grazing environment gives an indication of its usefulness as a nutritive and reliable source of forage.

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CHAPTER FOUR

ETIOLATED GROWTH OF KURA CLOVER

HYPOTHESIS TESTED

Etiolated regrowth of kura clover will be the same for plants seeded in May and July for treatments commencing at the end of the growing season in the establishment year.

Etiolated regrowth of kura clover will be the same for plants dug at the end of the growing season in the establishment year as the following spring.

Etiolated regrowth of kura clover will be the same for kura plants seeded in mixtures with either Kentucky bluegrass. meadow brome. orchard grass. timothy or kura plants seeded alone for treatments commencing at the end of the growing season in the establishment year.

INTRODUCTION

The initial growth of perennial forages in the spring and following harvests is fueled by energy that has previously been stored in the taproot, crown or at the base of the leaves and stems (Nelson, 1995). The higher the level of these reserves, the faster the regrowth and the higher the yields. Stored carbohydrates are also essential for the development of cold hardiness and successful persistence throughout the winter months (McKenzie et al., 1988).

Etiolated growth from a taproot is a method used to quantify the available root biomass that can be allocated toward vegetative regrowth (Reichel, 1993). Etiolated regrowth is growth in the absence of both light and nutrients and is therefore dependent on the non-structural carbohydrates in the root. Thus, the dry weight of the growth produced under these conditions in addition to the weight loss due to respiration equals the amount of non-structural carbohydrate in the root (Reichel, 1993).

Etiolated regrowth has been used to determine food reserves in alfalfa plants as they enter autumn and winter periods (McKenzie et al., 1988). McKenzie et al., (1988) found that the total mass of etiolated regrowth reached a maximum in the fall and declined throughout the winter, decreasing further as plants entered the spring. Chemical testing for stored carbohydrates found that non-structural carbohydrate levels increased in the fall, reaching a maximum in midwinter and then declined in the spring (McKenzie et al., 1988). The results suggested that not all non-structural carbohydrates stored in the roots are used for growth, a constant level of carbohydrates remained in the roots and crowns of the plants even after etiolated growth had stopped (McKenzie et al., 1988).

Kim (1996) examined the spreading ability of kura clover planted in existing grass swards, grass + nitrogen fertilizer, or killed grass swards, using 1 year-old stands of Kentucky bluegrass or smooth brome under two defoliation treatments. The kura plants were planted as plugs that had been started either from seed or from rhizome cuttings. In the two years following planting, kura plants in the killed grass plots spread 1 m in diameter whereas in the fertilized grass plots they spread 29 cm in the second year following planting. The defoliation height did not affect the ability of the kura to spread, however, the grass species that kura was sown into did. Kura plants spread more when sown into smooth brome plots as compared to Kentucky bluegrass. Kim (1996) concluded that the dense sod of Kentucky bluegrass inhibited the spreading ability of the kura via the suppression of rhizome development.

DESIGN AND METHODOLOGY

Etiolated regrowth trials were used as an indirect measure of the amount of non-structural carbohydrates stored in the roots of kura clover for the purpose of winter survival and the initiation of spring growth. Following the first fall frost in 1999 and 2000, five kura plants were dug from the eight solo seeded kura plots used in the kura:grass mixture trials, at random locations throughout the plot. The kura plants had been established in May or July of the same year. Plants seeded in May were harvested 8 weeks following seeding and allowed to regrow. Once the plants were removed from the soil the roots were washed and the length of the primary taproot was measured. The leaves of each plant were removed, counted and dried at 60 °C for three days. The number of meristems, or shoots that showed initiation of leaf production, were recorded as was the wet weight of the roots and crowns of each kura plant. The roots were then potted in perolite/vermiculite and placed in a growth chamber in the dark, at 20 °C for 12 hours /day and 18 °C for 12 hours /day. A similar procedure was followed in the spring using three plants from each of the eight solo seeded kura plots of the kura – grass mixture test.

Etiolated regrowth was harvested at 4 week intervals. At each harvest, the number of leaves were counted, leaves were removed and dried at 60 °C for 2 days, after which the dry weight was recorded. Upon the termination of leaf production, the roots and crowns were removed from the pots, washed and dried at 60 °C for 5 days.

