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NITROGEN FIXATION AND EFFECTS OF PRUNING ON *GLIRICIDIA SEPIUM* AND *LEUCAENA LEUCOCEPHALA*

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Abstract

This 7-year study examined genetic variability in N_2 fixation by *Gliricidia sepium* and the N_2-fixing capacity in *G. sepium and Leucaena leucocephala* as influenced by frequency of pruning, age, and shade from coconut. The ^{15}N-dilution method was used with the non-nodulating tree legume *Senna siamea* as the non-fixing reference. There were significant differences in total dry matter, N yield and N_2-fixation capacity among four *G. sepium* provenances. *Gliricidia* had higher values than *Leucaena* for dry matter, N yield, and amount of N fixed; %Ndfa was comparable in both species (47-55%). A substantial amount (18%) of fixed N_2 was present in the roots of both species. In a long-term study aimed at comparing the effect of pruning practices and age of trees, *G. sepium* grown under coconut outperformed *L. leucocephala* in terms of dry matter, N yield and amounts of N_2 fixation. Coconut saplings supplied with *G. sepium* and *L. leucocephala* prunings as green manure grew better than those supplied with *S. siamea;* the fraction of coconut-sapling N obtained from *Gliricidia* and *Leucaena* was 40 and 36%, respectively. These results suggest that *G. sepium*, which demonstrated a high potential for biomass production and N_2 fixation, is appropriate for interplanting with coconut palms. Also, *S. siamea* was found to be a suitable reference species.

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is a major plantation crop, playing a vital role in Sri Lanka's economy and contributing to the food security of its 18 million people. It has been reported that the majority of coconut-growing soils are inherently low in N, K and Mg, the major reason for the crop's low productivity – 7,000 nuts ha^{-1} yr^{-1} in many parts of the country. Declining fertility is due mainly to inadequate attention to the return of organic residues to plantation soils.

In Sri Lanka, numerous agroforestry systems associated with coconut, including crop and livestock components have been established during the past few decades, with a view to increasing productivity and maximizing income for coconut farmers. The interplanting of coconut with N_2-fixing trees (NFTs) has been successfully introduced to the coconut ecosystem. Such species are considered to be one of the most appropriate components in agroforestry systems because of their inherent ability to utilize atmospheric N_2 and grow well in soil low in mineral N. Among them, *Gliricidia sepium* (Jacq.) Walp. and *Leucaena leucocephala* (Lam) de Wit are recognized as having potential for production of green manure, animal fodder and for maintaining soil fertility [1,2]. By far, they are the most common species grown in association with coconut. The growth and biomass production of these two species and their effect on coconut production have been documented [3].

In view of increasing cost of N fertilizer, attention is now being focused on biological N_2 fixation (BNF) in tree legume species such as *G. sepium* and *L. leucocephala* in agroforestry systems. Estimates of BNF by these species have been reported under both glass-house and field conditions in young trees [2,4-6]. It has been shown that fixation in tree legumes is strongly influenced by genotype within species, by management practice [2,5] and tree age. However, these aspects are not well documented for older trees. This research was undertaken to strengthen our knowledge and understanding on the N_2-fixing potential in *G. sepium* and *L. leucocephala*, as influenced by genotype, management and age.

The objectives were as follows.

- To study genotypic variation in BNF.
- To quantify BNF capacity of tree-legume species under coconut canopy.
- To study the effect of tree management on BNF under coconut canopy.
- To study changes in BNF with tree age.
- To study N contribution from prunings of tree legumes to an associate crop.

2. MATERIALS AND METHODS

2.1. Experiment 1. N_2-fixation in four *Gliricidia sepium* genotypes

The experiment was conducted at the Coconut Research Institute (CRI) of Sri Lanka (08° 02'N, 79° 50'E, 2 m) from December 1990 to November 1991. The average annual rainfall at the site is 1,850 mm, with maximum and minimum air temperatures of 31 and 24°C, and relative humidity of 70 to 80%. The rainfall data for the experimental period are given in Table I. The soil was a sandy loam (Xanthic Ferralsols) and its chemical characteristics are given in Table II.

