EVALUATION OF NITROGEN TRANSFER FROM NODULATED ALFALFA TO ASSOCIATED GRASSES UNDER FIELD CONDITIONS[1]

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ABSTRACT - The contribution of N_2 - fixation to the nitrogen nutrition of alfalfa (*Medicago sativa* L.) and transference of N to associated grasses timothy (*Phleum pratense* L.) and bromegrass (*Bromus inermis* Leyss.) when grown in mixture, were estimated using the ^{15}N isotope dilution technique. The percentage of alfalfa N derived from atmosphere (% Ndfa) increased throughout the growing seasons ranging from 63 to 83%. The total amount of N_2 fixed by alfalfa has shown a pattern similar to % Ndfa with slight increases in the absolute amount of N_2 fixed by alfalfa in mixed stands. N transfer from alfalfa to an associated grass was evident, and contributed 26, 46 and 38% of the total annual N yield of grass and represented an absolute amount of about 5.0, 20.0 and 19.0 kg. ha^{-1} during the first, second and third year. The results showed that all grass species benefitted from N transfer from alfalfa, although the early maturity species with greater competitive ability are slightly more responsive.

Index terms: *Medicago sativa, Rhizobium meliloti, Phleum pratense, Bromus inermis,* ^{15}N isotope dilution.

AVALIAÇÃO DA TRANSFERÊNCIA DE NITROGÊNIO DA ALFAFA NODULADA PARA AS GRAMÍNEAS ASSOCIADAS EM CONDIÇÕES DE CAMPO

RESUMO - Um experimento de campo foi conduzido para avaliar a contribuição da fixação biológica de N_2 na nutrição nitrogenada da alfafa (*Medicago sativa* L.) e sobre a transferência de N para as gramíneas *Phleum pratense* L. e *Bromus inermis* Leyss., usando a técnica de diluição de isótopos (^{15}N). A percentagem do N em alfafa derivado da atmosfera (% Ndfa) aumentou durante os cortes e variou de 63 a 83%. A quantidade total de N_2 fixado apresentou um padrão similar a % Ndfa, com ligeiro incremento da quantidade absoluta de N_2 fixado pela alfafa em associação. A transferência de N da alfafa para a gramínea foi evidente e contribuiu com 26, 46 e 38% do rendimento total de N das gramíneas, representando uma quantidade de 5,0, 20,0 e 19,0 kg N.ha^{-1} no primeiro, segundo e terceiro ano, respectivamente. Os resultados mostraram que as gramíneas foram beneficiadas com a transferência de N, embora a espécie com maturidade precoce e maior habilidade de competição tenha sido ligeiramente mais responsiva.

Termos para indexação: *Medicago sativa, Rhizobium meliloti, Phleum pratense, Bromus inermis,* fixação de nitrogênio, diluição isotópica, ^{15}N.

INTRODUCTION

Nitrogen (N) is the most important nutrient to increase forage production. It can be supplied through N fertilizer or biological N_2 - fixation. With the rising cost of petrochemically derived N fertilizers and their adverse ecological effects on water pollution, more attention is being given to the possibility of using biological N_2 - fixation. Amongst the alternatives that are being sought, the increase of the level of free living N_2 - fixing organisms in the soil, the rotation, the intercropping and the mixture of non-fixing crops with legumes are the most applicable.

Grasses mixed with forage legumes are preferred to pure stands throughout the temperate zone, because they increase herbage yield and quality, and improve seasonal distribution of forage (Baylor 1974, De Anda et al. 1981, Broadbent et al. 1982). Although several explanations have been suggested, the most obvious is that legumes have the ability to fix all the N that they require and

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much of it is returned to the soil either during their growing period (excretion) or after harvesting (decomposition of nodule and root residues), and is absorbed by the subsequent or even by companion crops (Agboola & Fayemi 1972, Vallis et al. 1977, Haystead & Marriott 1979, Burity et al. 1985). This process improves N cycling and crop productivity which reduces the expensive input of industrial N fertilizers. Also, water utilization and weed control are improved by encluding legumes in the stands.

