Dinitrogen Fixation in Kura Clover and Birdsfoot Trefoil

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

ABSTRACT

The quantification of symbiotic N_2 fixation by legumes is essential to determine their impact on N budgets. Kura clover (*Trifolium ambiguum* M.B.) and birdsfoot trefoil (*Lotus corniculatus* L.) are two promising pasture legumes for which such estimates are scarce. Dinitrogen fixation by 2- and 3-yr-old stands of these species was determined at two locations in Minnesota using the ^{15}N isotope dilution method. Species were evaluated for forage and N yields, the percent of N derived from the atmosphere (%Ndfa), and the amount of fixed N_2 at each of four harvests. The results for each parameter varied with location, legume species, and harvest, as demonstrated by a three-way interaction among these terms. Across environments, both species produced the greatest amount of forage, total N, and fixed N yields at the first harvest. The yields decreased with successive forage harvests at one location but stabilized at the other. The %Ndfa for Kura clover was stable across harvests and environments. The %Ndfa for birdsfoot trefoil varied during the season, with a peak at the end of the season. The yearly total forage, total N, and fixed N yields of Kura clover were stable across environments, and the total N and fixed N yields were greater than those of birdsfoot trefoil at one location. Overall, Kura clover fixed about 155 kg N ha^{-1} yr^{-1}, with a %Ndfa of 57; birdsfoot trefoil had amounts of fixed N_2 that varied with locations (avg. of 145 kg N ha^{-1} yr^{-1}), and it obtained about 62% of its herbage N from fixation.

Because of their ability to fix N_2, forage legumes are an important N source in agroecosystems, often reducing N fertilizer needs in crop rotations and mixed grass-legume pastures (Mallarino et al., 1990). The amount of N_2 fixed by forage legumes varies greatly with species, management, soil N supply, and the presence of grasses, but it can reach 275 kg N ha^{-1} yr^{-1} in the north-central USA (Mallarino et al., 1990). An accurate determination of N_2 fixation by forage legumes is essential to assess their N contribution to agroecosystems.

Kura clover is a rhizomatous forage legume with potential as a long-lived pasture legume (Taylor and Smith, 1998). It is exceptionally persistent under grazing (Peterson et al., 1994a, 1994b) and harsh environmental conditions (Woodman et al., 1992), and it produces high quality forage (Peterson et al., 1994a). The use of Kura clover has been limited by slow establishment and low seed production, but it appears that these limitations could be lessened by establishment practices that minimize competition with weeds (Seguin et al., 1999), add N fertilizer (P. Seguin, unpublished data, 1999), and use appropriate rhizobial strains (Pryor et al., 1998). Selection for increased seedling vigor might also contribute to improving Kura clover establishment (DeHaan et al., 1997). Delayed and limited nodulation (Hely, 1963; Zorin et al., 1976; Patrick et al., 1994) and poor N_2 fixation in the seeding year (Watson et al., 1996) may also contribute to establishment problems. These limitations apparently do not restrict the growth of Kura clover once it is established, but there is currently no information available on the rates of N_2 fixation in established Kura clover.

Birdsfoot trefoil is another perennial forage legume that is adapted to a wide variety of soil types and produces high quality, nonbloating forage. However, it is characterized by a slow recovery rate after harvesting, and like Kura clover, a slow and difficult establishment (Grant and Marten, 1985). Under field conditions, the rates of N_2 fixation in birdsfoot trefoil vary between 50 and 205 kg ha^{-1} yr^{-1}, with a %Ndfa varying between 30 and 100 (Heichel et al., 1985; Mallarino et al., 1990; Farnham and George, 1994). The median values are 92 kg ha^{-1} yr^{-1} of fixed N_2 and a %Ndfa of 55 (Vance, 1998). These estimates are generally lower than those of other temperate forage legumes. This is attributable, in part, to the fact that birdsfoot trefoil has determinate nodules that do not regenerate after defoliation, requiring the plant to renodulate after each harvest (Vance et al., 1982).

Our objective was to determine N_2 fixation by established stands of Kura clover and birdsfoot trefoil under field conditions using the ^{15}N isotope dilution method.

