Journal of the Iowa Academy of Science: JIAS

Volume 98 | Number Article 5

1991

Comparison of Dinitrogen Fixation and Nitrogen Transfer Potentials of Four Red Clover Cultivars

D. E. Farnham
Iowa State University

A. P. Mallarino
Iowa State Universtiy, apmallar@iastate.edu

W. F. Wedin
Iowa State University

I. T. Carlson
Iowa State University, carlsoni@iastate.edu

Jour. Iowa Acad. Sci. 98(4):162-166, 1991

Comparison of Dinitrogen Fixation and Nitrogen Transfer Potentials of Four Red Clover Cultivars

D.E. FARNHAM*, A.P. MALLARINO, W.F. WEDIN, and I.T. CARLSON

Department of Agronomy, Iowa State University, Ames, IA 50011

The capacity to fix atmospheric N_2 and thus reduce the use of N fertilizer is an important reason for including legumes in forage mixtures. Selection for more efficient N_2 fixation could improve red clover (*Trifolium pratense* L.) (RC) production and its contribution to soil fertility. An isotope dilution study was conducted in two greenhouse experiments to compare the N_2 fixation and N transfer potentials of three commercial and one experimental RC cultivars. The experimental cultivar APR-8701 was selected for traits related to increased N_2 fixation (rate of acetylene reduction, root size and branch number, and nodule mass). 'Dawn' orchardgrass (*Dactylis glomerata* L.) (OG) was used as the grass in mixture and as the reference crop for the isotope dilution study. Four harvests were taken from each of the two experiments. Herbage was analyzed first for total N and then for isotopic composition by mass spectrometry. Under the conditions of this study, the particular traits selected for in the development of the cultivar APR-8701 resulted in a high N_2 fixation rate, ranging from 88.9 to 99.8 %N derived from the atmosphere (%Ndfa) in pure and mixed stands of both experiments, that parallelled the other three cultivars tested (ranging between 86.8 and 99.9 %Ndfa in pure and mixed stands of both experiments). However, APR-8701 was average when N transfer potentials were compared. Average %N in grass derived from the atmosphere (%Ngdfa) was 32.2 and 46.3% for OG grown with APR-8701 in experiments 1 and 2, respectively, and ranged between 30.5 and 50.1% for OG grown with the other three cultivars. We conclude that the experimental cultivar APR-8701 showed N_2 fixation rates similar to that of other superior, commercially available cultivars, however, APR-8701 only showed average N transfer capability.
INDEX DESCRIPTORS: dinitrogen fixation, nitrogen transfer, red clover, orchardgrass

Red clover (RC) has long been used in crop rotations as a forage and to provide N for succeeding nonleguminous crops. Reports on amounts of N_2 fixed by RC under field conditions range from 70 to 300 kg ha^{-1} yr^{-1} (e.g. Nutman, 1976; Rohweder et al., 1977; LaRue and Patterson, 1981; Heichel et al., 1985; Boller and Nösberger, 1987; Mallarino et al., 1990b). Little information is available, however, on amounts of N_2 fixed or transferred by different cultivars of RC. In a study comparing N_2 fixation (acetylene reduction) of four RC cultivars, Faris (1983) concluded that, due to large variation between cultivars, selection could be done for a characteristic such as rate of N_2 fixation. Heichel and Vance (1979) previously showed that this was possible with alfalfa (*Medicago sativa* L.).

Data compiled by Allos and Bartholomew (1959) suggest that the capacity to fix N_2 from the atmosphere is related to factors closely correlated with the size and gross weight of the plant. In addition, several environmental and management factors affect N_2 fixation by legumes. One important factor is the concentration of mineral N in the soil. Legume N_2 fixation often is inhibited by high mineral soil-N concentrations (McAuliffe et al., 1958) that are frequently found in agricultural soils. Thus, legume N_2 fixation usually is stimulated by grass growth when grass growth results in depletion of soil N because the reliance of legumes on N_2 fixation is increased (Brophy et al., 1987; Mallarino et al., 1990b). Previous research with other forage legumes (Haystead and Marriott, 1979; Ledgard et al., 1985; Butler, 1986; Ta and Faris, 1987) has shown that valuable preliminary information on N_2 fixation and N transfer can be obtained at relatively low cost from greenhouse studies. Information on potential for N transfer obtained from greenhouse studies would be particularly useful because of the intense intermingling of legume and grass roots and soil N depletion in pots.