Reports on transfer of N in the legume-grass mixture during the growing period are few and conflicting. Henzell (1962) measured transfer of N as the difference of N yield and found that only 0.6 to 1.7% of the total N_2 - fixed was transferred from tropical legumes to associated grasses. Ross et al. (1964) used gas lysimeters to estimate the amount of N transferred to be only 0.1 to 2,3% of fixed N_2. Haystead & Marriott (1979) used the isotope dilution method and found evidence of N transfer of 1.7% of the fixed N_2 from white clover (*Trifolium repens* L.) to ryegrass (*Lolium perenne* L.). However, a substantial transference of N was found between white clover and grass (*Lolium* spp.) (Broadbent et al. 1982), from alfalfa (*Medicago sativa* L.) to timothy (*Phleum pratense* L.) (Faris & Ta 1985), and from alfalfa to bromegrass (*Bromus inermis* Leyss.) and orchardgrass (*Dactilis glomerata* L.) (Burity et al. 1985). In those studies up to 80% of the N in ryegrass in mixed cultures was derived from the fixation process in white clover, and an average of 25% of the total N yield of timothy and bromegrass were contributed by alfalfa. Ledgard et al. (1985), utilized labelled plants of legume with ^{15}N by foliar absorption and found that 2.2% of the N in subterraneum clover was transferred to annual ryegrass, over a 29 days period.

A greater understanding of the contribution of symbiotic N_2 - fixation to the N economy of the legume-grass pastures is needed to more effectively exploit this aspect in the N nutrition of forage production. The present study was conducted to evaluate the amount of N transferred from N_2 - fixing alfalfa to associated grass and to measure N_2 - fixation rates of alfalfa grown alone and in mixture with grass, under field conditions utilizing the ^{15}N dilution technique.

MATERIAL AND METHODS

The experiment was conducted at Ottawa Research Station, Agriculture Canada, Ottawa, Canada, on a moderately drained sandy loam soil from 1983 to 1985. In the previous year the area was cultured with barley with no additional fertilizer. Following harvest, the stover was ploughed down. The soil had adequate levels of Mg and P as determined by soil analyses (Tabela 1). However, to increase the soil reaction as well as the K level, potassium and limestone were applied at a rate of 30 kg. ha^{-1} and 3.0 $ton.ha^{-1}$ as potassium chloride (KCl) and dolomitic limestone ($CaMg(CO_3)_2$, respectively. Alfalfa *(Medicago sativa* L.) cv. '520' seeds inoculated with a preparation of *Rhizobium meliloti* strains 102F20 and L-26 (Nitragin Co., Milwaukee, Wisconsin) were sown at the rate of 13 $kg.ha^{-1}$ in monoculture and in mixed culture at the rate of 11 kg. ha^{-1} with timothy (*Phleum pratense* L.) cvs. Salvo and Climax at 6.0 $kg.ha^{-1}$ and bromegrass (*Bromus inermis* Leyss.) cv. 'Tempo' at 9.0 $kg.ha^{-1}$. The same grass seeding rates in mixed stands were utilized in pure stands. Within each experimental unit (1.60 m x 6.0 m), one microplot was established approximately in the center of each plot. A plastic sheet box (1.0 m x 1.0 m) with 0.25 m in depth was inserted into the plot and levelled 1 cm above the surrounding ground level. A small amount, 1.0 g of $(^{15}NH_4)_2SO_4$ with 99% atom ^{15}N in solution (2.1 kg $N.ha^{-1}$), was equally applied to the surface of the soil in each microplot. An additional 0,25 g of $(^{15}NH_4)_2SO_4$ in solution was added to each microplot annually in the Spring after initial

TABLE 1. Chemical analysis of sand loam soil at Ottawa Research Station, including five essential elements, two micronutrientes and pH, spring 1983.

Nutrient	Concentration	
Nitrogen	0.09	(0.04) %
Phosphorus	96.7	(7.27) ppm
Potassium	98.0	(8.25) ppm
Magnesium	55.0	(10.9) ppm
Calcium	> 2000 ppm	
Zinc	30.2	(1.83) ppm
Manganese	39.0	(2.45) ppm
pH (water)	5.1	(0.22)

Values in parentheses are the standard error of the mean of the twenty replicate soil samples.

application, in order to keep the level of % of atom ^{15}N sufficiently high to be detected in the plant tissues.