Statistical analysis of the accumulated leaf biomass was used to compare the amount of non-structural carbohydrates available for winter survival and growth in the spring. Data were analyzed using the general linear model of SAS (1999) to test the analysis of variance between early and late seeded kura treatments. Means were separated using the Tukey test, significance was determined at $p<0.05$ (SAS Institute, 1999). Results of the analysis of variance tests are presented in tabular form and located in Appendix IV (Table IV-1).

In the fall of 2000, a similar test was initiated that took plants from the kura – grass mixture test established in 2000, and examined etiolated regrowth. In this test, kura plants were removed from the solo seeded kura plots as well as from the kura – Kentucky bluegrass, kura – meadow brome, kura- orchard grass and kura – timothy mixtures. In addition to the information gathered above, the number of rhizome initials were also counted. Plants were potted in vermiculite and placed in a growth chamber in the dark, at 20 °C for 12 hours /day and 18 °C for 12 hours /day. Plants were harvested at 4 week intervals as above and the dry weight of the leaves determined.

Statistical analysis was performed to determine if the companion grass affected the morphology of the kura plants including the non-structural carbohydrate storage. The general linear model procedure of SAS (1999) was utilized for analysis of variance to test the main effect of mixture. Treatment means were separated using a Tukey test and significance was tested at the 95% confidence interval (SAS Institute, 1999). Results of the analysis of variance tests are presented in tabular form and located in Appendix IV (Table IV-3 through Table IV-6).

RESULTS AND DISCUSSION

Early seeded (May) kura plants removed in the fall produced significantly more etiolated regrowth than plants that had been seeded later (July) in the season ($p<0.0001$) (Table 4-1). Individual kura plants averaged 0.562g or 0.034g of etiolated regrowth for early and late seeded plants, respectively. A similar trend was found for plants in the spring, early seeded kura plants produced significantly more etiolated mass than the late seeded plants (0.671g and 0.071g respectively). The length of time that individual plants demonstrated etiolated growth varied greatly within treatment groups and from fall to spring. The size of the plants that were removed from the plots also varied (Plate 4-1), due to random selection, which would affect the amount of carbohydrates that the plants had the capacity to store. The importance of early sowing was reflected in the poor development of the kura plants that were sown in July 2000, which had only one or two true leaves per plant at the time of the first fall frost and did not survive the following winter.

TABLE 4-1. Etiolated regrowth (g of dry weight) of kura clover plants established at either early or late seeding dates and removed from the soil in October or the following spring.

Fall	Early	12 – 36	0.562	a
	Late	12 – 20	0.034	b
Spring	Early	4 – 12	0.671	a
	Late	0 - 4	0.071	b

*Means within columns with the same letter are not significantly different at p<0.05.

TABLE 4-2. A comparison of the average number of meristems, leaves, rhizomes, leaf area and the etiolated growth of kura clover plants when grown in mixtures with either kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) or of kura plants grown alone (K) at the end of season in the establishment year.

Kura alone	4.55	a	12.66	a	175.93	2.0	a	0.456	a
K-KBG	3.00	b	8.74	a,b	100.35	1.47	a,b	0.564	a
K-MB	1.83	b	7.22	b	82.11	0.44	b	0.514	a
K-OG	1.41	b	5.18	b	83.93	0.18	b	0.357	a
K-T	2.32	b	8.53	a,b	171.93	1.05	a,b	0.321	a

*Means within columns with the same letter are not significantly different at p<0.05.

The plasticity of kura plants which allows them to adapt, is reinforced by the variance in physical characteristics of the plants when grown with different grass species. Growth of petioles and stems is initiated by meristems capable of vegetative reproduction. The number of meristems that the kura plants had was influenced by the companion grass species (Table 4-2). When grown in monoculture, kura plants averaged significantly more shoots per plants than when grown in binary mixtures. There were no significant differences in the number of meristems per plant for kura grown with either Kentucky bluegrass, meadow brome, orchard grass or timothy. Kura plants also had significantly more leaves per plant when grown alone versus kura grown with meadow brome or orchard grass but did not differ from the average number of leaves of kura plants sown with Kentucky bluegrass or timothy.

Although not significant. there were differences in the leaf area of the kura plants (Table 4-2). When kura is grown in monocultures the area available for light capture per plant was 175.93 cm^2. followed by kura grown with timothy (171.93 cm^2). kura grown with Kentucky bluegrass (100.35 cm^2). kura grown with orchard grass (83.93 cm^2) and finally. kura grown with meadow brome (82.11 cm^2). The shading effects of the more aggressive grass species. (i.e. MB and OG) appeared to result in kura plants with fewer leaves and therefore should reduce the plants ability to compete for light.