Our objective was to compare N_2 fixation and N transfer potentials of four RC cultivars, one an experimental cultivar selected mainly for increased N_2 fixation.

MATERIALS AND METHODS

Two similar greenhouse experiments were conducted at the Department of Agronomy facilities, Iowa State University, Ames, IA. There were eight treatments consisting of four RC cultivars grown in both pure stands and in binary mixtures with orchardgrass 'Dawn' (OG). Cultivars of RC were APR-8701 (experimental cultivar), Redland II, Arlington, and Mammoth. The RC cultivar APR-8701 was developed by AgriPro Biosciences, Incorporated (ABI). It was selected for an increased rate of acetylene reduction, larger roots with more branches, increased nodule mass, and improved winter hardiness as compared with more commonly used cultivars (J. Moutray, ABI, personal communication).

Plastic pots (15-cm diameter, with drainage holes in bottom) were filled with 1.65 L of a soil mix, consisting of 23% topsoil, 41% perlite, 23% peat, and 13% sand by volume. Pregerminated seedlings were planted into the pots on 25 Oct. 1988 for the first experiment (Exp. 1) and 28 Nov. 1988 for the second experiment (Exp. 2). Ten plants per pot were planted for the pure stands of legume and 12 plants per pot for the mixtures (a 50:50 ratio of legume to grass plants was used). Pure stands of OG, necessary for the isotope dilution study, were planted at 10 plants per pot, also. The soil in each pot (except pots with OG only) was inoculated by watering with a solution containing specific *Rhizobium* inoculant immediately after planting. After three weeks of growth, all pots were thinned to eight plants per pot, maintaining the 50:50 ratio in the mixtures. The plants were fertilized weekly with a "Hoagland's minus-N" micronutrient solution and biweekly with a P and K solution. The plants were watered periodically with distilled water, as required.

Dinitrogen fixation and N transfer were estimated by isotope dilution, using pure stands of OG as the reference crop. Three weeks after emergence, a solution of ^{15}N enriched (99.7 atom-% ^{15}N) ammonium sulfate (0.6 g ^{15}N L^{-1}) was injected into the center of all pots at a rate of 10 mL pot^{-1}. A 10.5 cm long stainless steel needle with opposing emission holes located alternately at 1-cm intervals was used to distribute the solution within the soil mixture. Plants from each pot were clipped 5 cm above the soil surface when most RC plants were at late-bud to early-bloom stage of development. Harvest dates for Exp. 1 were 22 Dec. 1988 and 15 Feb., 5 Apr., and 11 May 1989. Harvest dates for Exp. 2 were 23 Feb., 29 Mar., 26 Apr., and 11 May 1989. Harvested herbage from the mixtures was separated into legume and grass fractions. Plant material was dried at 65°C for 48 h in a forced-air oven, weighed, and ground to pass a 1-mm screen in a knife-type mill. A single reference value of ^{15}N %atom-excess averaged over all OG pots was used for N_2 fixation and transfer calculations on each

Journal Paper no. J-14411 of the Iowa Agric. and Home Econ. Exp. Stn., Ames. Project 2899. Supported in part by AgriPro Biosciences, Incorporated (ABI).
*Corresponding author.

harvest for each experiment. Total plant N was determined by the Kjeldahl method (Bremner, 1965). Isotopic composition of OG and legume herbage was determined by mass spectrometry (Fiedler and Proksch, 1975).