Plots were harvested when alfalfa plants reached the 50% bloom stage. Two harvests were obtained during the seeding year and subsequent year, while three harvests were performed in the third year. At each harvest, plants were cut 3 cm above the ground level. Plant material from all harvests was dried, weighed and then ground in a mill with a 0,5 mm screen (40 mesh). Total N concentration and ^{15}N enrichment was determined by the Kjeldahl and optical emission spectroscopy methods as described by Bremner (1965) and Preston et al. (1981), respectively.

The percentage of N in alfalfa derived from atmosphere (% Ndfa) was calculated on the assumption that the isotopic composition of the N derived from soil is similar in both crops and there are two sources of N available: soil N and N from the atmosphere. Using the ^{15}N dilution technique to distinguish the soil N uptake and the N_2-fixed, atom % ^{15}N excess in alfalfa is expressed as follows:

$$(\text{atom } \%\ ^{15}\text{N excess atmosphere} \times \text{Ndfa}) + [(1 - \text{Ndfa}) \times \text{atom } \%\ ^{15}\text{N excess soil}], \quad (1)$$

where Ndfa = fraction of N in alfalfa derived from atmosphere, Normally, atom % ^{15}N excess in the air = 0, and the non-fixing plant uptake only N from soil. Therefore, the percentage of N in alfalfa derived from N_2-fixation is:

$$\text{Ndfa} = \left(1 - \frac{\text{atom } \%\ ^{15}\text{N excess alfalfa}}{\text{atom } \%\ ^{15}\text{N excess monoculture control}}\right) \quad (2)$$

and the amount of N_2-fixed (NF),

$$\text{NF} = \text{Ndfa} \times \text{TN in alfalfa}, \quad (3)$$

Where atom % ^{15}N excess = atom % ^{15}N in labelled treatment – atom % ^{15}N in the atmosphere.

The amount of soil N and fixed N transferred from alfalfa to grass (Nt), when both were grown in association was estimated on the basis that there are two sources of N available to associated grass: N from associated alfalfa + soil N uptake. Using the ^{15}N dilution technique to assess Nt, the total N in grass in mixed is expressed as follows:

$$(\text{atom } \%\ ^{15}\text{N excess in grass association}) = (\text{atom } \%\ ^{15}\text{N excess alfalfa in association} \times F_t) + [\text{atom } \%\ ^{15}\text{N excess soil} \times (1 - F_t)], \quad (4)$$

Where F_t = fraction of N in grass plants originated from alfalfa. Since atom % ^{15}N excess in the soil is uptake by the control; therefore, the fraction of N derived from associated legume (F_t) is

$$F_t = \frac{(\text{atom } \%\ ^{15}\text{N excess grass in mixed stand}) - (\text{atom } \%\ ^{15}\text{N excess monoculture control})}{(\text{atom } \%\ ^{15}\text{N excess alfalfa in mixed stand}) - (\text{atom } \%\ ^{15}\text{N excess monoculture control})} \quad (5)$$

and the amount of N transferred

$$\text{Nt} = F_t \times \text{TN grass in mixed stand}. \quad (6)$$

An analysis of variance was carried out on all results. For each analysis, when a significant treatment effect was shown ($P \leqslant 0.05$), Duncan's Multiple Range tests were calculated.

RESULTS AND DISCUSSION

The proportion of alfalfa Ndfa ranged from 53.9 to 89.1% and there was usually slight seasonal variations throughout each entire growing season (Table 2). The crops obtained the greatest proportion of N from symbiosis after the seeding year, with and annual average of about 80% fixed N during the second and third year. In the seeding year, the % Ndfa of alfalfa plants grown in pure and mixed stands represented 62.78% in the initial harvest, while in subsequent harvest the proportion of N from symbiosis reached higher values. This difference was probably related to the N-stress in the initial phase of establisment of symbiotic process (Lawn & Brun 1974, Heichel et al. 1981. Eaglesham et al. 1983). The proportions of N derived from the air in these studies were much higher than those reported by Heichel et al. (1981). Moreover these values are similar to those of other studies, which indicate that from 70 to almost 100% of the legume shoot N can be derived from N_2-fixation (Bergersen & Turner 1983, Faris & Ta 1985, West & Wedin 1985). The absolute amounts of N_2-fixed by alfalfa in mono and mixed cultures are shown in Table 3. In general, there was seasonal variation in the amount of N_2-fixed. During the seeding year, alfalfa plants fixed a range per harvest from 40.70 to 42.81 kg $N.ha^{-1}$ and from 34.59 to 60.27 kg $N.ha^{-1}$ in pure and mixed stands, respectively. In the second and third year the alfalfa plants fixed large amounts of N_2 in the initial harvest. In the third year, the amount de-