The average number of rhizomes per kura plant was also affected by the companion grass species (Table 4-2). Similar to the number of meristems. leaves and leaf area. the greatest number of rhizomes per plant occurred when kura was grown in monoculture. This is similar to studies on white clover. which found that when grown in monoculture. the individual white clover plants were heavier and more complex in structure than when grown in binary mixtures (Sanderson and Elwinger. 1999). The number of rhizomes per kura plant from pure stands. was significantly greater than the number of rhizomes for kura plants grown with orchard grass and meadow brome where rhizome development was rarely found. Kura plants grown with Kentucky bluegrass or timothy. showed the development of rhizomes but were not significantly different from any other treatment. The greater number of rhizomes per plant for kura clover sown with Kentucky bluegrass is in contrast to Kim (1996) who concluded that the dense sod of KBG restricted the rhizome development of the kura. Peterson et al.. (1994) noted that the number of rhizome initials may be used as a measure of the plant vigor as rhizomes are an indicator of the amount of below ground growth. The reduction in vegetative structures in this study. reflected the biomass production of the kura plants when grown with various grass species. The greater number of leaves and meristems when kura is grown with KBG is consistent with the greater proportion of kura in a K-KBG stand found in the kura – grass mixture experiment. Studies in Wisconsin also showed that kura yielded more dry matter when sown with KBG than with smooth brome (Kim. 1996).

The etiolated growth of kura plants was not significantly affected by the presence or absence of a companion grass species (Table 4-2) however. plants growing with OG and T had less than 60% of the levels of plants growing with KBG. The small sample size (20 plants per treatment). and large plant to plant variation in carbohydrate levels may have accounted for the lack of significant differences between the treatment groups. Etiolated growth of kura plants ranged from 0.321 – 0.564 g of dry matter when sown with timothy or Kentucky bluegrass respectively. Kura plants were smaller. with fewer leaves and rhizomes when grown in mixtures with grasses vesus when grown alone. The smaller plant size did not affect the plants ability to store carbohydrates. This suggests that the ability of kura to make and store carbohydrates is not compromised when in binary mixtures, as demonstrated by the etiolated regrowth of the kura plants. Thus, the reduced ability to capture light, fewer leaves and meristems that were found to occur in mixtures with grass did not suppress the plants ability to prepare for winter. Peterson et al., (1994) found that for established stands of kura clover, storage of total non-structural carbohydrates was highest in the taproot during the spring and shifted to the rhizomes during the late summer and fall of the year.

A. Kura plants at the time of removal from the soil (October 1999) from early seeded plots, and...
B. From late seeded plots.
C. Etiolated regrowth of kura (growth without light or nutrients).
D. Kura plants in the growth cabinet.

E.

CONCLUSIONS

One of the vital characteristics that is required for a perennial plant to become incorporated into widespread use in central Alberta is the ability to over winter. As species are selected for improved productivity. the winter hardiness of plants is often compromised. such as in alfalfa (Alberta Forage Manual. 1998). Kura clover is known to have long – term persistence in pasture communities (Taylor and Smith. 1998) however. for it to become useful in Alberta it must also demonstrate the ability to survive the winter months. Etiolated regrowth can give an indication of the carbohydrate reserves within plant roots and crowns that can be mobilized for winter metabolism and the initiation of spring growth (Reichel. 1993). The ability to store carbohydrates does not guaranty the survival of the plant. If exposed to killing temperatures. death will result independent of the level of stored carbohydrate.

In this study. kura clover sown in the spring had more carbohydrates available for winter survival than kura plants sown mid-way through the growing season. Sowing kura with a companion grass altered the morphology of the kura plants but did not significantly affect etiolated regrowth. The number of meristems. leaves and rhizomes were reduced when kura was grown in binary mixtures as opposed to kura grown in monoculture. The results of this study suggest that if planted early. kura clover has the ability to store sufficient carbohydrates to survive an Alberta winter.

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CHAPTER FIVE

CONCLUSIONS

It is well known that inclusion of legume species in pasture mixtures is beneficial in several ways. The ability of legumes to fix nitrogen can provide a source of biological nitrogen to surrounding plants reducing the need to apply chemical fertilizer. Including a legume species with grasses can help even out seasonal distribution of the forage source resulting in more uniform season long production. The presence of legume species in a mixture. can increase the crude protein level of the sward.