The formulas used to perform the various calculations in this study are as follows:

$$\%Ndfa = \left(1 - \frac{\text{atom-\% } ^{15}N \text{ excess in legume}}{\text{atom-\% } ^{15}N \text{ excess in OG ref.}}\right) \times 100$$

$$\%Ngdfa = \left(1 - \frac{\text{atom-\% } ^{15}N \text{ excess in grass}}{\text{atom-\% } ^{15}N \text{ excess in OG ref.}}\right) \times 100$$

$$FNY = (\text{Legume DM}) \times (\%N \text{ in legume}) \times (\%Ndfa)$$

$$TNY = (\text{Grass DM}) \times (\%N \text{ in grass}) \times (\%Ngdfa)$$

where: %Ndfa is the percentage N in the legume derived from the atmosphere (N_2 fixation), %Ngdfa is the percentage N in the associated grass derived from the atmosphere (N transfer), FNY is the fixed N yield expressed on a "per pot" basis, and TNY is the transferred N yield expressed on a "per pot" basis (Fried and Middleboe, 1977; Rennie, 1984).

Analyses of variance were performed separately for each experiment and for pure stands and mixtures. The pots were arranged in completely randomized designs with four replications. The pure stands of OG (reference crop) were used in the calculations to estimate N_2 fixation and N transfer but were not included in statistical analyses. Each experimental unit consisted of two pots (whose herbage fractions were combined). The pots were rerandomized every 10 days to minimize any location effects that might have occurred across the greenhouse bench. Subsequently, an experimental unit (two pots) will be referred to as a "pot."

RESULTS AND DISCUSSION

Dry Matter Yields

Cultivars of RC grown in pure stands differed in herbage DM yield in both experiments (Table 1). In Exp. 1, DM yields for Mammoth usually were less than for other cultivars. Yields of DM for APR-8701, Arlington, or Redland II did not differ significantly. The low DM yield of Mammoth in Exp. 1 was not surprising because it is commonly regarded as a low-yielding cultivar. Mammoth plants were shorter and smaller leaved than any of the other three cultivars. In Exp. 2 (four-harvest average), pure stands of APR-8701 yielded more herbage DM than did either Mammoth or Redland II. In individual harvests, however, APR-8701 out-yielded the other cultivars in harvest 2 only. Differences in DM yield among cultivars grown in mixed stands were insignificant in both experiments, except for harvest 2 of Exp. 2 (Table 1). Trends in most harvests were similar to trends in pure stands, however, with APR-8701 showing greater DM yield than the other cultivars.

Yields of OG grown in association with RC (Table 2) were much less than for RC. Red clover dominated the pots in all harvests, and OG DM yield was noticeably greater in the first two harvests than in other harvests. A possible explanation for this outcome is that, once the available soil-N was depleted, OG growth was drastically limited for N. Further reduction in DM yield of OG resulting from reduced light interception by the grass as a consequence of increased legume foliage cannot be discounted. The low OG yields observed in this study, together with the low N concentration of OG herbage (not shown), suggest that available soil-N was depleted in the early stages of the study. This depletion of available soil-N was of positive consequences for this study because it forced the legume to rely on fixed-N for its source of N. The depletion of available soil-N may have forced OG to rely mainly on N transfer, also. Thus, differences between cultivars in N_2 fixation and N transfer probably were maximized in this study.

Table 1. Herbage dry matter yields of four red clover cultivars grown in pure and mixed stands with orchardgrass.

			Cultivar[a]				
Exp[b]	SR[c]	Har[d]	APRC	ARL	RII	MAM	LSD[e]
			g pot^{-1}				
1	A	1	3.1	2.7	3.0	2.3	0.8
1	A	2	7.8	7.3	7.2	6.3	1.2
1	A	3	11.6	11.0	10.6	8.9	1.6
1	A	4	10.7	10.3	11.4	9.4	1.1
1	A	AVG	8.3	7.8	8.0	6.7	0.8
1	B	1	1.9	1.2	1.8	1.9	NS
1	B	2	7.6	6.2	6.4	6.3	NS
1	B	3	12.0	10.6	10.5	9.8	NS
1	B	4	12.0	10.0	10.0	10.3	NS
1	B	AVG	8.4	7.0	7.2	7.1	NS
2	A	1	13.7	14.4	14.1	14.0	NS
2	A	2	17.1	15.5	14.3	14.8	2.2
2	A	3	12.7	11.6	11.8	12.4	NS
2	A	4	9.7	9.6	9.4	8.9	NS
2	A	AVG	13.3	12.8	12.4	12.5	0.7
2	B	1	8.3	9.9	6.4	7.4	NS
2	B	2	14.8	14.3	12.6	10.7	3.9
2	B	3	10.6	10.6	10.1	10.4	NS
2	B	4	9.5	8.3	8.2	9.3	NS
2	B	AVG	10.8	10.8	9.3	9.4	NS

