Pertanika 9(1), 17 – 22 (1986)

Nitrogen Fixation by *Leucaena leucocephala* as Measured by N-15 Dilution Technique*

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Key words: Nitrogen fixation; N-15 enrichment; N-15 atom excess.

ABSTRAK

Satu kajian ladang telah dijalankan untuk menentukan jumlah nitrogen yang dapat diikat oleh ***Leucaena leucocephala*** *dengan menggunakan teknik pencairan isotop yang telah dicadangkan oleh Fried dan Broeshart (1975). Tiga varieti Leucaena (Accession 55/65, Cunningham dan Peruvian) telah ditanam secara tulen dan campuran dengan rumput (****Setaria anceps*** *var. splendida). Penyelidikan ini telah menunjukkan Leucaena boleh mengikat sehingga 78% daripada jumlah nitrogen yang ada di bahagian atas tumbuhan dalam masa tiga bulan. Leucaena yang ditanam secara tulen didapati boleh mengikat lebih banyak nitrogen daripada yang ditanam bercampur dengan rumput.*

ABSTRACT

A field trial was conducted to measure the amount of nitrogen fixed by ***Leucaena leucocephala*** *using the isotope dilution technique proposed by Fried and Broeshart (1975). Three varieties of Leucaena (Accession 55/65, Cunningham and Peruvian) were planted both as a sole crop and mixed with* ***Setaria anceps*** *var. splendida grass. It was found that Leucaena can fix up to 78% of the nitrogen present in the plant tops within the period of three months. Leucaena grown as a sole crop tends to fix more nitrogen than those grown mixed with the grass.*

INTRODUCTION

Current interest of agronomists in producing good pasture for cattle is in the establishment of grass-legume mixture. *Leucaena leucocephala* is found to be one of the most promising tropical grazing legumes inspite of its tree-like habit and difficulties it can cause in animals due to its mimosine content (Hutton and Gray, 1959; Hill, 1971). It is known to produce very high fresh matter per hectare which contain about 4.3% nitrogen (Takeshahi and Ripperton, 1949). Little work has been done to quantify the amount of atmospheric nitrogen fixed by this legume.

This experiment was set up to quantify the nitrogen fixed by leucaena plants using the N-15 dilution technique as proposed by Fried and Broeshart (1975, 1981).

MATERIALS AND METHODS

The experiment was conducted in 1983 on a colluvial soil in Universiti Pertanian Malaysia, Serdang, Selangor. The soil had a pH of 4.3 (1 : 2.5 soil to water ratio) and a total N content of 0.5%. Three tons of dolomitic limestone were applied to the field before starting the experiment. Phosphorus was given as Christmas Island Rock Phosphate (15% P) at the rate of 130 kg

*This project was carried out under the FAO/IAEA Coordinated Research Programme on the Use of Nuclear Techniques in Improving Pasture Management, Contract No. 3330/1G.

P/ha. Potassium was applied as Muriate of Potash at 100 kg K/ha.

Three varieties of *Leucaena leucocephala* (Accession 55/65, Cunningham and Peruvian) seedlings were established in polybags in the greenhouse. Three-month old seedlings were planted in the field at a distance of 0.3 m × 0.5 m either pure or mixed with the grass (*Setaria anceps* var. splendida) which was planted 15 cm × 15 cm apart usig 20 cm cuttings. A pure grass plot was also established. The grass was used as the reference or standard crop. Each plot size was 3 m × 3 m and the treatments were arranged in a randomised block design with six replications.

Subplots of 1 m × 1 m were marked within each plot and used for N-15 application. Sulphate of ammonia with 10% atom excess N-15 was applied at the rate of 2 g N/m^2 while ordinary sulphate of ammonia was applied at the same rate in the areas outside the subplot.

Two harvests were made (July and November, 1983). The leucaena plants were cut at 50 cm above the ground level and the leaves subsampled, overdried at 60°C, until constant weight is achieved and then ground for total N and N-15 analyses. The grass was harvested at 15 cm above the ground, subsampled, dried and ground for the same analyses.

Total N was determined in the plant tissues using the modified Kjeldahl procedure (Cottenie *et al.*, 1978) and N-15 in the samples were prepared by using the Dumas method (Faust, 1967) and determined by emission spectrometry for the first harvest and mass spectrometry for the second harvest.

