

## Vegetative establishment of Kura clover

C. C. Sheaffer<sup>1</sup>, R. D. Mathison<sup>2</sup>, and P. Seguin<sup>3</sup>

<sup>1</sup>Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA; <sup>2</sup>North Central Research and Outreach Center, University of Minnesota, Grand Rapids, MN 55744, USA; and <sup>3</sup>Department of Plant Science, Macdonald Campus, McGill University, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9.

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Sheaffer, C. C., Mathison, R. D. and Seguin, P. 2008. **Vegetative establishment of Kura clover.** Can. J. Plant Sci. **88**: 921–924. Kura clover (*Trifolium ambiguum* M. Bieb.) is a rhizomatous perennial legume that is often challenging to establish from seed. Our objectives were to evaluate the effect of planting date (early April, late April, and mid-May), plant material (rhizomes and rhizomes with buds), and rhizome planting density (11, 44, and 178 plants m<sup>-2</sup>) on establishment of Kura clover. Establishment of the clover was enhanced by early April planting using rhizomes with buds. Kura clover populations increased with increased rhizome planting densities. Establishment of Kura clover at a rhizome density of 178 rhizome m<sup>-2</sup> has the potential to provide yield and stands similar to broadcast seeding.

**Key words:** Kura Clover (*Trifolium ambiguum* M. Bieb.), vegetative propagation

Sheaffer, C. C., Mathison, R. D. et Seguin, P. 2008. **Implantation végétative du trèfle Kura.** Can. J. Plant Sci. **88**: 921–924. Le trèfle Kura (*Trifolium ambiguum* M. Bieb.) est une légumineuse vivace à rhizome souvent difficile à implanter par semis. Les auteurs ont vérifié l'incidence de la date de plantation (début d'avril, fin d'avril et mi-mai), du matériel végétal (rhizomes et rhizomes avec bourgeons) et de la densité des semis (11, 44 et 178 plants par m<sup>2</sup>) sur l'établissement de la plante. Le trèfle Kura s'établit mieux quand on plante des rhizomes avec bourgeons au début d'avril. Les peuplements augmentent avec la densité des semis. Une densité de 178 rhizomes au mètre carré pourrait donner un rendement et des peuplements similaires à ceux obtenus par semis à la volée.

**Mots clés:** Trèfle Kura (*Trifolium ambiguum* M. Bieb.), multiplication végétative

Kura clover is a rhizomatous perennial legume that is well suited to grazing in the northern United States of America and parts of Canada. It has excellent persistence under frequent defoliation, the ability to spread, and produces favorable yields of high quality forage (Sheaffer et al. 2003). Clipped forage yields have ranged from 4 to 11 Mg ha<sup>-1</sup> and its persistence has exceeded that of alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.) and other commonly grown legumes (Taylor and Smith 1998). Its potential as a grazing legume has been demonstrated for over 20 yr in Minnesota, but Kura clover use still is limited due to establishment difficulties, which are attributable to poor seedling vigor. It is intolerant of competition for light during establishment, in part due to a translocation of energy to the crowns and roots (Genrich et al. 1998). Seguin et al. (1999) reported seeding year yields of less than 400 kg ha<sup>-1</sup>. In contrast, the excellent persistence of Kura clover is associated with an extensive crown-rhizome-root system. Peterson et al. (1994) reported that a 5-yr-old stand of Kura clover had a total below-ground mass of 7140 kg ha<sup>-1</sup>.

Kura clover has been vegetatively established into New Zealand grasslands using rhizomes (Scott and Mason 1992). Also, Teutsch et al. (2004) in Kentucky

successfully established Kura clover on reclaimed mined land in Kentucky using rhizome and crown sprigs. Based on the successful vegetative establishment of Kura clover and other species [e.g., zigzag clover (*Trifolium medium* L.); Flanagan and Gershoy 1964] it appears that rhizomes may be a viable alternative method for propagation of Kura clover, but additional information on plant establishment is needed. Our objectives were to determine the effects of planting date, type of plant material, and planting densities on the establishment of Kura clover using rhizomes.

Experiment 1 evaluated the effect of planting date and rhizome characteristics on establishment. It was conducted in 1990 and repeated in 1991 at St. Paul, MN (45°00'N; 93°06'W) on a Waukegan silt loam (fine-silty over sandy, mixed, mesic Typic Hapludoll). Soil P, K, and pH were maintained at greater than 80 kg ha<sup>-1</sup>, 350 kg ha<sup>-1</sup>, and 6.5, respectively.