[a]APRC = APR-8701; ARL = Arlington; RII = Redland II; MAM = Mammoth.
[b]Exp = experiment.
[c]SR = stand ratio; A = pure stand, B = 50:50 mixture of legume and grass.
[d]Har = harvest.
[e]LSD = least significant difference, $P \leq 0.05$. NS = no significant main effect.

Table 2. Herbage dry matter yields of orchardgrass grown in association with four red clover cultivars.

		Cultivar[a]				
Exp[b]	Har[c]	APRC	ARL	RII	MAM	LSD[d]
		g pot^{-1}				
1	1	0.32	0.38	0.32	0.38	NS
1	2	0.26	0.39	0.28	0.29	NS
1	3	0.21	0.29	0.22	0.24	NS
1	4	0.12	0.15	0.13	0.15	NS
1	AVG	0.22	0.30	0.24	0.27	NS
2	1	2.11	1.57	2.14	2.20	0.61
2	2	0.26	0.24	0.30	0.39	0.12
2	3	0.22	0.26	0.22	0.27	NS
2	4	0.17	0.18	0.18	0.19	NS
2	AVG	0.69	0.56	0.71	0.76	0.18

[a]APRC = APR-8701; ARL = Arlington; RII = Redland II; MAM = Mammoth.
[b]Exp = experiment.
[c]Har = harvest.
[d]LSD = least significant difference, $P \leq 0.05$. NS = no significant main effect.

Yields of OG grown in association with the four cultivars of RC did not differ in Exp. 1 (Table 2). In Exp. 2, however, OG grown in association with Mammoth had significantly greater ($P \leq 0.05$) average DM yield than OG grown with Arlington. Yields of OG associated with the other two RC cultivars were intermediate. Similar trends also were significant in the first two harvests of this experiment.

Dinitrogen Fixation

Values of %Ndfa for the four RC cultivars were very high in both pure and mixed stands and ranged from 86.8 to 99.9% (Table 3). These seemingly large values of %Ndfa were most likely the result of depletion of soil N in the pots that stimulated N_2 fixation. Similar results have been demonstrated in field studies (Boller and Nösberger, 1987; Mallarino et al., 1990a). Significant differences ($P \leq 0.05$) in %Ndfa between cultivars in most harvests of either experiment were either nonexistent or biologically meaningless. Where differences were significant, %Ndfa was higher than 96% and the largest difference was 1.2%.

Values of %Ndfa were lower in the first harvest of both pure and mixed stands compared with other harvests (Table 3). This result has been observed elsewhere (Brophy et al., 1987; Mallarino et al., 1990a) and may be explained by slow development of the photosynthetic and N_2-fixing systems and (or) higher availability of mineral N early in the experiment. Values of %Ndfa for RC were higher for mixtures than for pure stands in the first two harvests of both experiments. This result was most likely due to the depletion of soil N by the grass in the mixed stands, which stimulated N_2 fixation by the legume. This result has been reported in other studies (Brophy et al., 1987). Redland II usually had lower values of %Ndfa than other cultivars in the first harvest. Although this difference was not statistically significant, it suggests a slower development of the N_2-fixing system in this cultivar.

Table 3. Percentage N derived from the atmosphere in herbage of four red clover cultivars grown in pure and mixed stands with orchardgrass.