TABLE 2. Percent of nitrogen derived from atmosphere (% Ndfa) of alfalfa grown in mono and mixed cultures at different harvests, 1983 to 1985.

Year	treatment	Nitrogen derived from atmosphere Harvest 1 (%)	2	3	Mean
1983	Alfalfa	60.78 b	64.30 b		62.54
	Alfalfa with timothy (1)[+]	53.94 b	71.61 b		62.77
	Alfalfa with timothy (2)	61.78 b	86.58 a		74.18
	Alfalfa with bromegrass	74.64 a	81.35 a		77.99
	Mean[++]	62.78 b	75.96 a		69.37
	±SE	3.16	3.00		2.86
1984	Alfalfa	79.68 a	74.26 b		76.95
	Alfalfa with timothy (1)	81.68 a	68.89 b		75.65
	Alfalfa with timothy (2)	84.07 a	86.01 a		85.04
	Alfalfa with bromegrass	86.41 a	86.41 a		86.41
	Mean[++]	82.89 a	79.14 b		81.01
	±SE	2.67	2.72		2.53
1985	Alfalfa	77.18 a	75.35 a	79.47 a	77.56
	Alfalfa with timothy (1)	79.09 a	75.94 a	89.09 a	81.38
	Alfalfa with timothy (2)	81.18 a	75.64 a	82.26 a	79.70
	Alfalfa with bromegrass	70.16 a	82.35 a	82.04 a	78.18
	Mean[++]	77.07 b	77.33 b	83.21 a	79.20
	±SE	4.43	2.93	1.93	3.56

Means in a column followed by the same letter are not significantly different at the 5% level of probability, according to Duncan's Multiple Range Test.

[+] Timothy (1) cultivar Climax
Timothy (2) cultivar Salvo

[++] Means compared horizontally

clined significantly after first cut and reached values comparable to the seeding year, in harvest 3. There was only a slight variation when the N_2-fixed by alfalfa in mono and mixed cultures were compared. The few significant differences detected in the % Ndfa (independent yield criteria) and the amount of N_2-fixed (dependent yield criteria) from alfalfa grown in mixtures as compared with that in pure stand showed the validity of the dilution technique to assess N_2-fixation in mixed swards.

In this report, isotope dilution leads to an estimation of N_2-fixation that sometimes underestimates or overestimates when N_2-fixed was measured in mixed cultures in comparison with N_2-fixed by alfalfa in monoculture. This under and/or overestimation were not as severe as that claimed by Broadbent et al. (1982) who concluded that the ^{15}N dilution method can not be used with a legume-grass mixture because of underground transfer of N from legume to grass. However, the recent studies reviewed by Chalk (1985) have shown that the isotope technique can be applied to the measurement of N_2-fixation in associated pastures, because grass and legume roots are intimately mixed, and therefore sample the same soil N pool. Fried et al. (1983) also emphasized this aspect with respect to intercropping. Moreover, a suitable reference plant in pure stand is required to estimate the relative contribution of indigenous and soil N to the N nutrition of the fixing plant. In the present study, mixing alfalfa with grass did not lead to erroneous estimates of N_2-fixation because suitable controls were utilized with close uptake of labelled ^{15}N (Table 4), and only slight differences were found when the amounts of N_2-fixed were calculated.

Regarding annual N_2-fixation, alfalfa fixed a mean of 93.05 kg $N.ha^{-1}$ during the year of

TABLE 3. Amount of nitrogen fixed by alfalfa plants when grown in mono and mixed cultures at different harvests, 1983 to 1985.