Forage production provides the foundation for the beef. dairy. equine. sheep and managed wildlife industries of Alberta. At present. there is no legume that aptly fits the wide gradient of soils and moisture levels found in a variety of environments within the province. A significant problem in managed pasture ecosystems in Alberta. is the lack of a persistent legume species. particularly in intensively managed pastures. Studies from the United States. Australia and New Zealand have shown that kura clover (*Trifolium ambiguum*) can continue to be productive in grass – legume swards for >20 years under a range of grazing intensities. Kura clover has the potential to become a valuable component of grass – legume mixtures for grazing systems but has not previously been tested under Alberta growing conditions.

In order for kura to become adopted into use. several important questions must be answered. In this study. by integrating the results of the harvest frequency. grass – kura mixtures. and etiolated regrowth tests. a foundation of knowledge has been obtained from which we can better direct future studies.

Firstly. to become accepted for use. kura must demonstrate the ability to persist throughout the winter months. During the trial. kura plants survived three winters. each with varying climatic conditions. Over the winter of 2000. very little snow cover was present and temperatures fluctuated greatly. however. the kura plants showed little damage and no plants were lost due to winter kill. Additionally. the ability of kura to prepare itself for winter survival is strongly supported by our data on etiolated phytomass production and the length of time over which plants demonstrated etiolated regrowth. Plants in their seedling year. sown in May and removed from the soil in October. produced phytomass for 12 – 36 weeks without nutrients or light while plants sown in July produced phytomass for 12 – 20 weeks under the same conditions. The results of our test reflect those of prior studies. that found young kura plants concentrate their initial development on producing underground structures (Taylor and Smith. 1998: Speer and Allinson. 1985).

Because grazing pure stands may cause bloat in ruminants. it is necessary to plant kura in binary mixtures with grass species. In order for kura to become widely utilized in

Alberta. grass species must be identified. that are appropriate as companion grasses. Four commonly used grasses (Kentucky bluegrass. meadow brome. orchard grass and timothy) were chosen for their variation in growth habit and range of competitive abilities. and sown in mixture with kura clover. Kura in the seedling stage was negatively affected by competition from grasses. Meadow brome and orchard grass showed rapid development in the establishment year and subsequently. kura plants experienced significant amounts of shading. Under a four cut harvest frequency kura contributed very little to forage production when sown with MB or OG. In contrast. when sown in mixtures with Kentucky bluegrass. kura clover contributed up to 60% of the phytomass that was produced in the year following seeding. The large gradient of productivity of the clover directly results from the level of interspecific competition with the grass species. The resiliance of kura plants in spite of the high level of competition. is reflected through changes in species composition between the first and second production years when an increase in the proportion of kura in the mixtures occurred. This is supported by previous work. which determined the productivity of kura in mixed stands increased over time (Kim. 1996: Cuomo et al.. 2001: Zemenchik et al.. 2001).

The suppression of clover productivity when sown with MB or OG was reflected in the morphological changes in plants when compared to kura grown in monocultures. In monoculture. kura plants had significantly more meristems per plant than when grown in binary mixtures. Kura plants from solo seeded plots. also had significantly more leaves and rhizomes than plants taken from mixtures of K-MB and K-OG. a further indication of the effects that aggressive grass species have on establishing kura plants.

Of the species studied. Kentucky bluegrass was the most compatible with the kura under the conditions of this study. Mixtures of K-KBG did not produce the greatest yield when compared with mixtures of K-MB. K-OG or K-T. however by the second production year K-KBG stands yielded significantly more than K-OG and K-T. Additionally. when sown with Kentucky bluegrass kura clover exhibited better establishment. with larger plants having more stems and leaves than in other kura–grass mixtures. Since both kura and KBG expend energy developing rhizomes. the overall competition between the two species may be reduced by the more evenly matched productivity levels.

If kura is to be included in pasture mixtures. some benefit must be realized. The inclusion if legumes in grass swards can provide a natural source of nitrogen to the sward as well as increases in the overall protein level of the forage. Kura clover was found to have high nutritional value with crude protein levels above 20%. NDF levels from 26 – 38% and ADF levels of 18 – 28% when sown in monoculture. The use of binary kura-grass mixtures improved the crude protein value of the harvested forage when kura formed a significant percentage of the phytomass. and reduced the NDF and ADF values. These changes should improve the digestibility of the forage. and increase animal intake and thereby improve animal gain. Previous work comparing the forage quality of kura to several other legumes. found that kura had digestibility greater than alfalfa (Allinson et al.. 1985).