The treatments consisted of four *G. sepium* genotypes viz. provenances 14/84, 17/84 (Guatemala), 12/86 (Costa Rica) obtained from the Oxford Forestry Institute, UK, and the local land-race (designated LL). The non N_2-fixing legume tree species *Senna siamea* (syn. *Cassia siamea*) was used as the reference for the ^{15}N-dilution method [7]. *Senna* seeds were obtained from Tree Seed Centre, Thailand. The experiment had a randomized complete-block design with four replicates.

TABLE I. ANNUAL RAINFALL AND ITS DISTRIBUTION

Year	Total rainfall (mm)	Distribution (No. of rainy days)
1991 (Site 1)[a]	1,676	129
1992 (Site 2)[b]	1,868	86
1993	1,764	110
1994	1,718	115
1995	1,630	86
1996	1,199	95

[a](Experiment 1).
[b](Experiments 2 and 3).

TABLE II. SOIL CHARACTERISTICS (EXPT. 1)

Depth (cm)	pH[a]	Total N (%)	Organic C (%)	Bray P (mg kg^{-1})	Exchangeable cations K (meq 100^{-1} g)	Ca	Mg
0-20	5.41	0.096	0.89	8.43	0.326	0.40	< 0.001
20-40	5.28	0.097	0.85	7.29	0.215	0.30	< 0.001

[a]1:5, soil:H_2O.

Four-week-old inoculated seedlings (inoculum supplied by the BNF Resource Centre, Bangkok, Thailand), raised in polythene bags, were transplanted into holes (30×30×30 cm) made at a spacing of 2×1 m (equivalent to 5,000 plants ha^{-1})). After planting, the holes were refilled with top soil containing a basal fertilizer mixture (kg ha^{-1}): triple superphosphate 100, KCl 50, S 10, Cu 5.4, B 2.7, Zn 5.0 and Mo 0.5.

Each replicated block (plot) measuring 8×5 m consisted of four *G. sepium* provenances in the centre row and two rows of *S. siamea* on either side with the 2×1 m spacing, surrounded by a border row of *S. siamea*. Within each plot, an isotope sub-plot measuring 6×4 m was demarcated and the area under each tree within the sub-plot was contained by trenching and installation of galvanized sheets to a 45-cm depth. Three months after planting, a solution containing urea enriched with 5 atom % ^{15}N excess was sprayed (200 mL m^{-2}) onto the isotope sub-plot, at a rate equivalent to 20 kg N ha^{-1}. Unlabelled urea was applied at the same rate to the border trees outside the sub-plot.

The trees within the isotope sub-plot were harvested 11 months after transplanting, in November 1991. After removing the above-ground plant parts, the root system of each tree was exposed by washing the soil within the enclosed area and the whole plant was taken for sampling. Plants were separated into leaves, branches, stems and roots, chopped into 5-cm pieces and then weighed fresh. A 300-g sub-sample was taken for drying at 70°C until constant weight in a forced-draft oven, then milled to a fine powder.

Total-N content and ^{15}N enrichment in each plant part were determined [8] on an automatic N analyzer (1500 Carlo-Erba) coupled to a VG-Isogas mass spectrometer at the FAO/IAEA Soil Science Unit, Austrian Research Centre, Seibersdorf. The isotope-dilution equation [7] was used to estimate the percent N derived from fixation (i.e. from the atmosphere, %Ndfa) for individual plant parts. Subsequently, BNF in the whole plant was calculated from the weighted atom % ^{15}N excess (WAE) in the fixing and reference plants [9] as follows:

$$WAE = \frac{[(AE_L \times TN_L) + (AE_S \times TN_S) + (AE_B \times TN_B) + (AE_R \times TN_R)]}{[TN_{(L+S+B+R)}]}$$

Where

AE is atom %^{15}N excess,
TN is total N,
L is leaves,
S is stems,
B is branches,
R is roots.

The proportion of total N derived from atmospheric N_2 (%Ndfa) was then calculated as follows:

$$\%Ndfa = \left(1 - \frac{\text{WAE in } N_2 \text{ fixer}}{\text{WAE in } S.\ siamea}\right) \times 100$$

And the amount of N_2 fixed (NF) was calculated as follows:

$$NF = \frac{\%Ndfa}{100} \times \text{Total N (g plant}^{-1}\text{)}$$

The significance of differences between mean values was determined by analysis of variance by the General Linear Model of SAS [10].