RESULTS AND DISCUSSION

Dry Matter Yield

The average dry matter yield of the grass and Leucaena is shown in Table 1. About 5.8 tons of dry grass per hectare was obtained for the first harvest and 7.4 tons per hectare in the second harvest. There was no significant difference in the dry matter yield between grass grown as a sole crop or mixed with Leucaena except for the grass grown with Cunningham variety, for both harvests. These plots gave the lowest yield.

The dry matter yield for the three varieties of Leucaena grown as a pure stand were found to be not significantly different. A much higher yield was obtained in the second harvest due to these plants being better established in the field. A significant decrease in dry matter yield was observed when the Leucaena was grown mixed with grass for both harvests, especially for Accession 55/65 and Cunningham varieties. The Peruvian variety seemed to be more vigirous in

TABLE 1
Average dry matter yield of grass and Leucaena (kg/ha)

Treatment	Grass		Leucaena	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Grass	5807 [b]	7424 [b]	—	—
Accession 55/65	—	—	2332 [b]	7870 [b]
Cunningham	—	—	2215 [b]	7716 [b]
Peruvian	—	—	2711 [b]	7563 [b]
Accession 55/65 + Grass	5252 [b]	8181 [b]	1334 [a]	3489 [a]
Cunningham + Grass	2900 [a]	4256 [a]	1396 [a]	5568 [a]
Peruvian + Grass	4018 [b]	6785 [b]	1698 [b]	6711 [b]

Means in the same column followed by the same letter are not significant at P = 0.05.

its growth, as shown by its non-significant difference in the dry matter yield when grown mixed with the grass.

Nitrogen Content

The N content of the grass ranged from 1.5% to 1.7% in the first harvest and 0.9% to 1.3% in the second harvest (Table 2). This difference in N content in the grass is due to the N fertilizer being applied only once at the beginning of the experiment. No subsequent supply of the N was made prior to the second harvest. The N concentration in the grass grown as a pure or mixed stand was not significantly different.

The N concentration in Leucaena averaged 3.52% in the first harvest and 3.76% in the second harvest. No significant difference was observed in the N concentration of the Leucaena grown as a sole or mixed crop for both harvests.

A significantly lower N yield was observed for the grass grown with *Leucaena* var. Cunningham for both harvests. This was due to the lower dry matter yield of the grass obtained from these plots (Table 3). The pure Leucaena plots for all the three varieties gave significantly higher N yields for both harvests compared to the mixed plots.

In the first harvest, total N yield for mixed plots and pure grass was higher than pure Leucaena plots. But in the second harvest, the

TABLE 2
Average N concentration (%) in grass and Leucaena

Treatment	Grass		Leucaena	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Grass	1.78[a]	0.97[a]	—	—
Accession 55/65	—	—	3.71[a]	3.76[a]
Cunningham	—	—	3.37[a]	4.06[a]
Peruvian	—	—	3.56[a]	3.67[a]
Accession 55/65 + Grass	1.55[a]	0.96[a]	3.37[a]	4.20[a]
Cunningham + Grass	1.45[a]	1.30[a]	3.57[a]	3.72[a]
Peruvian + Grass	1.74[a]	1.02[a]	3.56[a]	3.15[a]

Means in the same column followed by the same letter are not significant at P = 0.05.

TABLE 3
Average N yield of grass and Leucaena (kg/ha)

Treatment	Grass		Leucaena	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Grass	103.36[b]	72.00[b]	—	—
Accession 55/65	—	—	86.51[b]	295.91[b]
Cunningham	—	—	74.65[b]	313.27[b]
Peruvian	—	—	96.51[b]	277.56[b]
Accession 55/65 + Grass	81.46[b]	78.54[b]	44.93[a]	146.54[a]
Cunningham + Grass	42.05[a]	55.32[a]	49.84[a]	207.13[a]
Peruvian + Grass	69.87[b]	69.21[b]	54.11[a]	211.39[a]

Means in the same column followed by the same letter are not significant at P = 0.05.

trend was reversed, where pure Leucaena plots gave higher total N than mixed or pure grass plots, except for the Peruvian variety which gave about the same total N yield in both pure and mixed plots.

