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## Nitrogen Fertilization and Rhizobial Inoculation Effects on Kura Clover Growth

Philippe Seguin, Craig C. Sheaffer,\* Nancy J. Ehlke, Michael P. Russelle, and Peter H. Graham

### ABSTRACT

**Kura clover (*Trifolium ambiguum* M.B.) is a persistent, rhizomatous forage legume; however, its use is currently limited by slow establishment. We determined the effects of rhizobial inoculation and N fertilization on kura clover growth and N<sub>2</sub> fixation in the seeding year. Kura clover was seeded with or without a commercial rhizobial inoculant and with and without N fertilization in three environments. Fertilization treatments consisted of 100 kg N ha<sup>-1</sup> either applied at seeding or split in 10 kg N ha<sup>-1</sup> applications every other week after seeding. Nitrogen fertilization increased seeding-year herbage accumulation in all locations, but the response to fertilizer N was greater on a loamy sand with low organic matter and available N than on a silt loam with high soil organic matter. Rhizobial inoculation failed to consistently improve seeding-year herbage accumulation compared with no inoculation; a positive response was observed in only one of three environments. Dry matter accumulation responses of root and rhizome to N fertilization and rhizobial inoculation were similar to that of herbage. Dinitrogen fixation in the seeding year varied between 9 and 25 kg ha<sup>-1</sup> fixed N, depending on the environment. Seeding-year inoculation increased postseeding year herbage yield. Also, when a positive response to N fertilization occurred in the seeding year, the response was maintained in the postseeding year. A commercial rhizobial inoculant was ineffective in establishing adequate nodulation in the seeding year in a N-limited soil, indicating the need to identify more effective rhizobia for kura clover.**

P. Seguin, Dep. of Plant Sci., McGill Univ., Macdonald Campus, 2111 Lakeshore Rd., Sainte Anne-de-Bellevue, QC, Canada H9X 3V9; C.C. Sheaffer and N.J. Ehlke, Dep. of Agron. and Plant Genet., Univ. of Minnesota, 1991 Buford Circle, 411 Borlaug Hall, St. Paul, MN 55108-6026; and M.P. Russelle, USDA-ARS, U.S. Dairy Forage Res. Cent. (Minnesota cluster) and Dep. of Soil, Water, and Climate, and P.H. Graham, Dep. of Soil, Water, and Climate, Univ. of Minnesota, 1991 Buford Circle, St. Paul, MN 55108-6028. Journal Paper no. 00-1-13-0159 of the Minnesota Agric. Exp. Stn. Received 6 Sept. 2000. \*Corresponding author (sheaf001@tc.umn.edu).

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EXCEPTIONAL PERSISTENCE and high quality forage have generated interest in the use of kura clover for permanent pastures (Peterson et al., 1994a,b) and for soil conservation (Alley et al., 1999; Zemenchik et al., 2000). Despite this interest, kura clover use is limited due to slow establishment. Establishment problems are related in part to kura clover's propensity to partition a large percentage of its fixed C to roots and rhizomes (Genrich et al., 1998; Woodman, 1999). While this characteristic promotes clonal development and persistence of kura clover, it also makes establishing plants very susceptible to competition from other species. Kura clover establishment is thus maximized by limiting competition from other species. However, even with appropriate management, forage yields in the seeding year have been small compared with other forage legumes, often <400 kg ha<sup>-1</sup> dry matter (DM) (Seguin et al., 1999).

Nitrogen fertilization of legumes is usually associated with a reduction in nodulation and N<sub>2</sub> fixation (Streeter, 1988), but there is variation in this response depending on the host–*Rhizobium* spp. association. Legumes with slow nodulation development and/or low N<sub>2</sub> fixation levels may benefit from moderate N fertilization, even past the onset of N<sub>2</sub> fixation. For example, with arrowleaf clover (*T. vesiculosum* Savi.), greater lotus (*Lotus pedunculatus* Cav.), and soybean [*Glycine max* (L.) Merr.], N fertilization increased herbage or grain yields and, in some cases, increased nodulation and N<sub>2</sub> fixation (Hojjati et al., 1978; Wedderburn, 1983; Schom-

**Abbreviations:** DAS, days after seeding; DM, dry matter; %Ndfa, percent of N derived from the atmosphere.

berg and Weaver, 1990; Abdel Wahab and Abd-Alla, 1996). However, for plants with a relatively high  $N_2$  fixation potential in the establishment year, such as alfalfa (*Medicago sativa* L.), N fertilization reduced nodulation and  $N_2$  fixation and did not affect herbage or N yields (Heichel and Vance, 1979; Eardly et al., 1985).