Year treatment	N_2-fixed Harvest 1 (kg/ha)	2	3	Total+++
1983 Alfalfa	42.75 ab	40.65 a		83.40 b
Alfalfa with timothy (1)+	34.59 b	46.17 a		80.76 b
Alfalfa with timothy (2)	40.41 ab	51.19 a		91.60 ab
Alfalfa with bromegrass	56.17 a	60.27 a		116.44 a
Mean++	43.48 a	49.57 a		93.05 b
± SE	6.12	8.53		8.41
1984 Alfalfa	154.01 a	115.61 a		269.62 a
Alfalfa with timothy (1)	123.38 a	123.68 a		247.06 a
Alfalfa with timothy (2)	137.39 a	131.83 a		269.22 a
Alfalfa with bromegrass	121.44 a	124.56 a		246.00 a
Mean++	134.05 a	123.94 a		258.00 a
± SE	25.29	35.49		40.81
1985 Alfalfa	122.44 a	63.97 a	44.30 a	230.71 a
Alfalfa with timothy (1)	133.14 a	53.60 a	65.01 a	251.75 a
Alfalfa with timothy (2)	118.04 a	60.52 a	45.50 a	223.96 a
Alfalfa with bromegrass	102.66 a	55.55 a	42.15 a	200.36 a
Mean++	119.06 a	55.91 b	49.21 b	226.70 a
± SE	26.30	13.85	10.95	41.38

Means in a column followed by the same letter are not significantly different at the 5% level of probability, according to Duncan's Multiple Range Test.
\+ Timothy (1) cultivar Climax
Timothy (2) cultivar Salvo
++ Means compared horizontally
+++ Total annual (means) compared vertically

establishment, and 258.00 and 226.70 kg $N.ha^{-1}$ during the second and third year of the stands, respectively (Table 3). Bell & Nutman (1971) reported N_2-fixation of 220 kg $N.ha^{-1}$ $year^{-1}$ for effectively nodulated alfalfa plants with up to 78% of plant N derived from the atmosphere. Heichel et al. (1981) found an average of 148 kg $N.ha^{-1}$ in the establishment year with 43% of the total N yield derived from fixation. Recently, West & Wedin (1985) reported an annual amount N_2-fixed of 70 kg $N.ha^{-1}$ with a range of 15-136 kg N. ha^{-1} in the seeding year and 15-122 kg $N.ha^{-1}$ in 2-year-old alfalfa in mixture with highly correlated with the percentage of alfalfa in the sward and with alfalfa dry matter yield (West & Wedin 1985), and it is closely linked to the growth rate in alfalfa (Heichel et al. 1981).

In all instances, grass atom % ^{15}N excess was lower in the mixtures than in pure stands (Table 4), which indicates that N_2-fixed by alfalfa was transferred to grass. The ^{15}N enrichment of the grasses exhibited increase or decline throughout the season. The observed increases or reductions reflected the additional applications of label ^{15}N in the Spring and the losses of ^{15}N from the available soil N pool via shoot removal (uptake) and other processes (immobilization and leaching). However, the similarity of the enrichment trends indicates that the seasonal soil N uptake pattern of the grass species used were similar, with only slight variations in the seeding year. The atom % ^{15}N excess for the grass in pure stands provided satisfactory evidence of this fact.

The proportion of N transferred to the grass

TABLE 4. Atom % ^{15}N excess of grasses grown in mono and mixed cultures at different harvests, 1983 to 1985.

Year treatment	Harvest 1	Harvest 2 (Atom % ^{15}N excess)	Harvest 3
1983 Timothy (1) with alfalfa	0.7726 cd	0.6660 c	
Timothy (2) with alfalfa	0.6273 e	1.0234 abc	
Bromegrass with alfalfa	1.0950 b	0.9549 bc	
Timothy (1)[+]	0.8453 c	0.8323 c	
Timothy (2)	0.6985 dc	1.4839 ab	
Bromegrass	1.2350 a	1.5437 a	
Mean[++]	0.8789 b	1.0480 a	
± SE	0.0538	0.2037	
1984 Timothy (1) with alfalfa	0.7950 bcd	0.2338 b	
Timothy (2) with alfalfa	0.7143 cd	0.3536 b	
Bromegrass with alfalfa	0.5868 d	0.3654 b	
Timothy (1)	1.0498 abc	0.3378 b	
Timothy (2)	1.1740 a	0.6986 a	
Bromegrass	1.0973 ab	0.6978 a	
Mean[++]	0.9028 a	0.4478 b	
± SE	0.1402	0.0560	
1985 Timothy (1) with alfalfa	0.2964 b	0.3122 abc	0.2687 cd
Timothy (2) with alfalfa	0.4229 a	0.2415 bc	0.1788 e
Bromegrass with alfalfa	0.3622 b	0.2350 c	0.2435 de
Timothy (1)	0.4163 a	0.3675 a	0.3955 ab
Timothy (2)	0.5833 a	0.3303 ab	0.3400 bc
Bromegrass	0.4481 a	0.3433 a	0.4400 a
Mean[++]	0.4225 a	0.3049 a	0.3110 a
± SE	0.0237	0.0343	0.0281