MATERIALS AND METHODS

Four different field experiments were conducted in 1998. Two experiments, one seeded in 1996 and the other in 1997, were each conducted at two locations: Becker and Rosemount, MN. This resulted in two experiments of different stand age (2- and 3-yr-old) at both locations. At the onset of the study in 1998, the Hubbard loamy sand (sandy, mixed, frigid Entic Hapludoll) at Becker had a pH of 7.0 and NO_3^-–N, Bray-1 extractable P, and exchangeable K levels of 3, 25, and 145 mg kg^{-1}, respectively, for the 1996 seeding. For the 1997 seeding, the pH was 7.2 and the NO_3^-–N, P, and K values were 1, 18, and 70 mg kg^{-1}, respectively. The soil organic matter content averaged 33 g kg^{-1} at Becker. At Rosemount, the soil was a Waukegan silt loam (fine-silty over sandy, mixed, mesic Typic Hapludoll) with a pH of 6.8 and NO_3^-–N, P, and K values of 2, 9, and 128 mg kg^{-1}, respectively, for the 1996 seeding. For the 1997 seeding, the pH was 7.0 and the NO_3^-–N, P, and K values were 2, 10, and 148 mg kg^{-1}, respectively. The soil organic matter content was 46 and 42 g kg^{-1} for the 1996 and 1997 seedings, respectively. The Becker site received 150 mm of irrigation, whereas Rosemount was rainfed. The total monthly precipitation and average temperature were near the 30-yr average at Becker, but Rosemount received 17 cm of precipitation above average in the spring. The air temperatures were near average at Rosemount.

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Abbreviation: %Ndfa, percent of N derived from the atmosphere.

Kura clover (cv. Endura) and birdsfoot trefoil (cv. Norcen) were sown in 3- by 6-m plots in 15-cm rows at a rate of 8 kg ha^{-1} on 30 Apr. 1996 and 28 Apr. 1997 at Becker and 21 May 1996 and 5 May 1997 at Rosemount. The seeds were inoculated with appropriate peat-based commercial rhizobial inoculants (LiphaTech, Milwaukee, WI)[1]. 'Ineffective Agate' alfalfa (*Medicago sativa* L.) and annual ryegrass (*Lolium multiflorum* Lam.) were used as non N_2-fixing reference species. Alfalfa was seeded at a rate of 12 kg ha^{-1} on the same date as Kura clover and birdsfoot trefoil, and annual ryegrass was seeded on 15 Apr. 1998 at a rate of 17 kg ha^{-1}. In 1998, the average stand densities of Kura clover, birdsfoot trefoil, alfalfa, and annual ryegrass were 150, 90, 50, and 80 plants m^{-2}, respectively. Plots were subjected to four harvests on 2 June, 7 July, 10 Aug., and 7 Oct. 1998 at Becker and 22 May, 29 June, 5 Aug., and 6 Oct. 1998 at Rosemount.

Dinitrogen fixation was estimated using the isotope dilution method. On 17 Apr. 1998, a 1-m^2 microplot in each plot was uniformly sprayed with 3 L of water containing 0.3 g N (99.7 atom % ^{15}N) of $(NH_4)_2SO_4$. Because Kura clover is rhizomatous, the microplots were surrounded to a 30-cm depth with aluminum flashing to reduce the uptake of nonlabeled soil N from outside the enriched areas. At each harvest, the center 0.36 m^2 of each microplot was harvested to a 5-cm stubble height. These samples were used for N and ^{15}N analyses. Other samples from a different 0.6- by 15-m area were harvested in each plot with a flail harvester to determine forage yields, and a 500-g subsample was then used to determine the dry matter content. All samples were dried in a forced-air oven at 60°C for 48 h. Samples from the ^{15}N-labeled microplots were ground to pass through a 0.5-mm screen and then analyzed by mass spectroscopy using an Integra-N integrated ^{15}N analyzer (PDZ-Europa, Cheshire, United Kingdom) to determine the total N and isotopic composition of the samples. Dinitrogen fixation was expressed as the %Ndfa and as the amount of N fixed on an area basis. The calculations used were:

$$\%\text{Ndfa} = \left[1 - \left(\frac{\text{atom \% } ^{15}\text{N excess in the nfp}}{\text{atom \% } ^{15}\text{N excess in the rp}}\right)\right] \times 100$$

$$\text{Ndfa (kg ha}^{-1}) = \left[1 - \left(\frac{\text{atom \% } ^{15}\text{N excess in the nfp}}{\text{atom \% } ^{15}\text{N excess in the rp}}\right)\right] \times (\text{DM}_{\text{nfp}}) \times (\%\text{N}_{\text{nfp}})/100$$

where nfp refers to N_2 fixing plants, rp refers to reference plants, DM refers to dry matter yields, %N refers to N concentration. For the first two harvests, Ineffective Agate alfalfa was used as the reference, and for the last two, annual ryegrass was used. This change of the reference plant was needed because the regrowth of Ineffective Agate alfalfa was poor after the last two harvests for all stands at Rosemount and for 2-yr-old stands at Becker. The %Ndfa was calculated at Becker for the last two harvests of 3-yr-old stands using either Ineffective Agate alfalfa or ryegrass as a reference. The percentages were not different ($P = 0.95$), supporting the acceptability of this change in the reference plant.

The plots of each of the four experiments were arranged in a randomized complete block design with six replicates. All of the data were subjected to an analysis of variance (ANOVA) using the General Linear Models (GLM) procedure in SAS (SAS Inst., 1985) to identify significant treatment effects and interactions. Homoscedasticity among the four experiments was verified using the chi-square test (Gomez and Gomez, 1984). The data were then analyzed in a combined analysis (McIntosh, 1983) that regrouped the locations, stand ages, legume species, and harvests in a combined strip-plot design, with the legume species and harvests as spatial and temporal strips, respectively (Gomez and Gomez, 1984). The stand ages, legume species, and harvests were considered fixed effects while the location was considered random. The yearly totals and means were analyzed in a combined analysis with the locations, stand ages, and legume species in a randomized complete block design. Appropriate *F*-tests in each case were calculated following McIntosh (1983). The differences between the treatments were ascertained using LSD and *F*-tests.

[1] Names are necessary to report factually on available data; however, the USDA and the University of Minnesota neither guarantee nor warrant the standard of the product, and the use of the name by the USDA and the University of Minnesota implies no approval of the product to the exclusion of others that may also be suitable.

RESULTS AND DISCUSSION

Dry Matter and Total Nitrogen Yields

Both the forage and total N yields showed a strongly significant location × legume species × harvest interaction (Table 1). In addition, the forage yields showed a significant stand age effect: Three-year-old stands of each species produced more than 2-yr-old stands (discussed below). When illustrated, the significant location × legume species × harvest interaction demonstrates that the response trends for dry matter and N yield were similar (Fig. 1 and 2).

At Becker, the forage and N yields of each species decreased during the year, with each successive harvest yielding less than the previous one. The effect was more pronounced with birdsfoot trefoil, which produced 60% of its yearly total forage yield at the first harvest compared with 35% for Kura clover (Fig. 1). The N yield followed a similar pattern as the forage yield, with birdsfoot trefoil and Kura clover producing 55 and 35% of the yearly total at the first harvest (Fig. 2). Birdsfoot trefoil had greater forage and N yields at the first harvest than Kura clover while the reverse was observed at the other harvest dates.

At Rosemount, most of the forage production of both

Table 1. Mean squares from the combined analysis of variance for the forage yield, N yield, percent of N derived from the atmosphere (%Ndfa), and amount of Fixed N_2 for 2- and 3-yr-old Kura clover and birdsfoot trefoil stands at Becker and Rosemount, MN.