Due to very specific rhizobial requirements, kura clover will not nodulate effectively with rhizobia that nodulate other commonly grown *Trifolium* spp. (Parker and Allen, 1952; Keim, 1954). Inoculation of kura clover in North America and New Zealand is recommended because few soils outside of its center of origin in the Caucasus contain effective indigenous rhizobia (Hely, 1957). Even when inoculated, there are indications that kura clover has very low  $N_2$  fixation in the establishment year (Watson et al., 1996; Genrich et al., 1998); thus, its initial growth might be N limited. Patrick and Lowther (1995) reported a strong correlation between nodulation and establishment of kura clover. They increased kura clover nodulation and establishment by increasing the inoculation rate above recommended rates. These studies suggest that kura clover establishment and initial growth could be N limited due to the poor efficiency of rhizobial strains currently used; if true, N fertilization could potentially benefit initial growth of kura clover. The effects of N fertilization on seeding-year growth and nodulation of kura clover have not been investigated. Our objective was to determine the effects of rhizobial inoculation and N fertilization on kura clover DM accumulation and  $N_2$  fixation in the seeding year. We hypothesized that the commonly reported poor performance of kura clover in the establishment year is due to N limitation.

## MATERIALS AND METHODS

Experiments were initiated in May 1998 and 1999 at Becker, MN and in May 1999 at St. Paul, MN. At Becker in 1998, the soil, a Hubbard loamy sand (sandy, mixed, frigid Entic Hapludoll), had pH,  $NO_3^-$ -N, and soil organic matter levels of 6.6, 1.5 mg kg<sup>-1</sup>, and 18 g kg<sup>-1</sup>, respectively. In 1999, soil pH,  $NO_3^-$ -N, and soil organic matter levels were 7.2, 2.1 mg kg<sup>-1</sup>, and 16 g kg<sup>-1</sup>, respectively. At St. Paul, the Waukegan silt loam (fine-silty over sandy, mixed, Typic Hapludoll) had pH,  $NO_3^-$ -N, and soil organic matter levels of 6.4, 16.7 mg kg<sup>-1</sup>, and 53 g kg<sup>-1</sup>, respectively. Soil exchangeable K (>400 kg ha<sup>-1</sup>) and extractable P (>50 kg ha<sup>-1</sup>) levels optimum for legume growth were achieved at all locations by fertilization before seeding. At Becker, plot areas were planted with rye (*Secale cereale* L.) in the preceding growing seasons while in St. Paul, the plot area was planted with corn (*Zea mays* L.). Temperature and precipitation at Becker were near average in 1998, but at both Becker and St. Paul in 1999, temperature and precipitation were above the 30-yr average. In the spring of 2000, precipitation was below average at both locations. In May 1999, precipitation was 46 and 97 mm above average at Becker and St. Paul, respectively, while in the spring of 2000, it was 25 and 50 mm below average at St. Paul and Becker, respectively. Becker plots were irrigated to meet evapotranspiration demand not met by rainfall, whereas St. Paul plots were rainfed, which resulted in a water deficit in the spring 2000.

Rhizobial inoculation and N fertilization treatments were

arranged in a randomized complete block design with split-plot restriction and five replications. Whole plots were inoculation treatments and subplots were N fertilization treatments. Seeds were either inoculated (20 g peat kg<sup>-1</sup> seed) or not inoculated with a peat-based commercial rhizobial inoculant (Liphatech, WI).<sup>1</sup> The inoculant was mixed with seeds just before seeding. This inoculant was a mixture of three *R. leguminosarum* biovar *trifolii* strains (i.e., 162C11, 162C13, and 162C14). In 1998 at Becker, there were two N fertilization treatments: 0 and 100 kg N ha<sup>-1</sup>, with all N applied 1 d after seeding (DAS). In 1999, at both locations, a third N fertilization treatment, the split application of 100 kg N ha<sup>-1</sup>, was added. In this treatment, 10 applications of 10 kg N ha<sup>-1</sup> started 1 DAS and then continued every other week.

Kura clover ('Endura') was sown in a prepared seedbed in 3- by 6-m plots with 15-cm rows at a rate of 10 kg ha<sup>-1</sup>. This resulted in populations of 350, 305, and 295 plants m<sup>-2</sup> at Becker in 1998, Becker in 1999, and St. Paul in 1999, respectively. Seeding occurred on 4 May 1998 and 3 May 1999 at Becker and on 25 May 1999 at St. Paul. Weed control included preplant incorporation of trifluralin [2,6-dinitro-*N*, *N*-dipropyl-4-(trifluoromethyl) benzaniline] (0.7 kg a.i. ha<sup>-1</sup>) and hand weeding. When detected, potato leafhoppers (*Empoasca fabae* Harris) were controlled by applications of 1.0 kg a.i. ha<sup>-1</sup> permethrin [*m*-phenoxybenzyl-*cis*, *trans*-3-(2,2-dichlorovinyl)-2, *d*-dimethylcyclopropanecarboxylate].