Understanding the growth pattern of kura under several harvest frequencies should allow for planning timing and intensity of grazing periods. Kura was found to be productive under a variety of defoliation treatments. Total phytomass production was similar whether the clover was harvested 2. 3. 4. or 5 times per season. The most uniform distribution of growth occurred when kura was harvested four times per season. at monthly intervals. The ability of kura to produce a consistent amount of forage under a variety of harvest frequencies demonstrated the adaptibility of the plants. By understanding the response of kura to multiple harvests. a companion grass species with a complimentary growth pattern can be selected according to the needs of the grazier.

Based on the results of this study. kura demonstrated the ability to survive the growing conditions in central Alberta. Kura showed persistence under a range of harvest frequencies. Kura clover also exhibited the ability to establish itself when grown with a companion grass species. However. in order for kura to become readily accepted for use in pasture mixtures several things must be further investigated.

Most importantly. the seeding rates of the grass species were those recommended for central Alberta. The rates used in this study were based on seeding mixtures with alfalfa. The ability of kura to establish itself in binary mixtures may be enhanced by reducing the seeding rates of the companion grasses to reduce competition.

The response of kura clover to both mixture and harvest frequency treatments was based on mowing as a simulation of grazing. Kura clearly demonstrated potential for use as a pasture legume. however. the response of plants to the grazing animal remains to be determined.

For kura to become adopted for use in western Canada there must exist a local seed source. At the present time. no information exists regarding the ability of kura clover to produce seed in Alberta. Flowering and seed production were observed in our study. in the first production year under a single harvest system. Thus. it appears possible for kura clover to produce viable seed in Alberta and further study is required to determine optimal management strategies to maximize seed yield.

The results of our study give an indication of the potential of kura clover for use in Alberta. The increase in forage quality of kura – grass mixtures, productivity under multiple harvest systems and good winter survival are positive indicators regarding the potential benefits that could be realized by adopting kura for use in Alberta.

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APPENDIX

APPENDIX I

TABLE I-1. Annual dry matter yield (kg/ha) of mixtures of kura clover with either Kentucky bluegrass, meadow brome, orchard grass or timothy or kura clover grown alone in the establishment year (1999) at early or late seeding dates for the significant interaction of seeding date by mixture.

Early	kura alone	1373.46 ± 277.78 b,c
Late	kura alone	0.00 ± 0.00 d **
Early	kura – Kentucky bluegrass	2218.75 ± 864.20 b
Late	kura – Kentucky bluegrass	108.02 ± 30.86 c,d
Early	kura – meadow brome	4166.67 ± 956.79 a
Late	kura – meadow brome	879.63 ± 401.23 b,d
Early	kura – orchard grass	4351.85 ± 1512.35 a
Late	kura – orchard grass	1080.25 ± 956.79 b,d
Early	kura - timothy	4197.53 ± 771.60 a
Late	kura - timothy	935.19 ± 37.04 b,d

*N.B. kura was below the cutting height of 2.5 cm

**Rows with the same alphabetical letter are not significantly different when $p<0.05$.

TABLE I-2. Annual dry matter yield (kg/ha) of mixtures of kura clover with either Kentucky bluegrass, meadow brome, orchard grass or timothy or kura clover grown alone in the establishment year (2000) at early or late seeding dates for the significant interaction of seeding date by mixture.

Early	kura alone	2455.88 ± 1502.23 b
Late	kura alone	69.44 ± 138.89 c
Early	kura – Kentucky bluegrass	2723.77 ± 1365.34 b
Late	kura – Kentucky bluegrass	115.74 ± 134.24 c
Early	kura – meadow brome	4591.05 ± 975.48 a
Late	kura – meadow brome	185.19 ± 186.89 c
Early	kura – orchard grass	5964.51 ± 647.11 a
Late	kura – orchard grass	339.51 ± 140.31 c
Early	kura - timothy	5084.88 ± 1108.64 a
Late	kura - timothy	115.74 ± 68.44 c

*Rows with the same alphabetical letter are not significantly different when $p<0.05$.