Source of variation	df	Mean squares			
		Forage yield	N yield	%Ndfa	Fixed N_2
Location (L)	1	523 312	40	158	484
Stand age (A)	1	2 836 459**	3 596	61	2 047
L × A	1	370	197	663	962
Error *a*	20	207 135	217	363	307
Species (S)	1	2 198 214	1 632	1 475	246
S × L	1	952 722	972	197	1 014*
S × A	1	69	42	82	76
S × L × A	1	596 065	422	792	134
Error *b*	20	238 388	241	213	187
Harvest (H)	3	56 587 248*	53 729**	1 797	16 950*
H × L	3	3 360 624***	2 248***	335	643**
H × A	3	602 624	1 180	1 091	1 790
H × L × A	3	286 249	160	263	279
Error *c*	60	110 283	111	160	147
H × S	3	3 814 899	2 132	1 331*	508
H × S × L	3	2 306 510***	1 732***	139*	661**
H × S × A	3	436 310	1 409	35	705*
H × S × L × A	3	105 865	186	35	42
Error *d*	60	137 819	158	43	92

* Significant at the 0.05 level.
** Significant at the 0.01 level.
*** Significant at the 0.001 level.

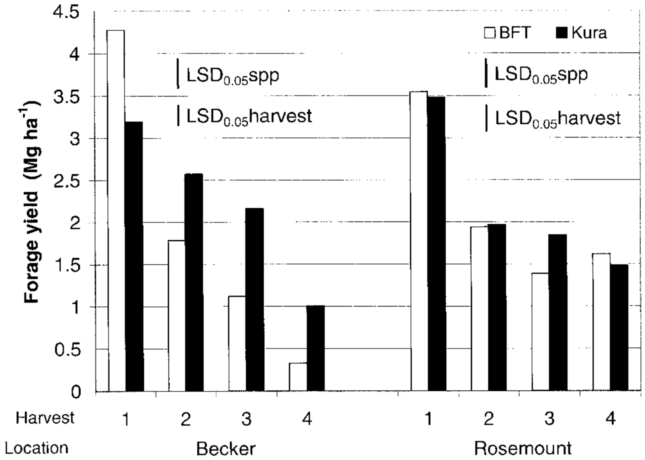

Fig. 1. Forage yields of Kura clover and birdsfoot trefoil stands harvested four times at two locations in Minnesota. The data represent the location × species × harvest interaction. For a given location, LSD(0.05)spp allows comparisons between the species within a harvest; LSD(0.05)harvest allows comparisons between the harvests of one species.

species also occurred at the first harvest. Birdsfoot trefoil and Kura clover produced 45 and 40%, respectively, of their yearly total at the first harvest. Unlike at Becker, the forage yields for both species at the other 3 harvests were similar. In addition, both species produced a similar quantity of forage at each harvest, except for the third harvest when Kura clover produced more than birdsfoot trefoil. The trends for N yields were similar, except that Kura clover produced lower N yields at each successive harvest.

An uneven forage-yield distribution is characteristic of Kura clover and birdsfoot trefoil (Heichel et al., 1985; Peterson et al., 1994a). For Kura clover under high to medium defoliation frequencies, Peterson et al. (1994a) attributed this to a phenotypic plasticity response, where plants respond to defoliation by reducing the petiole length and leaf size. This ensures stand persistence, but

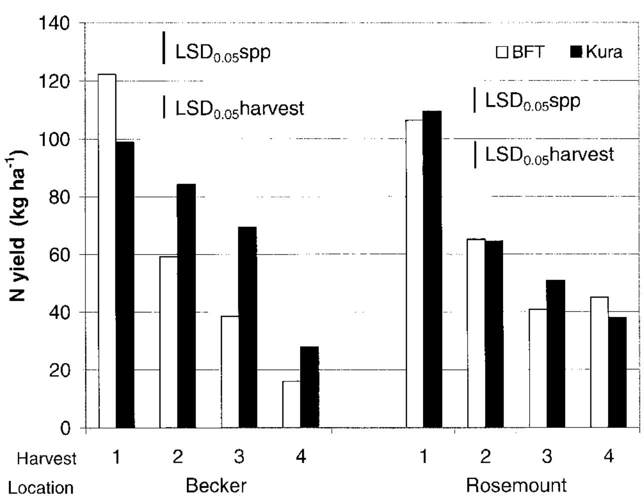

Fig. 2. Nitrogen yields of Kura clover and birdsfoot trefoil stands harvested four times at two locations in Minnesota. The data represent the location × species × harvest interaction. For a given location, LSD(0.05)spp allows comparisons between the species within a harvest; LSD(0.05)harvest allows comparisons between the harvests of one species.