Plots were sampled every 25 d from seeding to early October, resulting in five samplings at St. Paul and six at Becker. Plants were dug to a 20-cm depth from 0.5 m of two adjacent nonborder rows (0.20 m<sup>2</sup>), washed in water to remove soil, and blotted dry with towels. Plant number was recorded to determine plant density. Twenty-five plants were then randomly selected, and nodule number was recorded for each plant. Plant herbage, root, and rhizome fractions were separated and dried for 48 h at 60°C to determine dry mass. Dried herbage was ground to pass a 0.5-mm screen and then analyzed for N concentration using the micro-Kjeldahl procedure (AOAC, 1975). The difference method was used to calculate  $N_2$  fixation and percent of N derived from the atmosphere (%Ndfa) at time of maximal herbage DM accumulation using the noninoculated kura clover plots as the nonfixing reference crop (Zhu et al., 1998). In late May of the postseeding year, plants were dug from a 1-m<sup>2</sup> area to a 20-cm depth at Becker and to a 10-cm depth at St. Paul. Low soil moisture levels at St. Paul in 2000 made digging and root recovery difficult. Plants were washed in water; separated into herbage, root, and rhizome components; and dried for 48 h at 60°C to determine mass on a DM basis.

Dry matter accumulation data were first analyzed as a split-split-plot model (Gomez and Gomez, 1984) using the PROC ANOVA procedure of SAS (SAS Inst., 1988). Main plots were inoculation treatments, split plots were N fertilization treatments, and split-split plots were sampling date. Because several interactions were significant (Table 1), data were also analyzed as a split plot for each sampling date. This same model was used to analyze maximum herbage, root, and rhizome yields; N yield and  $N_2$  fixation at a particular time; and yields in the postseeding year. Location effects were not tested because locations had different numbers of treatments, sampling dates, or both.

<sup>1</sup> The University of Minnesota and the USDA neither guarantee nor warrant the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that may also be suitable.

**Table 1. Analysis of variance for seeding-year dry matter (DM) accumulation of morphological components of kura clover when inoculated or not with rhizobial inoculant and fertilized with three N treatments in three environments.**

Plant part		Becker 1998	Becker 1999	St. Paul 1999
Herbage	Inoculation status (I)†	NS	*	NS
	N fertilization (N)‡	**	***	*
	I × N	*	NS	NS
	Date (D)	***	***	***
	D × I	NS	***	NS
	D × N	**	***	**
	D × I × N	NS	NS	NS
Root	I	*	NS	NS
	N	**	***	NS
	I × N	NS	NS	NS
	D	***	***	***
	D × I	***	NS	NS
	D × N	***	***	NS
	D × I × N	NS	NS	NS
Rhizome	I	NS	NS	NS
	N	NS	*	NS
	I × N	NS	NS	NS
	D	***	***	***
	D × I	**	***	NS
	D × N	**	***	NS
	D × I × N	*	NS	NS
Nodules, no. per plant	I	**	**	*
	N	*	*	*
	I × N	*	*	NS
	D	***	***	***
	D × I	***	***	***
	D × N	NS	**	NS
	D × I × N	**	**	NS

\* Significant at the 0.05 level.  
 \*\* Significant at the 0.01 level.  
 \*\*\* Significant at the 0.001 level.  
 † Inoculated (20 g peat kg<sup>-1</sup> seed) or not inoculated.  
 ‡ N fertilization: no N fertilizer applied, 100 kg N ha<sup>-1</sup> applied at seeding, or split application of 100 kg N ha<sup>-1</sup> at 10 kg N every other week after seeding.

**RESULTS AND DISCUSSION**

**Herbage, Root, and Rhizome Dry Matter Accumulation and Maximum Yields**

Kura clover DM accumulation varied greatly among environments (Fig. 1). In most cases, DM accumulations of herbage and root were linear although quadratic trends were sometimes observed. Herbage and root DM accumulations were four to six times greater at St. Paul than at Becker. Location differences were likely related to greater initial soil fertility and organic matter level at St. Paul.