Exp[b]	SR[c]	Har[d]	Cultivar[a] APRC	ARL	RII	MAM	LSD[e]
			-------------------- % --------------------				
1	A	1	88.9	88.2	87.7	87.2	NS
1	A	2	95.9	95.4	95.0	96.2	NS
1	A	3	99.8	99.7	99.7	99.6	0.1
1	A	4	99.3	99.2	99.3	99.3	NS
1	A	AVG	96.0	95.6	95.3	95.7	NS
1	B	1	90.0	88.3	86.8	90.9	NS
1	B	2	97.1	96.5	97.7	97.3	1.0
1	B	3	99.9	99.6	99.8	99.5	NS
1	B	4	99.7	99.6	99.6	99.7	0.1
1	B	AVG	96.7	96.0	96.0	96.9	NS
2	A	1	93.2	93.6	91.7	94.2	NS
2	A	2	99.3	99.1	98.6	99.0	0.4
2	A	3	99.7	99.6	99.5	99.6	NS
2	A	4	99.6	99.5	99.6	99.4	NS
2	A	AVG	97.9	97.9	97.4	98.0	NS
2	B	1	94.1	95.8	95.6	95.3	NS
2	B	2	99.4	99.5	99.6	99.4	0.2
2	B	3	99.8	99.8	99.9	99.9	NS
2	B	4	99.8	99.8	99.8	99.9	0.1
2	B	AVG	98.3	98.7	98.8	98.6	NS

[a]APRC = APR-8701; ARL = Arlington; RII = Redland II; MAM = Mammoth.
[b]Exp = experiment.
[c]SR = stand ratio; A = pure stand, B = 50:50 mixture of legume and grass.
[d]Har = harvest.
[e]LSD = least significant difference, $P \leq 0.05$. NS = no significant main effect.

Table 4. Yield of fixed N in herbage of four red clover cultivars grown in pure and mixed stands with orchardgrass.

Exp[b]	SR[c]	Har[d]	Cultivar[a] APRC	ARL	RII	MAM	LSD[e]
			------------- mg pot^{-1} --------------				
1	A	1	111	270	103	79	NS
1	A	2	246	234	250	234	NS
1	A	3	455	385	378	329	74
1	A	4	407	378	406	368	34
1	A	AVG	305	317	284	252	NS
1	B	1	70	40	58	72	NS
1	B	2	262	204	156	222	NS
1	B	3	415	390	363	372	NS
1	B	4	468	383	390	434	53
1	B	AVG	289	254	242	275	NS
2	A	1	460	467	473	507	NS
2	A	2	635	552	460	564	157
2	A	3	495	416	446	487	56
2	A	4	407	397	385	385	NS
2	A	AVG	499	458	441	486	46
2	B	1	266	269	167	239	NS
2	B	2	535	466	379	429	133
2	B	3	396	389	355	400	NS
2	B	4	383	332	324	398	NS
2	B	AVG	395	364	306	366	83

[a]APRC = APR-8701; ARL = Arlington; RII = Redland II; MAM = Mammoth.
[b]Exp = experiment.
[c]SR = stand ratio; A = pure stand, B = 50:50 mixture of legume and grass.
[d]Har = harvest.
[e]LSD = least significant difference, $P \leq 0.05$. NS = no significant main effect.

On occasion, cultivars of RC differed in FNY in both pure and mixed stands (Table 4), a result that contrasts to results for %Ndfa. The cultivar APR-8701 usually showed the greatest values of FNY in both pure and mixed stands, although differences among cultivars neither occurred in all harvests nor were always consistent. Differences in FNY among other cultivars either were insignificant or inconsistent among harvests. There were wide differences in FNY values among harvests (Table 4). This result can be explained by trends in %Ndfa and DM yields. Both DM yield and %Ndfa were low in harvest 1; thus, FNYs in harvest 1 were lower than in other harvests. Because of minimal differences in %Ndfa among cultivars, FNY was strongly influenced by DM yields.

Nitrogen Transfer to Associated Orchardgrass

Orchardgrass grown in association with Arlington frequently had higher %Ngdfa than with any other cultivar; differences were not significant, however, in two harvests of each experiment (Table 5). Orchardgrass grown with APR-8701 and Mammoth had significantly lower %Ngdfa in two harvests of Exp. 1. In two harvests of Exp. 2, however, OG grown with Redland II had lower %Ngdfa than the other three cultivars.