Means in a column followed by the same letter are not significantly different at the 5% level of probability, according to Duncan's Multiple Range Test.

[+] Timothy (1) cultivar Climax
Timothy (2) cultivar Salvo

[++] Means compared horizontally

varied throughout the growing seasons in 1983 and 1984 (Table 5). In 1985, the differences among harvests were always small and none reached significance. The % of N in grass transferred from alfalfa (% Nt) ranged from an average of 16.02% in the initial harvest to 48.97% in the final harvest. The % Nt of the three grass species was similar, although timothy cultivar 'Climax' showed a significant disadvantage in later growth in 1984. However, in general all grass species benefitted from N transfer from alfalfa during the entire period. Up to 57% of the total nitrogen transferred to grass in mixed swards was derived from the N_2-fixation process of alfalfa. The % Nt was significantly lower during the first growth (harvest 1), but it increased substantially thereafter. The results did indicate that some N transference had occurred before the first cutting, even in the seeding year. Thus the transference of N from alfalfa was not primarily due to the sloughing off of the nodules from alfalfa and to a decay of its roots system after harvest as suggested by Butler & Bathurst (1956), Dilz & Mulder (1962). It may have involved a considerable degree of N excretion during the growing season. Burity et al. (1985) reported that 20 to 25% of total N_2-fixed by alfalfa was transferred to associated grass prior to shoot removal. Chujo & Daimon (1984) reported that a growth acceleration of grass species in association with red clover in the early stage of development is probably due to the absorption of N compounds excreted directly from the root sys-

TABLE 5. Percentage of nitrogen in grasses transferred from associated alfalfa at different harvests, 1983 to 1985.

Year	Treatment	Nitrogen in grass transferred from alfalfa Harvest 1	2 (%)	3
1983	Timothy (1)[+] with alfalfa	16.11 a	30.84 b	
	Timothy (2) with alfalfa	16.47 a	33.32 ab	
	Bromegrass with alfalfa	15.48 a	46.90 a	
	Mean[++]	16.02 b	37.02 a	
	± SE	5.50	8.31	
1984	Timothy (1) with alfalfa	29.79 a	43.91 b	
	Timothy (2) with alfalfa	44.71 a	55.38 a	
	Bromegrass with alfalfa	50.80 a	56.85 a	
	Mean[++]	41.76 b	52.05 a	
	± SE	6.48	5.10	
1985	Timothy (1) with alfalfa	35.94 a	23.70 a	35.55 a
	Timothy (2) with alfalfa	26.92 a	34.36 a	56.62 a
	Bromegrass with alfalfa	35.94 a	37.70 a	54.72 a
	Mean[++]	32.64 a	31.92 a	48.97 a
	± SE	4.50	10.84	13.41

Means in a column followed by the same letter are not significantly different at the 5% level of probability, according to Duncan's Multiple Range Test.
[+] Timothy (1) cultivar Climax
Timothy (2) cultivar Salvo
[++] Means compared horizontally

tem of the legume. However, due to the apparent operation of the excretion mechanism mainly when the plants are subjected to stress. Henzell (1962), Haystead & Marriott (1979) concluded that a more important pathway of transference in a legume-grass pasture involves the sloughing off and decomposition of legume nodule and root tissues.