TABLE I-3. Total annual dry matter yield (kg/ha) of kura clover in mixture with either Kentucky bluegrass, meadow brome, orchard grass, timothy or kura clover grown alone in the first production year for plots established at either early or late seeding dates in the year 1999 for the significant interaction of seeding date by mixture.

early kura alone	12600.31 ± 1327.78 b,c
late kura alone	10007.72± 1290.43 d
early kura – Kentucky bluegrass	12908.95 ± 783.33 b,c
late kura – Kentucky bluegrass	11481.48 ±358.02 c,d
early kura – meadow brome	14429.01 ± 796.3 a,b
late kura – meadow brome	15771.6 ± 181.73 a
early kura – orchard grass	12901.23 ± 1317.28 b,c
late kura – orchard grass	13981.48 ± 524.38 a,b
early kura – timothy	11427.47 ± 205.25 c,d
late kura - timothy	13078.7 ± 853.4 b,c

*Rows with the same alphabetical letter are not significantly different when $p<0.05$.

TABLE I-4. Total annual dry matter yield (kg/ha) of mixtures of kura clover with either Kentucky bluegrass, meadow brome, orchard grass or timothy or kura grown alone in the first production year following establishment at either early or late seeding dates (2000) for the significant interaction of seeding date by mixture.

Early	kura alone	4243.83 ± 696.91 d,e
Late	kura alone	1512.35 ± 1694.41 e
Early	kura – Kentucky bluegrass	5023.15 ± 388.24 c,d
Late	kura – Kentucky bluegrass	6250.00 ± 473.81 b,c,d
Early	kura – meadow brome	8101.85 ± 315.25 a,b
Late	kura - meadow brome	10308.64 ± 1166.57 a
Early	kura – orchard grass	4336.42 ± 504.32 d,e
Late	kura – orchard grass	6658.95 ± 501.72 b,c,d
Early	kura timothy	6165.12 ± 891.97 b,c,d
Late	kura - timothy	7870.37 ± 2882.24 a,b,c

*Rows with the same alphabetical letter are not significantly different when $p<0.05$.

TABLE I-5. Annual dry matter yield (kg/ha) of kura clover in mixture with either Kentucky bluegrass, meadow brome, orchard grass or timothy or grown alone in the second production year of plots established in the year 1999 for the significant main effect of mixture.

kura	6940.59 ± 987.65	b
kura – Kentucky bluegrass	6828.70 ± 1049.38	b
kura – meadow brome	10174.37 ± 339.51	a
kura – orchard grass	5760.03 ± 493.83	c
kura - timothy	4290.12 ± 339.51	d

* Rows with the same alphabetical letter are not significantly different when $p<0.05$.

APPENDIX II

TABLE II-1. Analysis of Variance for the total annual yield of kura clover grown alone or in mixtures with either Kentucky bluegrass, meadow brome, orchard grass or timothy in the establishment year for plots sown in 1999 at early or late seeding dates.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	12	11.96	0.997	30.05	0.0001
Error	27	0.896	0.033		
Corrected Total	39	12.86			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.93	29.16	0.182	0.625	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	3	0.943	0.314	9.47	0.0002
Seeding date	1	7.40	7.405	45.22	0.0025
Mixture	4	2.96	0.74	4.52	0.0865
Sd*mix	4	0.655	0.164	4.93	0.0041

TABLE II-2. Analysis of Variance for the total annual yield of kura clover grown alone or in mixtures with either Kentucky bluegrass, meadow brome, orchard grass or timothy in the establishment year for plots sown in 2000 at early or late seeding dates.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	12	21.61	1.800	38.24	0.0001
Error	27	1.27	0.047		
Corrected Total	39	22.88			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.944	30.77	0.217	0.705	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	3	0.875	0.291	6.20	0.0024
Seeding date	1	16.99	16.99	40.94	0.0031
Mixture	4	2.07	0.519	1.25	0.4161
Sd*mix	4	1.66	0.415	8.81	0.0001

TABLE II-3. Analysis of Variance for the total annual yield of kura clover grown alone (K) or in mixtures with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the first production year (2000) for plots established in 1999 at early or late seeding dates

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	27	18.20	0.674	30.32	0.0001
Error	132	2.93	0.002		
Corrected Total	159	21.14			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.861	14.306	0.149	1.042	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Seeding date	1	0.000002	0.00002	0.00	0.9918
Mixture	4	1.815	0.453	2.43	0.2056
Harvest	3	11.33	3.777	169.87	0.0001
Sd*mix	4	0.747	0.186	8.41	0.0001
Mix*harvest	12	3.550	0.295	13.30	0.0001