Nitrogen fertilization affected herbage DM accumulation in all environments (Table 1). A fertilization × sampling date interaction occurred because the increase in kura clover DM accumulation caused by N fertilization was greater at later sampling dates (Fig. 1). In contrast, rhizobial inoculation increased DM accumulation only at Becker in 1999. An interaction of inoculation with sampling date occurred because the response to inoculation only appeared late in the season. A N fertilization × rhizobial inoculation interaction only occurred at Becker in 1998 where herbage DM accumulation was greater with a combination of N fertilizer and rhizobial inoculant than with other treatments.

Maximum herbage yield was reached at different times depending on the environment and the inocula-

**Table 2. Main effects of rhizobial inoculation and N fertilization treatments on maximum seeding-year yields of kura clover in three environments. Means for inoculation treatment effects are averaged over N treatments, and means for N treatment effects are averaged over inoculation treatments.**

Plant part	Treatment	Maximum DM† yield				
		Becker 1998	Becker 1999	St. Paul 1999		
kg ha <sup>-1</sup>						
Herbage	Inoculation (I)	Not inoculated	505	1445	5281	
		Inoculated‡	1121	1890	5210	
		LSD(0.05)	NS§	401	NS	
	N fertilization (N)¶	0	575	817	4499	
		100	1049	1508	5760	
		S100	–	2678	5476	
		LSD(0.05)	NS	797	NS	
		I × N	NS	NS	NS	
	Root	I	Not inoculated	1014	1932	4449
			Inoculated	1752	2024	4121
LSD(0.05)			NS	NS	NS	
N		0	1000	1037	3667	
		100	1766	1952	4571	
		S100	–	2945	4617	
		LSD(0.05)	527	560	NS	
		I × N	NS	NS	NS	
Rhizome		I	Not inoculated	28	75	110
			Inoculated	106	145	122
	LSD(0.05)		NS	NS	NS	
	N	0	28	33	89	
		100	107	129	134	
		S100	–	167	125	
		LSD(0.05)	NS	110	NS	
		I × N	NS	NS	NS	

† DM, dry matter.  
 ‡ Inoculated, 20 g peat kg<sup>-1</sup> seed.  
 § NS, not significant at the 0.05 level.  
 ¶ N fertilization: 0, no N fertilizer applied; 100, 100 kg N ha<sup>-1</sup> applied at seeding; S100, split application of 100 kg N ha<sup>-1</sup> at 10 kg N every other week after seeding.

tion and N fertilization treatment (Fig. 1). In 1998 at Becker and in 1999 at St. Paul, maximum herbage yield was reached 150 DAS with all treatments while in 1999 at Becker, maximum yield varied between 100 and 150 DAS, depending on treatments. At the time of maximum DM accumulation, there were few significant N fertilization or inoculation effects and no N fertilization × inoculation interaction (Table 2). Only at Becker in 1999 did both N fertilization and inoculation significantly increase ( $P \leq 0.05$ ) maximum herbage yield compared with nonfertilized and uninoculated treatments, respectively. The responses to N fertilization at Becker in 1998 and St. Paul in 1999 and the response to rhizobial inoculation at Becker in 1998 were significant but only at  $P \leq 0.1$ . Maximum herbage yields of kura clover observed for the inoculated nonfertilized treatment were similar to those reported by Genrich et al. (1998) under similar conditions at Becker, but in St. Paul, yields that we observed were almost two times greater.

Root DM accumulation was increased by N fertilization at Becker in 1998 and 1999, and as with herbage yield, N fertilization × sampling date interactions occurred at Becker because of the increasing response to