TABLE III. SOIL CHARACTERISTICS (EXPTS. 2, 3 AND 4)

Depth (cm)	pH^a	Total N (%)	Organic C (%)	Bray P ($mg\ kg^{-1}$)	Exchangeable cations ($meq\ 100^{-1}\ g$)		
					K	Ca	Mg
0-20	5.24	0.082	0.85	12.8	0.104	0.849	<0.339
20-40	5.52	0.057	0.78	11.4	0.052	0.818	<0.285

[a]1:5, soil:H_2O.

2.2. Experiment 2. N_2 fixation by *G. sepium* and *L. leucocephala* under coconut

This study was conducted in a 50-year-old plantation (planted 8.5×8.5 m) at CRI, for a period of 12 months, commencing 15 May, 1992.

The photosynthetically active radiation (PAR) transmitted to the under-storey on a sunny day was 70%. The average annual rainfall at the site is 1,600 mm and the maximum and minimum air temperatures were 31 and 23°C, respectively. Rainfall data during the experimental period are in Table I. The soil was a lateritic gravel (ferric acrisols) and its chemical characteristics are given in Table III.

The treatments consisted of the NFTs *G. sepium* (provenance 12/86)and *L. leucocephala* (K 636) and the non-fixing reference legume *S. siamea*, arranged in a randomized complete-block design with four replicates. Within each replicate, a 6×2 m sub-plot was enclosed by cutting a trench and placing galvanized sheets to a 45-cm depth on all sides and between trees.

The seedlings were raised in polythene bags for 8 weeks, the NFTs inoculated with appropriate rhizobium cultures. They were transplanted to the field in planting holes (30×30×30 cm) in single rows, 2 m apart, between the rows of coconut palms (640 trees ha^{-1}). Prior to transplanting, a fertilizer mixture consisting of triple superphosphate (equivalent to 100 kg ha^{-1}), KCl (50 kg ha^{-1}), B (2.7), Zn (5.0) and Mo (0.5), was applied to each planting hole and mixed with top soil.

Three months after establishment, a solution containing ammonium sulphate enriched at 5 atom % ^{15}N excess was applied to the isotope sub-plots at the rate of 20 kg N ha^{-1} as 200 mL m^{-2} followed by thorough watering to ensure uniform distribution. Unlabelled ammonium sulphate was applied to border rows at the same rate. The plots were weeded thrice and the weeds left to decompose on the surface.

Twelve months after planting, four trees of each species were harvested. The above-ground parts were sampled first by cutting the main stem 10 cm above ground level and separating branches, leaves and stems. When uprooting trees, the soil within each enclosed area was excavated to a depth of 90 cm and most of the root biomass was recovered by hand-sorting. Each component plant part was chopped into 5-cm pieces and weighed fresh. A 300-g sub-sample of each was dried in a forced-draft oven at 70°C to constant weight for determination of dry weight. All dried plant samples were milled, analyzed for N content and ^{15}N enrichment, and determinations of %Ndfa and NF made, as described above.

2.3. Experiment 3. Pruning-frequency and tree-age effects on N_2 fixation by *G. sepium* and *L. leucocephala* under coconut.

The details of the experiment site, species, date, method of planting and fertilizer applied were as described for Experiment 2. Each 18×2 m sub-plot consisting of nine legume trees, three of each species, was enclosed by cutting a trench and placing a two-layered black polythene sheet to a depth of 90 cm along the border. There were three pruning regimes (4-monthly, 6-monthly, and once per year designated "unpruned") arranged with the three species in a 3^3 factorial randomized complete-block design with four replicates.

Twelve months after establishment in the field, all trees were cut back to 1 m. Each replicate consisted of three plots and one pruning treatment was assigned per plot. Following the initial cut-back in the sub-plots and thereafter at completion of each pruning interval, a solution of ammonium sulphate enriched with 5 atom % ^{15}N excess was applied at 20 kg N ha^{-1} to the soil in two or three split doses. Trees that were pruned every 4 months were applied with fertilizer solution in three doses each year, and those pruned every 6 months and unpruned trees were applied with fertilizer solution twice per year. The border trees outside the isotope sub-plot were applied with unlabelled ammonium sulphate at the same rate. All plots were weeded thrice per year.

At each harvest, the above-ground biomass of each tree was collected and separated into leaves and branches and weighed fresh. Thereafter, 300-g sub-samples were taken from the three trees of each species within a sub-plot. Each sub-sample was dried and milled for estimation of total N and ^{15}N enrichment.