The experimental design for the trial was a randomized complete block replicated four times with treatments in a split-split-plot arrangement. Whole plots were years (1990 and 1991). Subplots were three planting dates, and sub-subplots (10 m long by 1 m wide) were two propagation methods. The three planting dates were Apr. 11, Apr. 29, and May 15. Propagation methods

consisted of rhizomes at least 7.5 cm long, with two or more nodes, or rhizomes that included a developed green bud. Rhizomes were dug from a 6-yr-old stand of Rhizo Kura clover, stored in refrigeration at 6°C, and planted the following day. On Apr. 11, Kura clover was breaking dormancy and had one true trifoliolate leaf. By May 15, Kura clover had about seven leaves per plant and was suitable for grazing.

Each propagation method treatment consisted of 20 rhizomes, which were planted about 0.4 m apart in 10 m long rows which were 1 m apart. In both years, rhizomes were established into a closely grazed perennial sod consisting of Kentucky bluegrass (*Poa pratense* L.), quackgrass (*Elytrigia repens* L.), and smooth brome-grass (*Bromus inermis* Leyss) following removal of a 10-cm-diameter piece of sod. Rhizomes were inoculated with appropriate rhizobial inoculant immediately before planting. Initial growth of the grass sod was suppressed using 0.56 kg ha<sup>-1</sup> sethoxydim {2-[1-(ethoxyimino) butyl]-5-[2-(ethylthio) propyl]-3-hydroxy-2-cyclohexen-1-one} and later by sheep grazing whenever the grass began to form a canopy over the developing Kura clover plants. In October of the establishment year and in October of the following year, numbers of live plants from each row were counted. In addition, the spread between a parent and a scion was measured.

Experiment 2 was conducted to determine the effect of rhizome planting density on Kura clover population and yield. Research was conducted from 1996 to 1998 at Rosemount, MN (44°43'N; 93°06'W) on a Waukegan silt loam (fine-silty over sandy, mixed typic Hapludoll) and at Grand Rapids (47°22'N; 93°52'W) in northern Minnesota on a cowhorn very fine sand (coarse-loamy, mixed nonacid Aeric Haplaquepts) with pH, P and K levels greater than 6.5, 60 kg ha<sup>-1</sup>, and 300 kg ha<sup>-1</sup>, respectively.

The experimental design was a randomized complete block with three replicates. Treatments were three equidistant Kura clover rhizome spacings of 30.4, 15.2, and 76 cm that provided 11, 44, and 178 plants m<sup>-2</sup>, respectively, and for comparison, broadcast seeding at 10 kg ha<sup>-1</sup> that provided 500 seeds m<sup>-2</sup>. This broadcast seeding rate is recommended in the Midwest for establishment of pure stands of Kura clover (Sheaffer et al. 2003). Plots were 1.5 by 5 m. Rhizomes contained at least two nodes and a developed green bud. Rhizomes were dug from a 3-yr-old stand of Rhizo Kura clover, stored in refrigeration at 6°C and planted the following day. Seeds were broadcast and incorporated to a 1-cm depth. Rhizomes were planted horizontally to a 2.5 cm depth. Planting into a tilled seedbed occurred on 1996 May 16 at Rosemount and Grand Rapids. Both seeds and rhizomes were inoculated with appropriate rhizobial inoculant. Trifluralin [2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzenamine] at 0.5 a.i. kg ha<sup>-1</sup> was applied before planting and incorporated for weed control.

At Rosemount, stand density was determined on 1996 Sep. 15, 1997 Jun. 01 and 1998 Jun. 01 by counting plants in a 1-m<sup>2</sup> area of each plot. Kura clover yield was measured in 1997 and 1998 by harvesting a 1 by 3 m strip on about May 25, Jul. 01, and Sep. 01. At each harvest, a 0.5-kg subsample was dried for 72 h, and weighed to determine dry matter yield. An additional 1-kg subsample was collected from each plot, separated into Kura clover and weed fractions, and dried at 54°C to determine the contribution of each fraction to yield. Because Kura clover constituted over 95% of the yield, only Kura clover yield data were statistically analyzed. At Grand Rapids, stand density and forage yield from two harvests (May 15 and Aug. 01) were determined only in the year following seeding. Dates of activities and procedures were similar to those at Rosemount.