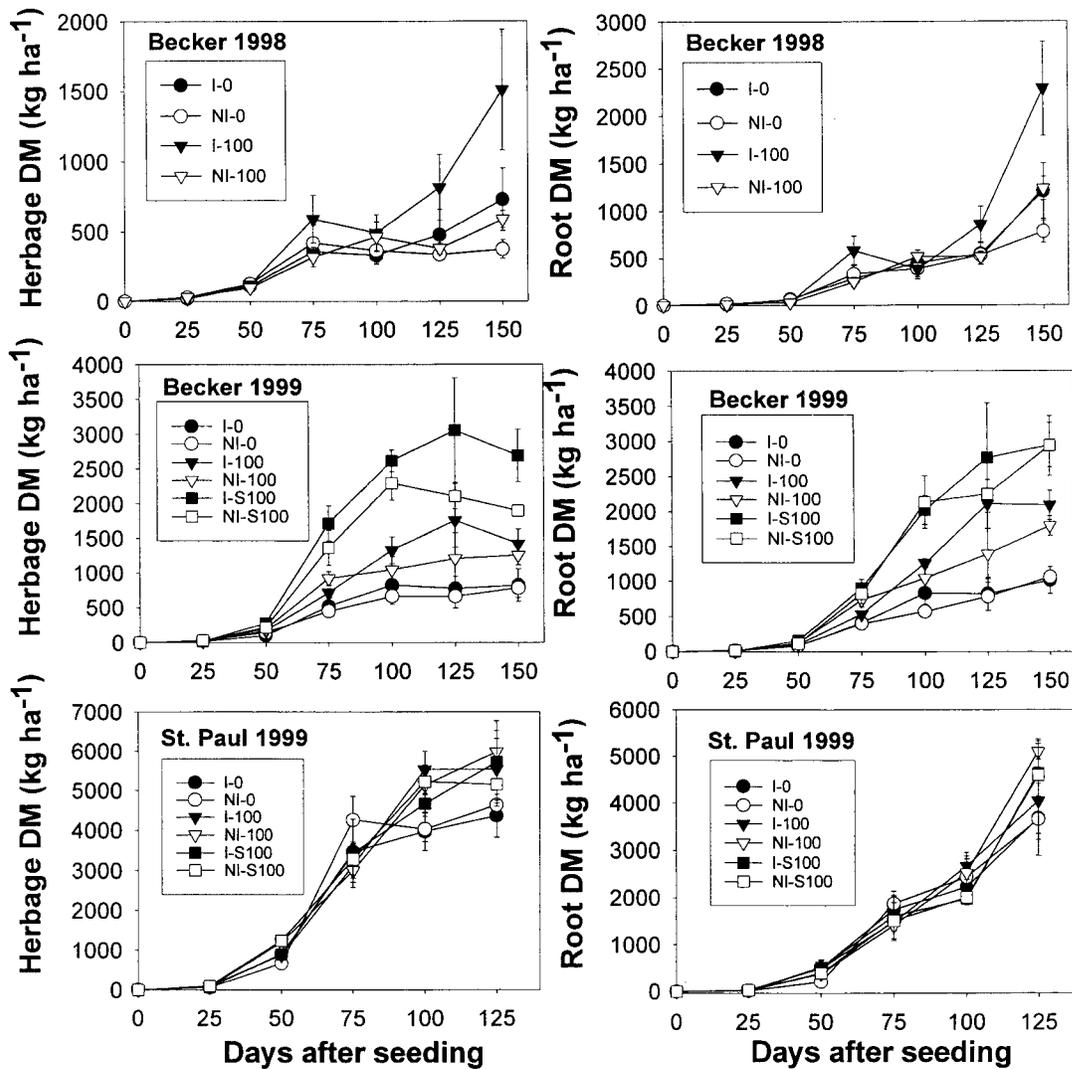


Fig. 1. Seeding-year growth of kura clover herbage and root when inoculated (I) or not inoculated (NI) with rhizobial inoculant and fertilized with three different N treatments in three environments in Minnesota. 0, no fertilization; 100, 100 kg N ha<sup>-1</sup> applied at seeding; and S100, split application of 100 kg N ha<sup>-1</sup> at 10 kg N every other week after seeding. Vertical bars indicate the standard deviation.

N fertilization with time (Table 1). Rhizobial inoculation only increased root DM accumulation at Becker in 1998, the inoculation × date interaction illustrating that the response increased with time. There was no fertilization × inoculation interaction at any location. Maximum root yield occurred at 150 DAS in all environments with all treatments and was increased by N fertilization at Becker in 1998 and 1999 (Table 2). As with herbage yield, maximum root yield observed in our study for inoculated, nonfertilized kura clover was relatively similar to yields reported by Genrich et al. (1998) at Becker, but at St. Paul, yields that we observed were greater. Because herbage and roots were affected similarly by treatments, root/shoot ratio was unaffected (data not presented).

Rhizome DM accumulation in this study was slow, with rhizomes initially observed at about 100 DAS; in contrast, Genrich et al. (1998) reported rhizome initiation at 65 DAS. Rhizobial inoculation and N fertilization thus only increased rhizome DM accumulation late in the season as demonstrated by the inoculation × sam-

pling date and N fertilization × sampling date interactions at Becker in 1998 and 1999 (Table 1). At the end of the season, when maximum rhizome yield occurred, N fertilization increased rhizome yield at Becker in 1999, but inoculation effects were not significant in any environments (Table 2).