Table 2. Mean squares from the combined analysis of variance for the annual total forage yield, N yield, amount of fixed N_2, and avg. percent of N derived from the atmosphere (%Ndfa), for 2- and 3-year-old Kura clover and birdsfoot trefoil stands at Becker and Rosemount, MN.

Source of variation	df	Mean squares			
		Forage yield	N yield	%Ndfa	Fixed N_2
Location (L)	1	2 093 270	2 086	40	1 934
Stand age (A)	1	11 345 847**	30 411	15	8 195
L × A	1	1 479	1 499	167	3 846
Error *a*	20	953 550	1 035	91	1 227
Species (S)	1	8 792 832	17 349	370	981
S × L	1	3 810 905	6 444*	49	4 051*
S × A	1	277	45	21	306
S × L × A	1	2 384 290	309	199	536
Error *b*	20	828 538	1 143	53	747

* Significant at the 0.05 level.
** Significant at the 0.01 level.

it also results in lower forage production. The factors in birdsfoot trefoil are likely low total nonstructural carbohydrate reserves and regrowth from axillary buds rather than from the crown like most other temperate forage legumes (Grant and Marten, 1985).

There was a significant stand age effect on the total annual forage yield (Table 2). At each location, 3-yr-old stands of both species were significantly more productive than 2-yr-old stands (8.91 vs. 7.94 Mg ha^{-1}). For both species, the forage yields of 3-yr-old stands were similar to those reported in other studies (e.g., Heichel, 1985; Sheaffer and Marten, 1991); however, the Kura clover yield of 2-yr-old stands in our study was substantially higher than the yield reported by Sheaffer and Marten (1991) of 4.70 Mg ha^{-1}yr^{-1}. This might be due, in part, to the fact that they used the cultivar Rhizo, which develops at a slower rate and initially produces less forage than Endura, the cultivar used in this study (Genrich et al., 1998). For the total annual N yield, there was a significant location × species interaction (Table 2). At Becker, the Kura clover N yield was 28% greater than that of birdsfoot trefoil, but at Rosemount, there was no significant species effect (Table 3).

Percent of Nitrogen Derived from the Atmosphere and Fixed Dinitrogen

The %Ndfa and amount of N_2 fixed per hectare varied with location, legume species, and harvests, as was indicated by the highly significant interaction among these

Table 3. Yearly total forage, N, and fixed N_2, and the avg. percent of N derived from the atmosphere (%Ndfa) for Kura clover and birdsfoot trefoil stands in their first and second production year at two locations in Minnesota.†

Location	Species	Forage yield	N yield	Fixed N_2	%Ndfa
		Mg ha^{-1}	kg ha^{-1}	kg ha^{-1}	%
Becker	Birdsfoot trefoil	7.50	215	131	61
	Kura clover	8.92	276	158	57
	F-test	NS	**	*	NS
Rosemount	Birdsfoot trefoil	8.49	251	162	64
	Kura clover	8.78	266	152	57
	F-test	NS	NS	NS	NS
	Avg.	8.42	252	151	60

* Significant at the 0.05 level.
** Significant at the 0.01 level.
† Results are the avg. of two stand ages.