TABLE II-4. Analysis of Variance for the annual yield of kura clover grown alone or in mixtures with either Kentucky bluegrass, meadow brome, orchard grass or timothy in the first production year at four harvests for plots established in 2000 at early or late seeding dates.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	39	14.33	0.367	13.92	0.0001
Error	120	3.169	0.026		
Corrected Total	159	17.508			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.818	33.598	0.162	0.483	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Seeding date	1	0.246	0.246	9.33	0.0028
Mixture	4	4.805	1.201	45.48	0.0001
Harvest	3	5.633	1.877	71.09	0.0001
Sd*mix	4	1.309	0.327	12.39	0.0001
Mix*harvest	12	1.412	0.117	4.46	0.0001

TABLE II-5. Analysis of Variance for the total annual yield of kura clover grown alone (K) or in mixtures with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the second production year (2001) for plots established in 1999 at early or late seeding dates.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	39	9.501	0.243	19.29	0.0001
Error	118	1.489	0.012		
Corrected Total	157	10.991			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.964	20.572	0.112	0.546	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Seeding date	1	0.125	0.125	9.95	0.0020
Mixture	4	3.373	0.843	66.79	0.0001
Sd*mix	4	0.022	0.005	0.45	0.7728
Mix*harvest	15	5.719	0.381	30.20	0.0001

TABLE II-6. Analysis of variance for species composition when kura clover was grown in mixtures with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the first production year (2000) for plots established in 1999 at early or late seeding dates.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	27	21.875	0.810	194.20	0.0001
Error	132	0.550	0.0041		
Corrected Total	159	22.426			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.975	20.315	0.064	0.317	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Seeding date	1	0.070	0.070	4.00	0.1162
Mixture	4	21.173	5.293	302.22	0.0001
Harvest	3	0.1933	0.064	15.45	0.0001
Sd*mix	4	0.070	0.0175	4.20	0.0031
Mix*harvest	12	0.365	0.0304	7.29	0.0001

TABLE II-7. Analysis of variance for species composition when kura clover was grown in mixtures with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the first production year (2001) for plots established in 2000 at early or late seeding dates.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	19	7.091	0.373	13.72	0.0001
Error	60	1.632	0.027		
Corrected Total	79	8.723			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.812	34.945	0.164	0.472	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Mixture	4	6.594	1.648	61.64	0.0001
Harvest	3	0.176	0.058	2.20	0.1413
Mix*harvest	12	0.320	0.026	0.98	0.4753

TABLE II-8. Analysis of variance for species composition when kura clover was grown in mixtures with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) in the second production year (2001) for plots established in 1999 at early or late seeding dates.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	24	19.824	0.826	62.53	0.0001
Error	135	1.783	0.013		
Corrected Total	159	21.607			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.917	34.55	0.114	0.3326	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Seeding date	1	0.224	0.224	6.53	0.0629
Mixture	4	18.944	4.736	138.80	0.0002
Sd*mix	4	0.137	0.034	2.60	0.0391
Mix*harvest	15	0.518	0.034	2.62	0.0017

TABLE II-9. Analysis of variance for the percentage of crude protein in the first production year at four harvests of kura clover grown in mixture with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) established in 1999.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	19	4922.97	259.10	109.65	0.0001
Error	58	137.05	2.362		
Corrected Total	77	5060.023			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.972	10.189	1.537	15.091	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Mixture	4	4675.947	1168.986	494.72	0.0001
Harvest	3	144.486	48.162	20.38	0.0001
Mix*harvest	12	86.956	7.246	3.07	0.0021

TABLE II-10. Analysis of variance for the percentage of crude protein in the first production year of either Kentucky bluegrass (KBG), meadow brome (MB), orchard grass (OG) or timothy (T) established in 1999.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	19	4148.50	218.34	40.68	0.0001
Error	55	295.17	5.36		
Corrected Total	74	4443.68			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.933	16.754	2.316	13.823	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Mixture	4	3934.189	983.547	183.26	0.0001
Harvest	3	56.884	18.961	3.53	0.0205
Mix*harvest	12	84.023	7.001	1.30	0.2426