Table 5. Percentage N derived from the atmosphere (%Ngdfa) in herbage of orchardgrass grown in mixture with four red clover cultivars.

Exp[b]	Har[c]	Cultivar[a] APRC	ARL	RII	MAM	LSD[d]
		%				
1	1	15.6	46.2	39.7	13.4	15.8
1	2	22.3	53.6	33.1	18.1	17.2
1	3	38.1	51.0	40.7	38.0	NS
1	4	53.2	49.6	46.6	52.6	NS
1	AVG	32.3	50.1	40.0	30.5	12.0
2	1	36.3	31.5	19.8	30.4	NS
2	2	42.3	48.4	29.1	40.0	18.0
2	3	47.4	56.3	41.8	51.7	14.1
2	4	59.1	57.6	52.5	58.0	NS
2	AVG	46.3	48.4	35.8	45.0	NS

[a]APRC = APR-8701; ARL = Arlington; RII = Redland II; MAM = Mammoth.
[b]Exp = experiment.
[c]Har = harvest.
[d]LSD = least significant difference, P≤0.05. NS = no significant main effect.

Table 6. Yield of transferred-N in herbage of orchardgrass when grown in mixture with four red clover cultivars.

Exp[b]	Har[c]	Cultivar[a] APRC	ARL	RII	MAM	LSD[d]
		mg pot^{-1}				
1	1	0.8	4.2	2.4	0.9	1.9
1	2	0.8	2.7	1.2	0.7	1.5
1	3	1.1	2.0	1.3	1.4	NS
1	4	1.4	1.5	1.3	1.6	NS
1	AVG	1.0	2.6	1.5	1.2	1.1
2	1	10.0	6.9	5.1	8.8	NS
2	2	1.9	2.0	1.4	2.8	NS
2	3	1.9	2.6	1.7	2.5	NS
2	4	2.2	2.0	2.0	2.2	NS
2	AVG	4.0	3.4	2.5	4.0	NS

[a]APRC = APR-8701; ARL = Arlington; RII = Redland II; MAM = Mammoth.
[b]Exp = experiment.
[c]Har = harvest.
[d]LSD = least significant difference, P≤0.05. NS = no significant main effect.

Orchardgrass grown in association with Arlington yielded the greatest amounts of transferred-N in Exp. 1. Similar trends were observed in the first three harvests (Table 6). Orchardgrass grown in association with Redland II, Mammoth, and APR-8701 did not differ in either average TNY or in TNY for individual harvests. In Exp. 2, there were no significant differences (Table 6), although there was a general trend for OG grown with Redland II to show the lowest TNY values. Yield of transferred N was greater in Exp. 2 than in Exp. 1. This result may be explained by the much greater DM yields of OG in Exp. 2 than in Exp. 1.

Nitrogen transfer, expressed as %Ngdfa, tended to increase with time (Table 5). This result agrees with reports from field (Brophy et al., 1987; Mallarino et al., 1990b) and greenhouse (Haystead and Marriott, 1979; Ta and Faris, 1987) studies with RC or other legumes. Reasons for this trend include increasing N_2 fixing capability by the legume and increasing root and nodule turnover. The often high values of %Ngdfa and TNY for OG grown in association with Arlington are not consistent with the usually low to average FNY shown for this cultivar. This observable contradiction is explained by the increasing clover dominance over time observed in our study. Other studies (Mallarino et al., 1990b) showed that %Ngdfa for several legumes increased with an increase in legume proportion, but TNY either did not change or it decreased.

SUMMARY AND CONCLUSIONS

The results indicate that %Ndfa was similar for the four RC cultivars in most harvests of this study. The experimental cultivar APR-8701 usually was among the cultivars yielding the greatest amounts of FNY. In general, trends for FNY values among cultivars followed closely trends for DM yields. This result suggests that, to have high N_2 fixation capability, a legume must also exhibit the capability to produce a large quantity of photosynthetic tissue.

The cultivar Arlington usually transferred the greatest amounts of N to associated grass. This finding indicates that high N_2 fixation capability may not always result in high N transfer to associated grass because Arlington did not show consistently greater FNY than other cultivars.