The apparently overall greater response of DM accumulation over the season and at time of maximum yield, especially for herbage, to N fertilization compared with rhizobial inoculation suggests that kura clover growth may have been N limited when inoculated. The response was especially greater at maximum DM accumulation at Becker than at St. Paul. At Becker, plants that were not N fertilized appeared chlorotic during the seeding year. The lower response to N fertilization at St. Paul, which has a silt loam soil with greater organic matter and that mineralizes more organic N than the loamy sand at Becker, suggests that for many soils, response to N fertilization may actually not occur. With adequate N nutrition, maximum herbage yields observed at

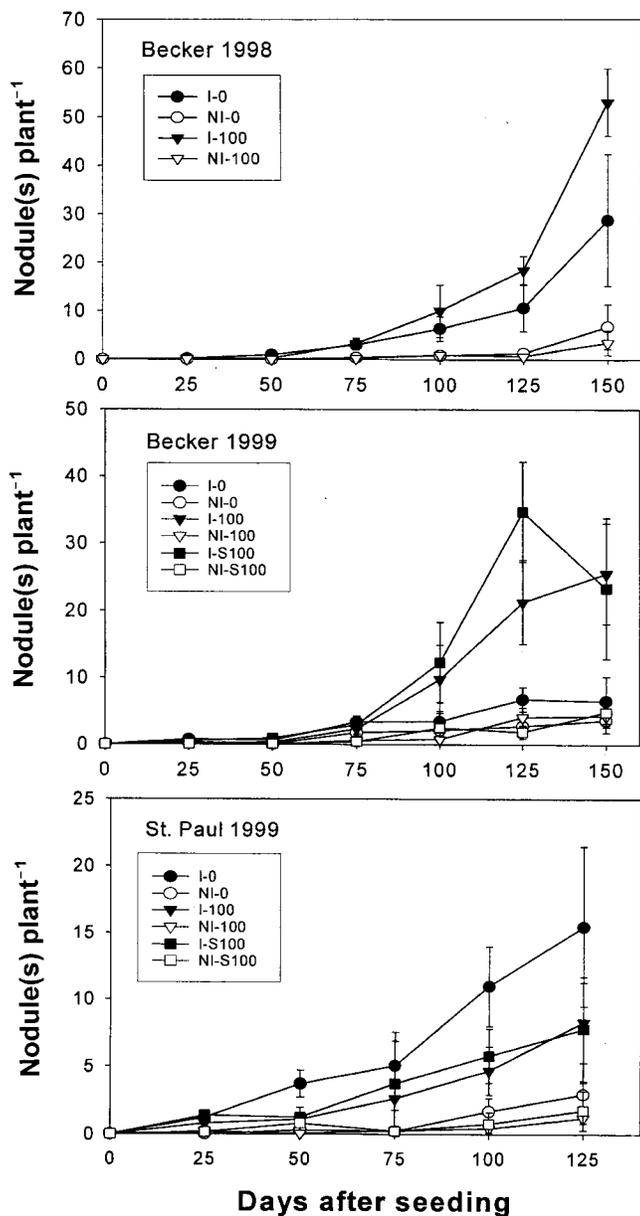


Fig. 2. Seeding-year nodulation of kura clover when inoculated (I) or not inoculated (NI) with rhizobial inoculant and fertilized with three different N treatments in three environments in Minnesota. 0, no fertilization; 100, 100 kg N ha<sup>-1</sup> applied at seeding; and S100, split application of 100 kg N ha<sup>-1</sup> at 10 kg N every other week after seeding. Vertical bars indicate the standard deviation.

Becker and St. Paul were comparable to seeding-year yields reported for other perennial forage legumes (Heichel et al., 1984, 1985; Hall et al., 1995). Also, the N fertilization response and the contrasting lack of inoculation response at Becker reveals the inefficiency of currently available kura clover rhizobia. In low-N soils, rhizobial inoculation will not be sufficient to maximize DM accumulation in kura clover.

### Nodulation, Nitrogen Yield, and Dinitrogen Fixation

Rhizobial inoculation increased nodulation in all environments, and a sampling date × inoculation interac-

Table 3. Effects of rhizobial inoculation and N fertilization on seeding-year N yield of kura clover, and N<sub>2</sub> fixation and percentage of N derived from the atmosphere (%Nd<sub>fa</sub>) of inoculated kura clover in three environments. For N yields, means for inoculation treatment effects are averaged over N treatments, and means for N treatment effects are averaged over inoculation treatments.