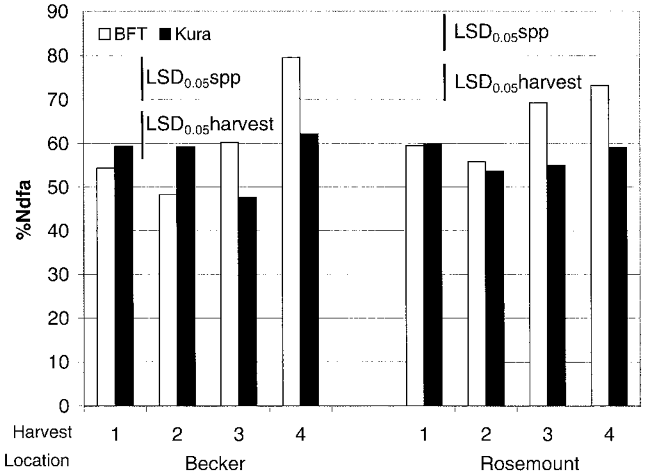

Fig. 3. Percent of N derived from the atmosphere (%Ndfa) for Kura clover and birdsfoot trefoil stands harvested four times at two locations in Minnesota. The data represent the location × species × harvest interaction. For a given location, LSD(0.05)spp allows comparisons between the species within a harvest; LSD(0.05)harvest allows comparisons between the harvests of one species.

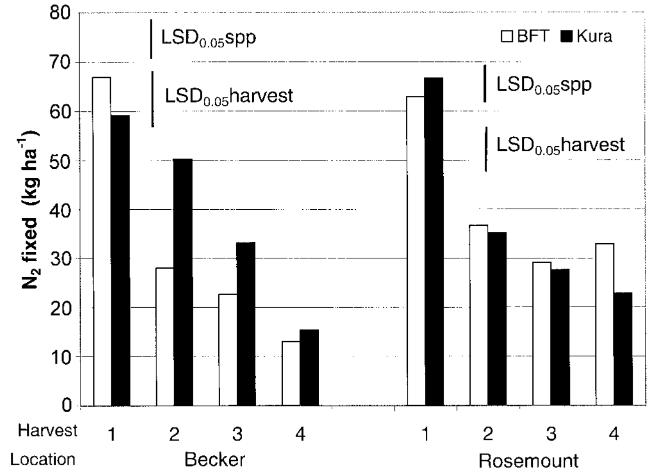

Fig. 4. Dinitrogen fixed by Kura clover and birdsfoot trefoil stands harvested four times at two locations in Minnesota. The data represent the location × species × harvest interaction. For a given location, LSD(0.05)spp allows comparisons between the species within a harvest; LSD(0.05)harvest allows comparisons between the harvests of one species.

three factors (Table 1). The %Ndfa at both locations followed a similar trend in that it was nearly constant for Kura clover (avg. of 57%) throughout the season, except for a drop down to 47% for the third harvest at Becker (Fig. 3). For birdsfoot trefoil, the %Ndfa also followed a trend that was similar at both locations in that it was lower at the first and second harvest and higher at subsequent harvests. This trend for birdsfoot trefoil is opposite to that observed by Heichel et al. (1985) for 2-yr-old stands, where the maximum %Ndfa occurred at the second of three harvests. Differences between their study and ours might result from environmental conditions because those authors reported unusually high rainfall in the spring. However, studies with other species such as alfalfa and red clover (*Trifolium pratense* L.) have demonstrated that in general, the %Ndfa is lowest in the spring (e.g., Heichel et al., 1984, 1985) due to suboptimal temperatures, high inorganic N availability, or both. An alternative explanation might be that our study used a fairly intensive defoliation scheme, with four defoliations during the season compared with two or three in the studies of Heichel et al. (1984, 1985), and thus affected the regrowth period available to the plants. Also, differences in the soil N supply between the studies could have affected the results. Concerning the species differences at individual harvests, birdsfoot trefoil had a greater %Ndfa than Kura clover at the last two harvests (avg. of 70 vs. 55%, respectively) at both locations. While the reverse was true for the second harvest at Becker, where the %Ndfa for Kura clover was 59% compared with 47% for birdsfoot trefoil, no differences were noted at the second harvest at Rosemount. However, these temporal differences between species were not reflected in the annual average %Ndfa (Table 2), and both species contained a %Ndfa of about 60% for the year (Table 3).