We conclude that under the conditions of this study, the particular traits selected for in the development of the experimental cultivar APR-8701 resulted in N_2 fixation rates similar to that of other superior, commercially available cultivars. The APR-8701 experimental cultivar, however, showed average N transfer to associated grass.

REFERENCES

ALLOS, H.F., and W.V. BARTHOLOMEW. 1959. Replacement of symbiotic fixation with available nitrogen. Soil Sci. 87:61-67.

BOLLER, B.C., and J. NOSBERGER. 1987. Symbiotically fixed nitrogen from field-grown white clover and red clover mixed with ryegrass at low levels of ^{15}N fertilization. Plant and Soil 104:219-226.

BREMNER, J.M. 1965. Total nitrogen. *In* C.A. Black (ed.) Methods of soil analysis. Part II. Agronomy 9:1149-1178. American Society of Agronomy, Madison, WI.

BROPHY, L.S., G.H. HEICHEL, and M.P. RUSSELLE. 1987. Nitrogen transfer from forage legumes to grass in a systematic planting design. Crop Sci. 27:753-758.

BUTLER, J.H.A. 1987. The effect of defoliation on growth and N_2 fixation by *Medicago* sp. grown alone or with ryegrass. Soil Biol. Biochem. 19:273-279.

FARIS, M.A. 1983. Variation in the rate of nitrogen fixation among red clover cultivars. Forage Notes 27:77-80.

FIEDLER, R., and G. PROKSCH. 1975. The determination of ^{15}N by emission and mass spectrometry in biochemical analysis: A review. Anal. Chim. Acta 78:1-62.

FRIED, M., and V. MIDDLEBOE. 1977. Measurement of amount of nitrogen fixed by a legume crop. Plant and Soil 47:713-715.

HAYSTEAD, A., and CAROL MARRIOTT. 1979. Transfer of legume nitrogen to associated grass. Soil Biol. Biochem. 11:99-104.

HIECHEL, G.H., and C.P. VANCE. 1979. Nitrate-N and *Rhizobium* strain roles in alfalfa seedling nodulation and growth. Crop Sci. 19:512-518.

HEICHEL, G.H., C.P. VANCE, D.K. BARNES, 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.

LARUE, T.A., and T.G. PATTERSON. 1981. How much nitrogen do legumes fix? Adv. Agron. 34:15-38.

LEDGARD, S.F., J.R. FRENEY, and J.R. SIMPSON. 1985. Assessing nitrogen transfer from legumes to associated grasses. Soil Biol. Biochem. 17:575-577.

MALLARINO, A.P., W.F. WEDIN, R.S. GOYENOLA, C.H. PERDOMO, and C.P. WEST. 1990a. Legume species and proportion effects of symbiotic dinitrogen fixation in legume-grass mixtures. Agron. J. 82:785-789.

MALLARINO, A.P., W.F. WEDIN, C.H. PERDOMO, R.S. GOYENOLA, and C.P. WEST. 1990b. Nitrogen transfer from white clover, red clover, and birdsfoot trefoil to associated grass. Agron. J. 82:790-795.

MCAULIFFE, C., D.S. CHAMBLEE, H. URIBE-ARANGO, and W.W. WOODHOUSE, Jr. 1958. Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by ^{15}N. Agron. J. 50:334-337.

NUTMAN, P.S. 1976. IBP field experiments on nitrogen fixation by nodulated legumes. p. 211-237. *In* P.S. Nutman (ed.) Symbiotic nitrogen fixation in plants. Cambridge University Press, Cambridge.

RENNIE, R.J. 1984. Comparison of N balance and ^{15}N isotope dilution to quantify N_2 fixation in field-grown legumes. Agron J. 76: 785-790.

ROHWEDER, D.A., W.D. SHRADER, and W.C. TEMPLETON, Jr. 1977. Legumes, what is their place in today's agriculture? Crop Soil 29:11-15.

TA, T.C., and M.A. FARIS. 1987. Species variation in the fixation and transfer of nitrogen from legumes to associated grasses. Plant and Soil 98:265-274.