Treatments	Becker 1998	Becker 1999	St. Paul 1999		
	kg ha <sup>-1</sup>				
N yield	Inoculation (I)				
	Not Inoculated	13	27	114	
	Inoculated†	37	34	122	
	LSD(0.05)	NS‡	NS	NS	
N fertilization (N)§	0	16	16	97	
	100	33	26	128	
	S100	–	50	129	
		LSD(0.05)	NS	15	NS
	I × N	NS	NS	NS	
N <sub>2</sub> fixation	0	14	2	18	
	100	36	7	23	
	S100	–	20	19	
		LSD(0.05)	NS	NS	NS
	I × N	NS	NS	NS	
%Nd <sub>fa</sub>	0	40	7	14	
	100	62	18	12	
	S100	–	26	10	
		LSD(0.05)	NS	NS	NS

† Inoculated, 20 g peat kg<sup>-1</sup> seed.

‡ NS, not significant at P < 0.05.

§ N fertilization: 0, no N fertilizer applied; 100, 100 kg N ha<sup>-1</sup> applied at seeding; S100, split application of 100 kg N ha<sup>-1</sup> at 10 kg N every other week after seeding.

tion occurred in all environments because the response to inoculation increased with time. For both years at Becker, two- and three-way interactions among inoculation, N fertilization, and sampling date occurred because nodulation was promoted not only by inoculation, but also by N fertilization and the response increased with time. In St. Paul, N fertilization reduced nodulation, and there was no inoculation × N fertilization interaction. The difference between locations in nodulation response to N fertilization may be attributable to the greater inherent soil N levels at St. Paul compared with Becker. It has been previously shown that at low initial soil N levels, moderate N fertilization may stimulate nodulation (Abdel Wahab and Abd-Alla, 1996). In this study, we hypothesize that nodulation stimulation by N fertilizer at Becker was attributable to increased herbage DM from the fertilization, resulting in increased photosynthates available for the nodulation process.

Nodulation response to inoculation appeared on average 50 DAS, and it took 100 DAS to observe more than 10 nodules per plant (Fig. 2), confirming that nodulation of kura clover is a very slow process (Hely, 1957; Genrich et al., 1998). Nodulation is typically observed 2 to 3 wk after inoculation in other species (Graham, 1998). This slow rate of nodulation may be responsible for kura clover's poor seeding-year response to inoculation. In all environments, nodulation of uninoculated plants was extremely limited, with no effective nodules observed. This confirms the absence of native rhizobia effectively nodulating kura clover in soils of our study.

Nitrogen yields (i.e., herbage yield × N concentration) at maximum herbage DM accumulation were only

increased by N fertilization at Becker in 1999 (Table 3). Nitrogen yields were not increased by N fertilization at other locations or by inoculation at any location.

Fixed N<sub>2</sub> and %Ndfa were low in all environments and were similar for the N fertilization treatments, demonstrating that N fertilization did not have a negative effect on N<sub>2</sub> fixation of kura clover (Table 3). The amount of N<sub>2</sub> fixed and %Ndfa were greater at Becker in 1998 than in the other environments. This might be attributable to greater nodulation observed in that environment (Fig. 2); a positive correlation was observed between nodulation and %Ndfa across environments ( $r = 0.40, P = 0.05$ ). Averaged over treatments, the amount of N<sub>2</sub> fixed was 9 kg N ha<sup>-1</sup> at Becker in 1999, 20 at St. Paul in 1999, and 25 at Becker in 1998 while the %Ndfa was 12% at St. Paul in 1999, 17% at Becker in 1999, and 51% at Becker in 1998. Such values are well below those generally reported under similar conditions for other perennial forage legumes in the seeding year. Heichel et al. (1984, 1985), using the <sup>15</sup>N dilution method, reported seeding-year N<sub>2</sub> fixation to be 112, 133, and 165 kg N ha<sup>-1</sup> and %Ndfa to be 40, 65, and 58% for birdsfoot trefoil (*L. corniculatus* L.), red clover (*T. pratense* L.), and alfalfa, respectively. Very low levels of seeding-year N<sub>2</sub> fixation in kura clover are consistent with our observations of very chlorotic, inoculated non-N-fertilized plants, especially at Becker, and with the poor response to rhizobial inoculation reported in all environments. Again, this can be explained by the very slow nodulation of kura clover.

Dinitrogen fixation values presented herein are the first reported estimates of seeding-year N<sub>2</sub> fixation in kura clover. They are consistent with the report by Watson et al. (1996) of almost nil dinitrogenase activity levels in kura clover nodules. Although kura clover N<sub>2</sub> fixation appears to be insignificant in the seeding year, Seguin et al. (2000) reported postseeding year N<sub>2</sub> fixation levels to be similar to those of birdsfoot trefoil.