% N-15 Atom Excess

Table 4 shows the % N-15 atom excess found in the grass and Leucaena samples. The N-15 atom excess in the first harvest was higher and was able to be detected using the emission spectrometer. For the second harvest, the N-15 atom excess for both the grass and Leucaena was too low to be detected by emission spectrometer.

As was expected, the N-15 enrichment of the grass was higher than the Leucaena samples. In the first harvest, the grass from the pure stand and mixed plots had the same isotopic composition. This indicated that there was no transfer of nitrogen from the legume to the grass, as was explained by Broadbent *et al.*, 1982. In the second harvest, there was a slight decrease in N-15 enrichment of the grass grown mixed with Leucaena as compared to the pure stand. This might indicate some transfer of fixed nitrogen from the Leucaena to the grass (Broadbent *et al.*, 1982).

The lower N-15 enrichment of the Leucaena samples in the pure and mixed stand showed the reliance of Leucaena on atmospheric nitrogen.

Percent Nitrogen Fixed by Leucaena

The percent of nitrogen derived from fixation (% Ndf fix) was calculated using the formula:

$$\% \text{ Ndf fix} = 1 - \frac{\% \text{ N-15 atom excess in fixing crop}}{\% \text{ N-15 atom excess in non-fixing crop}} \times 100$$

and the amount of nitrogen fixed by the Leucaena was derived from the formula:

$$N_2 \text{ fixed} = \frac{\% \text{ Ndf fix}}{100} \times \text{Total N in fixing crop}$$

The average percentage of nitrogen fixed by the Leucaena is shown in Table 5. A lower percentage of nitrogen fixed was observed for all the three varieties of Leucaena for the first harvest. This may be due to the Leucaena being only three months after establishment, and the dry matter yield was much lower (Table 1). For the second harvest, both pure and mixed plots of Leucaena did not show any difference in the % N derived from fixation. The Cunningham variety showed lower percentage of N derived from fixation in both pure and mixed plots compared to the other two varieties.

Table 6 shows the total amount of nitrogen fixed by Leucaena. Pure Leucaena plots tend to fix higher amounts of nitrogen in both harvests

TABLE 4
Mean N-15 excess of grass and Leucaena (%)

Treatment	Grass		Leucaena	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Grass	0.87	0.124	—	—
Accession 55/65	—	—	0.45	0.027
Cunningham	—	—	0.58	0.052
Peruvian	—	—	0.32	0.033
Accession 55/65 + Grass	0.80	0.090	0.38	0.027
Cunningham + Grass	0.89	0.107	0.46	0.050
Peruvian + Grass	0.87	0.080	0.59	0.025

TABLE 5
% Nitrogen derived from fixation in Leucaena

Variety	Pure		Mixed	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Accession 55/65	48.3	78.2	52.5	70.0
Cunningham	33.3	58.1	48.3	53.2
Peruvian	63.2	73.4	32.2	68.8

TABLE 6
Mean total nitrogen fixed by leucaena (kg/ha)

Variety	Pure		Mixed	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Accession 55/65	41.8	231.4	23.6	102.6
Cunningham	24.9	182.0	24.1	78.0
Peruvian	61.0	203.7	17.4	145.4

as compared to mixed plots. The total amount of nitrogen fixed by Leucaena during the second harvest was much higher than the first harvest. The highest amount was fixed by the Accession 55/65 variety which was planted as a sole crop.

CONCLUSION

Leucaena leucocephala was found to produce up to 7.8 tons of dry matter per hectare per harvest. Using the isotope dilution technique, it was calculated that up to 78% of the total nitrogen in the plant was fixed from the atmospheric nitrogen within a period of three months. The Cunningham variety showed a consistently lower percentage of nitrogen fixed in both pure and mixed stand for both harvests.

ACKNOWLEDGEMENT

Grateful appreciation is extended to the International Atomic Energy Agency for granting the research fund; Dr. S.K.A. Danso, its Scientific Secretary for the project; Malaysian Research and Development Institute (MARDI) for supplying Acession 55/65 Leucaena seeds, and Universiti Pertanian Malaysia for allowing the research to be conducted.

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(Received 6 March, 198