The pruning treatments were imposed over four years (1993/94, 1994/95, 1995/96, 1996/97) after the initial pruning In plots with unpruned trees, sub-samples of leaves and branches were taken for estimation of %N and ^{15}N enrichment on completion of a pruning cycle at the end of each year, while a set of unpruned trees in each species was used to estimate pruning biomass after the cut back to 1 m.

2.4. Experiment 4. Recovery of N from prunings applied to coconut seedlings

This follow-up study utilized the prunings labelled with ^{15}N in Experiment 3.

Six-month-old coconut seedlings (var. Tall × Tall) were established in the field on 15 May, 1995, by planting them in holes (45×45×45 cm) at a spacing of 1 m in the area between coconut-palm rows, which was divided into two main blocks, each of four plots of equal size (4×3 m) consisting of six coconut seedlings. The seedlings in three plots in one block were supplied with ^{15}N-labelled prunings from either *G. sepium, L. leucocephala* or *S. siamea,* at the rates of 108, 86 and 159 kg per plot, respectively, during the experimental period. In the other block, three similar plots were mulched with the same quantities of unlabelled prunings from three species. One plot in each block served as the control without mulching.

At the end of 12 months growth, the third leaf of each coconut seedling was sampled, dried and milled for determination of %N and ^{15}N enrichment. Subsequently, fresh and dry weights of the third frond were recorded. The growth of each coconut seedling within a plot was determined by measuring plant height, basal girth and canopy size. For statistical analysis, individual coconut seedlings in each plot within a block were considered as replicates. The experiment had a fully randomized design with four replicates. The N obtained by the coconut seedlings from the prunings was calculated as follows:

$$\%N \text{ derived from prunings} = \frac{\%^{15}N \text{ a.e. in coconut with prunings} - \%^{15}N \text{ a.e. in coconut control}}{\%^{15}N \textit{ in prunings.}} \times 100$$

The ^{15}N enrichments in the prunings were, *Leucaena* 0.189%, *Gliricidia* 0.162% and *Senna* 0.346%.

3. RESULTS

3.1. Experiment 1

3.1.1. Dry matter and N yields

There were significant differences ($P \leq 0.001$) among the *G. sepium* provenances (Tables IV and V). Provenance OFI 14/84 produced the highest dry matter (DM) and N yields followed by the local land-race (LL). Although the non-N_2-fixing reference *S. siamea* accumulated the highest total DM, its N yield was lower than that of provenance 14/84 but, almost double that of other two provenances 17/84 and 12/86. The above-ground plant parts accumulated 80-90% of the total DM and N yields; the roots had 10-20% irrespective of species and provenance (Tables IV and V). In terms of N-use efficiency (NUE), the amount of dry matter produced by the plant per unit of taken up, *S. siamea* was significantly ($P \leq 0.001$) more efficient than *G. sepium* provenance OFI 14/84 and the local land-race (Table V).

TABLE IV. DRY MATTER AND ITS DISTRIBUTION IN PLANT COMPONENTS OF FOUR *GLIRICIDIA SEPIUM* GENOTYPES AND *SENNA SIAMEA*, 1991 (EXPT. 1)

Genotype	Leaves	Branches	Stem (kg plant^{-1})	Roots	Total
OFI 14/84	0.80(16)[a]	0.60(12)	2.90(58)	0.72(14)	5.02
OFI 17/84	0.31(10)	0.57(18)	1.66(53)	0.61(19)	3.15
OFI 12/86	0.42(15)	0.46(16)	1.49(51)	0.53(18)	2.90
LL	0.97(22)	0.58(13)	1.99(44)	0.94(21)	4.48
Senna	0.88(15)	0.56(26)	2.65(44)	0.97(16)	6.06
Significance	*	**	*	NS	**
$LSD_{0.05}$	0.39	0.84	0.98	-	1.56
CV(%)	37	73	29	39	23

[a](%).