The amount of N_2 fixed per hectare varied with location, stand age, species, and harvest, as was demonstrated by the three-way interactions between these terms (Table 1). As the amount of fixed N_2 is calculated from the N yield and %Ndfa, the illustration of the location × legume species × harvest interaction demonstrates that the response was indeed a composite of those reported earlier for the N yields and %Ndfa (Fig. 4). At Becker, the N_2 fixed by Kura clover was lower at each subsequent harvest with 37, 32, 20, and 11% of the total season N_2 fixed at the first, second, third, and fourth harvest, respectively. In contrast, the intra-annual pattern of N_2 fixed by Kura clover at Rosemount was much more uneven, with 45% of the total season N_2 fixed at the first harvest. The N_2 fixation at the other harvests was similar (avg. of 29 kg ha^{-1} harvest^{-1}). The birdsfoot trefoil followed a trend similar to Kura clover at Rosemount, fixing 45% of the total fixed N by the first harvest; however, unlike Kura clover, this was also the case at Becker. The locations × species × harvest interaction occurred because at Becker, Kura clover fixed more N_2 than birdsfoot trefoil at the second and third harvest, whereas at Rosemount, birdsfoot trefoil fixed more N_2 than Kura clover at the last harvest.

The interaction between locations and species was also significant for the annual total fixed N_2 (Table 2). At Becker, Kura clover fixed significantly more N_2 than birdsfoot trefoil (158 vs. 130 kg ha^{-1} yr^{-1}, respectively), but at Rosemount, the N_2 fixation by Kura clover and birdsfoot trefoil was similar (avg. of 157 kg ha^{-1} yr^{-1}) (Table 3). Such levels of N_2 fixation suggest that both species fix less N than other commonly used temperate forage legumes. Based on a compilation of the literature, Vance (1998) reported median N_2 fixation levels of 180, 172, and 170 kg N ha^{-1} yr^{-1} for alfalfa, white clover (*Trifolium repens* L.), and red clover, respectively. That author also reported a value of 92 kg N ha^{-1} yr^{-1} for birdsfoot trefoil. The values observed in our study were substantially higher, but the differences are likely due to differences in the cultivars, management, environmental conditions, methodology used to determine N_2 fixation, soil N supply, and N demand.

The stand age × species × harvest interaction for fixed N_2 (Table 1) indicated that 2-yr-old Kura clover stands fixed similar amounts of N_2 at the first three harvests (avg. of 40 kg ha^{-1} $harvest^{-1}$) but less at the last harvest (23 kg N ha^{-1}). In 3-yr-old stands, 50% of the annual total was fixed by the first harvest (80 kg N ha^{-1}), with the amounts of fixed N_2 decreasing at subsequent harvests. Kura clover has limited N_2 fixing activity in the seeding year (Watson et al., 1996), and results here suggest that this might carry on to the beginning of the second year, where amounts of fixed N_2 appear relatively limited in the spring compared with the third year. The birdsfoot trefoil stands of both ages fixed most N_2 before the first harvest.

CONCLUSIONS AND SUMMARY

This study demonstrates that Kura clover and birdsfoot trefoil differ in their seasonal trends for forage, N production, and N_2 fixation, depending on the environment. Both species produced most of their forage, total N, and fixed N yields at the first of four harvests, with a subsequent reduction in yields. However, at one location, Kura clover forage, total N, and fixed N yields tended to decrease more gradually than those of birdsfoot trefoil, resulting in a more even distribution of yields. Kura clover had a constant %Ndfa during the season while that of birdsfoot trefoil varied, peaking at the end of the season. This resulted in a greater %Ndfa than Kura clover at the last two harvests. At one of the two locations, Kura clover had significantly higher annual total N and fixed N yields than birdsfoot trefoil. Kura clover had more stable total annual N_2 fixation and %Ndfa across environments than birdsfoot trefoil. In this study, the N_2 fixed by Kura clover was 155 kg N $ha^{-1}yr^{-1}$ in all environments, with a %Ndfa of 57. Birdsfoot trefoil also had about a %Ndfa of 62, but the amounts of fixed N_2 varied with locations.

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REFERENCES

DeHaan, L.R., N.J. Ehlke, and C.C. Sheaffer. 1997. Selection methods for increasing seedling vigor in Kura clover. p. 68. *In* 1997 Agron. abstr. ASA, Madison, WI.