Data from both experiments were subjected to analysis of variance and means were compared using LSD (0.05). In exp. 2, because of differences in data collected between locations, data from each location were analyzed independently.

### Experiment 1

Our results concur with those of previous work (Scott and Mason 1992; Teutsch et al. 2004) that indicated that Kura clover can be established vegetatively from rhizomes. However, we found that planting date and plant material affect establishment. Planting rhizome-bud sections resulted in greater establishment than rhizome sections without buds each year (Table 1). This was likely related to the increased potential for sections with buds to supply photosynthates for root establishment. In New Zealand, Scott and Mason (1992) reported no differences in establishment of rhizome and rhizome plus bud sections. Planting on Apr. 11 or 29 consistently resulted in greater establishment than planting on May 15. Plant material by planting date interactions were not significant ( $P > 0.05$ ). Lower establishment on May 15 may

**Table 1. Effect of planting material and planting date on Kura clover establishment (% survival) in the planting year and its spread following planting**

Treatment <sup>z</sup>	Establishment year		Spread	
	1990	1991	1991	1992
	(%)		(cm)	
Material				
Rhizome	17	38	12	13
Rhizome-bud	50	64	17	19
LSD (0.05)	11	13	NS <sup>y</sup>	NS
Planting date				
Apr. 11	55	61	32	23
Apr 29	26	69	14	16
May 15	19	24	5	8
LSD (0.05)	15	21	2	2

<sup>z</sup>Material and planting date main effects are presented because the material × planting date interaction was not significant ( $P > 0.05$ ).

<sup>y</sup>NS, not significant ( $P > 0.05$ ).

**Table 2. Effect of Kura establishment treatments on Kura clover populations and dry matter yields at Rosemount**

Treatment	Plant populations			Dry matter yield	
	1996 <sup>2</sup>	1997	1998	1997	1998
		(Plants m <sup>-2</sup> )		(Mg ha <sup>-1</sup> )	
11 rhizomes m <sup>-2</sup>	14	74	315	2.6	3.5
44 rhizomes m <sup>-2</sup>	60	190	360	3.4	3.4
178 rhizomes m <sup>-2</sup>	112	171	495	5.8	3.6
500 seeds m <sup>-2</sup>	173	225	459	7.6	4.3
LSD (0.05)	76	127	99	1.4	0.8

<sup>2</sup>Establishment of rhizomes and seed on 1996 May 16.

have been related to less favorable environmental conditions as average air temperatures were warmer than in April. However, planting date effects due to environment were confounded with those due to stage of development of the rhizome material. Plants dug and transplanted on May 15 had grown from 2 to 4 wk longer than those planted in April, which may have changed their ability to re-establish.

In the fall of the planting year, individual plants in all treatments had multiplied and had given rise to two or three daughter plants. By the fall of the year following planting, each original propagule had spread and resulted in new daughter plants. Spread was similar for the rhizome and rhizome-bud treatment in both years and was greatest for the Apr. 11 planting date. Although not noted from seeding year herbage production, Kura clover does have significant below-ground development in the seeding year (Forde et al. 1989). Genrich et al. (1998) reported that Kura clover plants established from seeds in a tilled seedbed had rhizomes with leaves that were 20 cm long in the fall of the seeding year.

## Experiment 2

At Rosemount, Kura clover populations in September following spring seeding predictably increased with increased rhizome planting densities (Table 2). Plant population was lower for the 11 rhizomes m<sup>-2</sup> planting density than for the other treatments. Plant population was greatest for the seed treatment, but only about 40% of the seed developed into plants. Plant populations for all treatments increased in 1997 and 1998. By 1998, populations exceeded 315 plants m<sup>-2</sup> for all treatments, but remained less for the 11 rhizomes m<sup>-2</sup> treatment than the other treatments. Forage yields in 1997 were greatest for the 178 rhizome m<sup>-2</sup> and the broadcast seed treatment, but were similar for all treatments in 1998. In this experiment at Rosemount, yields for seeded Kura clover in the year following seeding were uncharacteristically large. Seguin et al. (1999) reported post-seeding year Kura clover yields of about 2.3 Mg ha<sup>-1</sup> when Kura clover had been established with a herbicide or without a herbicide for weed control. Kura clover typically requires 3 yr before reaching a high level of production (Seguin 2007).