On an area basis, the amount of N transferred ranged from an annual total average of 4.54, 20.16 and 18.64 kg $N.ha^{-1}$ for the first, second and third year, respectively (Table 6.). The values are equivalent to 26.5, 46.9 and 37.8% of the total N yield of grasses in association. Transfer of N from legume to grass is important for the growth of the grass, mainly under low N conditions. This process represents a small percentage of the N_2-fixed by alfalfa and allow the grass plants to grow at a rate similar to grass in monoculture in soil with low availability of N (0.09%). Broadbent et al. (1982) reported extensive transfer of fixed N_2 to ryegrass. Where up to 80% of the N was found to be derived from symbiotic process in ladino clover. However, they found little transfer of N in a short term and suggest that several months are involved in the gradual mineralization of dead roots and nodule tissues from the legume through microbial activity.

Evidence of N transfer was observed in this study for all grass species. Nevertheless, there were differences among the grass species relatively to the amount of N transferred from alfalfa. Bromegrass was able to take up more transferred N than timothy in year 2 and 3. This may be due to the fact that bromegrass has a well developed root system, better adapted to drought conditions. Abnormal dry conditions that prevailed through the summers of 1984 and 1985, decreased the rate of the growth of the grass species less adapted to a drought environment (timothy) and influenced significantly their ability to take up available N. According to Henzell (1962) the increase of N transference may be a function of the competitive ability of the grass component in the association. Thus, the advantage could be attributed to a greater

TABLE 6. Amount of nitrogen transferred from alfalfa to the associated grass at different harvests, 1983 to 1985.

Year	Treatment	Amount N in grass transferred from alfalfa Harvest 1	2	3	Total[+++]
			kg		
1983	Timothy (1)[+] with alfalfa	1.22 a	2.24 b		3.46 a
	Timothy (2) with alfalfa	1.61 a	1.90 b		3.15 a
	Bromegrass with alfalfa	1.38 a	5.28 a		6.66 a
	Mean[++]	1.40 a	3.14 a		4.54
	± SE	0.49	1.72		2.56
1984	Timothy (1) with alfalfa	3.05 b	10.68 b		13.73 b
	Timothy (2) with alfalfa	4.73 ab	14.91 ab		19.64 ab
	Bromegrass with alfalfa	5.72 a	21.40 a		27.12 a
	Mean[++]	4.50 a	15.66 b		20.16
	± SE	0.58	7.59		8.17
1985	Timothy (1) with alfalfa	11.72 ab	1.37 b	1.81 b	14.90 b
	Timotny (2) with alfalfa	8.31 b	3.75 a	3.47 ab	15.53 b
	Bromegrass with alfalfa	15.57 a	5.89 a	4.03 a	25.49 a
	Mean[++]	11.86 a	3.67 b	3.10 b	18.64
	± SE	2.02	1.19	0.43	2.91

Means in a column followed by the same letter are not significantly different at the 5% level of probability, according to Duncan's Multiple Range Test.
[+] Timothy (1) cultivar Climax
Timothy (2) cultivar Salvo
[++] Means compared horizontally
[+++] Total transference (means) compared vertically

competitive ability of bromegrass cv. Tempo, an early maturity species. Timothy cvs. Salvo and climax, which are intermediate and later maturing cultivars, respectively, benefitted from the transference process to a lesser extent than bromegrass. Good contact between roots and large occupation of soil volume also make more efficient use of N released from associated legumes and soil N. De Anda et al. (1981) and Chujo & Daimon (1984) reported that the roots of grasses and legumes were tightly intertwined when they are grown together, and any N compounds excreted by legume root nodules would be rapidly absorbed by grass roots.

CONCLUSIONS

1. The experimental evidences indicated that throughout the entire seasons alfalfa plants were able to fix most of their total N requirement. The % Ndfa increased over the growing season and ranged from 63 to 83%.

2. Transfer of N from alfalfa to the grass paralleled the N_2-fixing capacity of alfalfa. During the initial season less than 5 kg $N.ha^{-1}$ was transferred from alfalfa to grass, but these values incrased to 20.0 kg $N.ha^{-1}$ and 19 kg $N.ha^{-1}$ in the second and third years, represented about 26.0, 46.0 and 38.0% of the total N yield of grass in association during those years.

3. Both grass species benefitted similarly from transfer by alfalfa during the entire period, although the species with early maturing and greater competitive ability were more responsive.

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