TABLE II-11. Analysis of variance for the percentage of crude protein in the first production year at four harvests of kura clover grown alone (K) or of kura clover in mixture with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	18	2631.85	146.21	35.65	0.0001
Error	42	172.27	4.10		
Corrected Total	60	2804.13			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.938	10.959	2.025	18.480	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Mixture	4	1953.241	488.310	119.05	0.0001
Harvest	3	388.254	129.418	31.55	0.0001
Mix*harvest	12	1216.491	110.59	26.96	0.0001

TABLE II-12. Analysis of variance for the percentage of NDF in the first production year (2000) at four harvests of kura clover grown in mixture with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	19	35227.58	1854.08	0.68	0.0001
Error	58	1185.92	20.44		
Corrected Total	77	36413.51			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.967	11.09	4.52	40.74	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Mixture	4	34154.22	8538.55	417.60	0.001
Harvest	3	486.30	129.418	31.55	0.0001
Mix*harvest	12	624.08	52.006	2.54	0.0090

TABLE II-13. Analysis of variance for the percentage of NDF in the first production year of either Kentucky bluegrass (KBG), meadow brome (MB), orchard grass (OG) or timothy (T) established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	19	38202.31	2010.64	40.17	0.0001
Error	55	2753.12	50.05		
Corrected Total	74	40955.44			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.932	16.93	7.07	71.78	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Mixture	4	36504.52	9126.13	182.32	0.0001
Harvest	3	560.00	186.66	3.73	0.0164
Mix*harvest	12	1225.3144	102.10	2.04	0.0376

TABLE II-14. Analysis of variance for the percentage of NDF in the first production year at four harvests of kura clover grown alone (K) or of kura clover in mixture with either Kentucky bluegrass (K-KBG), meadow brome (K-MB), orchard grass (K-OG) or timothy (K-T) established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	18	7416.86	412.04	6.70	0.0001
Error	41	2522.39	61.52		
Corrected Total	59	9939.25			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.746	25.17	7.81	30.50	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Mixture	4	2360.72	590.05	9.59	0.0001
Harvest	3	3631.72	1210.57	19.68	0.001
Mix*harvest	12	3675.48	334.13	5.43	0.0001

APPENDIX III

TABLE III-1. Analysis of Variance for total annual yield of kura clover under five harvest frequencies in the first production year for plots established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	4.59	1.14	11.46	0.0002
Error	15	1.50	0.100		
Corrected Total	19	6.09			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.753	13.82	0.316	2.28	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Frequency	4	4.59	1.14	11.46	0.0002

TABLE III-2. Analysis of Variance for total annual yield of kura clover under five harvest frequencies in the second production year for plots established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	8.35	2.08	11.57	0.0002
Error	15	2.71	0.18		
Corrected Total	19	11.06			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.755	18.03	0.425	2.35	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Frequency	4	8.35	2.08	11.57	0.0002

TABLE III-3. Analysis of Variance for crude protein of kura clover under four harvest frequencies in the first production year (2001) for plots established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	3	43.41	14.47	95.64	0.0001
Error	4	0.60	0.15		
Corrected Total	7	44.02			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.986	1.875	0.388	20.73	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Frequency	3	43.41	14.47	95.42	0.0004

TABLE III-4. Analysis of Variance for NDF of kura clover under four harvest frequencies in the first production year (2001) for plots established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	3	121.10	40.39	10.87	0.0216
Error	4	14.86	3.71		
Corrected Total	7	135.97			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.890	5.510	1.927	34.975	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Frequency	3	121.109	40.36	10.87	0.0216

TABLE III-6. Analysis of Variance for ADF of kura clover under four harvest frequencies in the first production year (2001) for plots established in 1999.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	3	152.36	50.789	25.46	0.0046
Error	4	7.977	1.994		
Corrected Total	7	160.345			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.95	5.17	1.41	27.29	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Frequency	3	152.367	50.789	25.46	0.0046

APPENDIX IV

TABLE IV-1. Analysis of variance for etiolated regrowth of kura clover plants removed in the fall in the year of planting or the following spring after establishment at early or late seeding dates or in mixture with either Kentudky bluegrass, meadow brome, orchard grass or timothy.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Model	6	2.619	0.436	11.98	0.0001
Error	28	1.020	0.036		
Corrected Total	34	3.64			
	R-square	**Coefficient of Variation**	**Root MSE**	**Total Mean**	
	0.719	54.37	0.190	0.351	
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	3	0.137	0.045	1.25	0.3089
Seeding date	1	2.09	2.09	57.38	0.0001
Mix	4	698.20	174.55	4.26	0.0032