TABLE V. TOTAL N YIELD, ITS DISTRIBUTION IN PLANT COMPONENTS AND N-USE EFFICIENCY (NUE) OF FOUR *G. SEPIUM* GENOTYPES AND *S. SIAMEA*, 1991 (EXPT. 1)

Genotype	Leaf	Branch	Stem (g N plant^{-1})	Root	Total	NUE (g DM g^{-1} N)
OFI 14/84	21.3(22)[a]	3.4(4)	65.2(68)	6.3(7)	96.1	53
OFI 17/84	3.0(8)	3.5(9)	26.4(69)	5.5(14)	38.3	85
OFI 12/86	5.5(14)	2.2(6)	26.7(69)	4.2(11)	38.5	78
LL	36.1(46)	4.9(6)	29.8(38)	7.6(10)	78.4	59
Senna	25.2(38)	6.1(9)	30.5(46)	4.3(7)	66.2	101
Significance	***	NS	*	NS	**	**
$LSD_{0.05}$	12.7	-	24.1	-	33.1	21
CV(%)	45	63	44	45	34	18

[a](%).

TABLE VI. DISTRIBUTION OF ATOM % ^{15}N EXCESS IN PLANT COMPONENTS AND THE MEAN WEIGHTED ^{15}N ATOM EXCESS (WAE) OF FOUR *G. SEPIUM* GENOTYPES AND *S. SIAMEA*, 1991 (EXPT. 1)

Genotype	Leaf	Branch	Stem	Root	WAE
		(atom % ^{15}N excess)			(whole plant)
OFI 14/84	0.094	0.137	0.135	0.151	0.120
OFI 17/84	0.137	0.168	0.177	0.152	0.160
OFI 12/86	0.150	0.219	0.166	0.167	0.163
LL	0.138	0.123	0.191	0.177	0.159
Senna	0.313	0.358	0.346	0.363	0.337
Significance	**	***	***	***	***
$LSD_{0.05}$	0.089	0.070	0.076	0.077	0.071
CV(%)	35	23	34	25	24

3.1.2. *Atom %^{15}N excess*

The ^{15}N enrichment in plant parts and weighted average for the whole plant in *S. siamea* was significantly higher ($P \leq 0.001$) than those of the *G. sepium* genotypes (Table VI). However, differences in atom %^{15}N excess among *G. sepium* provenances were not significant. Enrichment values in the leaves of *G. sepium* genotypes (mean 0.130), were lower than in the stems branches and roots (0.162-0.167). There was a similar trend with *S. siamea*.

3.1.3. *Nitrogen fixation*

On a whole-plant basis, provenance OFI 14/84 had the highest %Ndfa (64%), however owing to the high coefficient of variation, this was not significantly greater than for the other *G. sepium* genotypes (Table VII). In contrast, the amount of N fixed by OFI 14/84 (61.7 g. N $plant^{-1}$) was significantly higher than by OFI 1784 and 12/86, but not the local land-race (40 g N $plant^{-1}$). It is noteworthy that, except in a few instances, %Ndfa for any individual plant part was similar to that estimated for the whole plant (Table VII). Of the total N fixed in the whole plant, the amounts in leaves, branches, stems and roots represented 29, 5.8, 57 and 9.0%, irrespective of *G. sepium* provenance.

3.2. Experiment 2

3.2.1. *Dry Matter and its distribution*

Among the three tree-legume species, total DM accumulations by *G. sepium* and the reference *S. siamea* were comparable at around 2.8 kg $plant^{-1}$ with *L. leucocephala* producing only 1.7 kg $plant^{-1}$. However, the differences between species were not significant, owing to a high CV (Table VIII). Of the total DM, a high proportion (70-80%) was distributed in the above-ground plant parts, the accumulation of DM in roots accounting for 20-30% in all three species. Furthermore, the distribution of DM in above ground plant parts in *G. sepium* and *S. siamea* was similar, in contrast to *L. leucocephala* in which the trunk accumulated the highest proportion of the DM.