Farnham, D.E., and J.R. George. 1994. Harvest management effects on productivity, dinitrogen fixation, and nitrogen transfer in birdsfoot trefoil–orchardgrass communities. Crop Sci. 34:1650–1653.

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

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. John Wiley & Sons, New York.

Grant, W.F., and G.C. Marten. 1985. Birdsfoot trefoil. p. 98–108. *In* M.E. Heath et al. (ed.) Forages: The science of grassland agriculture. 4th ed. Iowa State Univ. Press, Ames.

Heichel, G.H., D.K. Barnes, C.P. Vance, and K.I. Henjum. 1984. N_2 fixation, and N and dry matter partitioning during a 4-year alfalfa stand. Crop Sci. 24:811–815.

Heichel, G.H., D.K. Barnes, C.P. Vance, and K.I. Henjum. 1985. Dinitrogen fixation, and N and dry matter distribution during 4-year stands of birdsfoot trefoil and red clover. Crop Sci. 25: 101–105.

Hely, F.W. 1963. Relationship between effective nodulation and time to initial nodulation in a diploid line of *Trifolium ambiguum* M. Bieb. Aust. J. Biol. Sci. 16:43–54.

Mallarino, A.P., W.F. Wedin, R.S. Goyenola, C.H. Perdomo, and C.P. West. 1990. Legume species and proportion effects on symbiotic dinitrogen fixation in legume–grass mixtures. Agron. J. 82:785–789.

McIntosh, M.S. 1983. Analysis of combined experiments. Agron. J. 75:153–155.

Patrick, H.N., W.L. Lowther, and K.D. Trainor. 1994. Inoculation for successful establishment of Caucasian clover. Proc. N.Z. Grassl. Assoc. 56:101–105.

Peterson, P.R., C.C. Sheaffer, R.M. Jordan, and C.J. Christians. 1994a. Response of Kura clover to sheep grazing and clipping: I. Yield and forage quality. Agron. J. 86:655–660.

Peterson, P.R., C.C. Sheaffer, R.M. Jordan, and C.J. Christians. 1994b. Response of Kura clover to sheep grazing and clipping: II. Belowground morphology, persistence, and total nonstructural carbohydrates. Agron. J. 86:660–667.

Pryor, H.N., W.L. Lowther, H.J. McIntyre, and C.W. Roson. 1998. An inoculant *Rhizobium* strain for improved establishment and growth of hexaploid Caucasian clover (*Trifolium ambiguum*). N.Z. J. Agric. Res. 41:179–189.

SAS Institute. 1985. SAS user's guide: Statistics. 5th ed. SAS Inst., Cary, NC.

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

Sheaffer, C.C., and G.C. Marten. 1991. Kura clover forage yield, forage quality, and stand dynamics. Can. J. Plant Sci. 71:1169–1172.

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

Vance, C.P. 1998. Legume symbiotic nitrogen fixation: Agronomic aspects. p. 509–530. *In* H.P. Spaink et al. (ed.) The *Rhizobiaceae*: Molecular biology of model plant-associated bacteria. Kluwer Academic Publ., Dordrecht, The Netherlands.

Vance, C.P., L.E.B. Johnson, S. Shade, and R.G. Groat. 1982. Birdsfoot trefoil (*Lotus corniculatus* L.) root nodules: Morphogenesis and the effect of forage harvest on structure and function. Can. J. Bot. 60:505–518.

Watson, R.N., F.J. Neville, N.L. Bell, and S.L. Harris. 1996. Caucasian clover as a pasture legume for dryland dairying in the coastal Bay of Plenty. Proc. N.Z. Grassl. Assoc. 58:183–188.

Woodman, R.F., J.M. Keoghan, and B.E. Allan. 1992. Pasture species for drought-prone lower slopes in the South Island high country. Proc. N.Z. Grassl. Assoc. 54:115–120.

Zorin, M., J. Brockwell, and W.J. Müller. 1976. Symbiotic characteristics of *Trifolium ambiguum* seedlings grown in tube culture as affecting subsequent symbiotic vigour. Aust. J. Exp. Agric. Anim. Husb. 16:854–862.