### Postseeding Year Yield

In contrast to the seeding year when rhizobial inoculation had limited impact on herbage DM accumulation and maximum DM yield of kura clover, in the postseeding year, spring herbage yields at Becker in 1999 and at St. Paul in 2000 were greatly increased by inoculation in the preceding year (Table 4). In contrast, seeding-year N fertilization increased postseeding year herbage yield only at Becker in 2000. There was no inoculation × N fertilization interaction in any environments. Results suggest that rhizobial inoculation in the seeding year benefits kura clover beginning in the year following seeding. This delay in inoculation response might be due to the slow nodulation process in kura clover.

Spring root yields in the postseeding year were increased by seeding-year inoculation at Becker in 1999 while seeding-year N fertilization increased yields at Becker in 1999 and 2000 (Table 4). At Becker in 1999, there was also a significant inoculation × N fertilization interaction due to a greater response to seeding-year N fertilization when plants were inoculated (data not

**Table 4. Main effects of rhizobial inoculation and N fertilization treatments on yields of kura clover in spring of the postseeding year (last week of May) in three environments. Means for inoculation treatment effects are averaged over N treatments, and means for N treatment effects are averaged over inoculation treatments.**

Plant part	Treatment	DM† yield		
		Becker 1999	Becker 2000	St. Paul 2000
		kg ha <sup>-1</sup>		
Herbage	<b>Inoculation (I)</b>			
	Not inoculated	334	1407	4800
	Inoculated‡	2191	2031	5703
	LSD(0.05)	155	NS§	597
	<b>N fertilization (N)¶</b>			
	0	1076	1189	5668
	100	1449	1905	5358
	S100	–	2064	4729
	LSD(0.05)	NS	595	NS
	I × N	NS	NS	NS
Root	<b>I</b>			
	Not inoculated	573	1362	1786
	Inoculated	1627	1684	1476
	LSD(0.05)	635	NS	NS
	<b>N</b>			
	0	927	934	1470
	100	1273	1468	1683
	S100	–	2167	1740
	LSD(0.05)	266	544	NS
	I × N	**#	NS	NS
Rhizome	<b>I</b>			
	Not inoculated	34	526	324
	Inoculated	325	831	172
	LSD(0.05)	226	NS	NS
	<b>N</b>			
	0	164	364	230
	100	195	732	238
	S100	–	940	277
	LSD(0.05)	NS	327	NS
	I × N	NS	NS	NS

† DM, dry matter.

‡ Inoculated, 20 g peat kg<sup>-1</sup> seed applied in the seeding year only.

§ NS, not significant at the 0.05 level.

¶ N fertilization: 0, no N fertilizer applied; 100, 100 kg N ha<sup>-1</sup> applied at seeding; S100, split application of 100 kg N ha<sup>-1</sup> at 10 kg N every other week after seeding. N fertilizer was applied only in the seeding year.

# The I \* N interaction indicates a greater response to N fertilization when inoculated than when not inoculated.

shown). The lack of treatment response at St. Paul, as well as the relatively low root yields observed at this location, may be explained by the low soil moisture at harvest that made root recovery difficult. Rhizome yields were increased by seeding-year inoculation at Becker in 1999 and by seeding-year N fertilization at Becker in 2000. There was no inoculation × N fertilization interaction in any of the environments.

When response to N fertilization was observed at time of maximum DM yield in the seeding year (Becker, 1999; Table 2), response was maintained in the postseeding year (Becker 2000; Table 4). This sustained response to N fertilization from the seeding to postseeding year emphasizes the importance of proper N nutrition during kura clover establishment.

### SUMMARY AND CONCLUSIONS

Our study suggests that the use of a current commercial rhizobial inoculant for kura clover has limited measurable benefits in the seeding year. Rhizobial inocula-

tion failed to consistently improve seeding-year herbage, root, and rhizome DM accumulation and N yields compared with no inoculation. This can be explained by very slow nodulation of kura clover plants and consequently, limited N<sub>2</sub> fixation. Rhizobial inoculation in the seeding year, however, benefited kura clover in the postseeding year. In contrast, N fertilization more consistently increased kura clover seeding-year herbage and root DM accumulation, suggesting that kura clover growth was often N limited in the seeding year. This response was more pronounced at Becker than at St. Paul, probably due to the lower soil N availability of the Becker site. Benefits of seeding-year N fertilization were sustained in the postseeding year. The high maximum herbage yields in the seeding year observed with adequate N nutrition in this study indicate that initial vigor of kura clover could be improved with better N nutrition. This could be achieved by applying N fertilizers or by identifying rhizobial strains that result in a more rapid and profuse nodulation of kura clover in the seeding year. The use of N fertilizers could increase weed competition and have potentially negative impacts on the environment.

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