At Grand Rapids, Kura clover was slower to establish and the dry matter yield was lower than at Rosemount. However, the results clearly show the potential value of establishment with a high population of rhizomes. The 178 rhizomes m<sup>-2</sup> treatment had greater dry matter yields and populations in the year after seeding than other treatments. Yields for two harvests in 1997 were 2.4, 0.7, and 0.4 Mg ha<sup>-1</sup> for the 178, 44, and 11 rhizomes m<sup>-2</sup> treatments, respectively. Yield for the seed treatment was 0.7 Mg ha<sup>-1</sup>. Plant populations were 154, 66, and 22 plants m<sup>-2</sup> for the 178, 44, and 11 rhizomes m<sup>-2</sup>, respectively, and 88 plants m<sup>-2</sup> for the seed treatment.

Kura clover is a perennial, winterhardy legume with dependable long-term persistence under grazing but its use is limited because of poor establishment from seeds. Establishment with rhizomes has potential to provide similar populations and forage yields as seeds. Establishment using rhizomes is, however, enhanced by early establishment (early April), by using rhizomes with buds, and by using high planting densities. Rhizome sections with buds can easily be obtained in Minnesota by digging Kura clover in early April. Stand establishment using seeds will remain the most conventional approach to Kura clover establishment; however, establishment using rhizomes is an alternative approach that could be considered. The use of rhizomes for establishment would require specialized equipment such as a mechanical sprig digger, and facilities for short term storage. Transportation costs might also limit distance between plant material source and establishment site. However, establishment using asexual reproduction has been successfully accomplished for Bermudagrass [*Cynodon dactylon* (L.) Pers.] and perennial peanut (*Arachis hypogaea* L.), and could become a standard practice in the northern USA for some species such as Kura clover.

**Flanagan, T. R. and Gershoy, A. 1964.** Soft-stem cuttings of zigzag clover, *Trifolium medium*. *Crop Sci.* 3: 417–418.

**Forde, M. B., Hay, M. J. M. and Brock J. L. 1989.** Development and growth characteristics of temperature perennial legumes. Pages 91–109 in G. C. Marten et al., ed. Persistence of forage legumes. Proc. Aust. New Zealand/United States workshop, Honolulu, HI. 1998 Jul. 18–22. ASA, CSSA, and SSSA, Madison, WI.

**Genrich, K. C., Sheaffer, C. C. and Ehlke, N. J. 1998.** Kura clover growth and development during the seeding year. *Crop Sci.* **38**: 735–741.

**Peterson, P. R., Sheaffer, C. C., Jordan, R. M. and Christians, C. J. 1994.** Responses of kura clover to sheep grazing and clipping: II. Below-ground morphology, persistence, and total nonstructural carbohydrates. *Agron. J.* **86**: 660–667.

**Scott, D. and Mason, C. R. 1992.** Potential for high country improvement from planting of rhizome fragments of spreading legumes. *Proc. NZ Grassl. Assoc.* **54**: 127–129.

**Seguin, P. 2007.** Kura clover forage yield contribution increases over time when seeded in mixture with grasses in southwestern Québec. *Forage and grazinglands*. doi:10.1094/FG-2007-1217-02-RS. [Online] Available: <http://www.plantmanagementnetwork.org/sub/fg/research/2007/kura/> [2008 May 23].

**Seguin, P., Sheaffer, C. C., Ehlke, N. J. and Becker, R. L. 1999.** Kura clover establishment methods. *J. Prod. Agric.* **12**: 483–487.

**Sheaffer, C. C., Marten, G. C., Jordan, R. M. and Ristau, E. A. 1992.** Forage potential of kura clover and birdsfoot trefoil when grazed by sheep. *Agron. J.* **84**: 176–180.

**Sheaffer, C. C., Ehlke, N. J., Albrecht, K. A. and Peterson, P. R. 2003.** Forage legumes. *Minnesota Agricultural Experiment Station Bull.* 608–2003.

**Taylor, N. L. and Smith, R. R. 1998.** Kura clover (*Trifolium ambiguum* M.B.) breeding, culture and utilization. *Adv. Agron.* **63**: 153–178.

**Teutsch, C. D., Collins, M. and Ditsch, D. C. 2004.** Impact of incorporation, mulch, and root coating on the establishment of kura clover from rhizome segments on mine spoils in southeastern Kentucky. *Forage and grazinglands*. doi: 10.1094/FG-2004-1117-01-RS. [Online]. Available: <http://www.plantmanagementnetwork.org/pub/fg/research/2004/kura/> [2008 May 23].