TABLE VII. ESTIMATES OF PERCENTAGE (%NDFA) AND AMOUNT OF N_2 FIXATION (IN PARENTHESIS) BY FOUR *G. SEPIUM* GENOTYPES, 1991 (EXPT. 1)

Genotype	Leaf	Branch	Stem	Root	Total
			%Ndfa (g N plant^{-1})		
OFI 14/84	70 ± 7[a] (14.9 ± 5)	61 ± 9 (2.1 ± 1)	62 ± 4 (40.2 ± 12)	57 ± 11 (3.6 ± 1)	64 (61.7)
OFI 17/84	56 ± 12 (1.7 ± 1)	51 ± 15 (1.8 ± 1)	45 ± 19 (11.3 ± 6)	56 ± 14 (3.1 ± 1)	53 (18.4)
OFI 12/86	52 ± 2 (2.9 ± 1)	36 ± 19 (0.8 ± 1)	48 ± 18 (11.8 ± 2)	52 ± 11 (2.2 ± 1)	52 (17.8)
LL	56 ± 9 (20.2 ± 4)	63 ± 18 (3.3 ± 2)	42 ± 24 (15.4 ± 6)	47 ± 24 (3.6 ± 2)	53 (40.4)
Significance					NS (*)
$LSD_{0.05}$					25.7

[a] Mean ± SE.

TABLE VIII. DRY MATTER AND ITS DISTRIBUTION IN PLANT COMPONENTS OF *G. SEPIUM, L. LEUCOCEPHALA* AND *S. SIAMEA* IN THE ESTABLISHMENT YEAR, 1992/93 (EXPT. 2)

Species	Leaf	Branch	Trunk	Root	Total	% DM in root
			(g plant^{-1})			
L. leuco.	287	148	835	478	1,747	27
G. sepia	486	736	675	905	2,801	32
S. siamea	687	871	661	603	2,822	22
Significance	*	*	NS	NS	NS	NS
$LSD_{0.05}$	323	536	-	-	-	-
CV(%)	40	57	45	45	40	19

3.2.2. N yield and its distribution

A trend similar to that for dry matter occurred in total-N yield, *G. sepium* being the highest with 36 g N plant^{-1}, followed by the reference *S. siamea*, at 27 g N plant^{-1} (Table IX), and *L. leucocephala* accumulated the least N. However, these values were not significantly different due to the high CV. Among the above-ground plant parts, leaves accounted for the highest proportion of the N in all three species. The NFTs had relatively more N in the root than did the reference plants. Also, the NUE in the reference plant *S. siamea* was significantly ($P \leq 0.001$) higher than those of the NFT species, which had similar values (Table IX).

3.2.3. *Atom %^{15}N excess*

The ^{15}N enrichments within individual plant parts and their whole-plant weighted averages differed significantly among the three legumes. As expected, the concentration of ^{15}N in the component parts of the reference species, *S. siamea* was higher ($P \leq 0.001$) than in the two N_2 fixing species, *G. sepium* and *L. leucocephala* (Table X) indicating that significant fixation had occurred. Whereas the ^{15}N enrichments in the leaves of the NFTs tended to be lower than in the branches, stems and roots, in *S. siamea* the enrichments were more uniform across organs.

TABLE IX. TOTAL N YIELD, ITS DISTRIBUTION IN DIFFERENT PLANT COMPONENTS AND N-USE EFFICIENCY (NUE) OF *G. SEPIUM*, *L. LEUCOCEPHALA* AND *S. SIAMEA* IN THE ESTABLISHMENT YEAR, 1992/93 (EXPT. 2).

Species	Leaf	Branch	Trunk (g N plant^{-1})	Root	Total	% of total in root	NUE (g DM g^{-1} N)
L. leuco.	9.8	1.0	6.1	5.6	22.6	25	78
G. sepium	15.3	5.0	4.1	11.8	36.2	32	75
S. siamea	15.6	4.1	2.5	4.7	26.9	17	107
Significance	NS	*	NS	*	NS	**	**
$LSD_{0.05}$	-	2.83	-	5.45	-	7.9	17
CV(%)	40	51	48	46	40	20	12

TABLE X. ATOM % ^{15}N EXCESS IN PLANT COMPONENTS AND THE WEIGHTED ATOM % ^{15}N EXCESS (WAE) FOR THE WHOLE PLANT IN *G. SEPIUM*, *L. LEUCOCEPHALA* AND *S. SIAMEA* IN THE ESTABLISHMENT YEAR, 1992/93 (EXPT. 2)

Species	Leaves	Branches	Trunk (Atom %^{15}N excess)	Roots	WAE
L. leuco.	0.083	0.089	0.108	0.133	0.102
G. sepium	0.088	0.114	0.135	0.152	0.118
S. siamea	0.223	0.246	0.219	0.227	0.224
Significance	**	**	**	*	**
$LSD_{0.05}$	0.07	0.05	0.05	0.07	0.06
CV(%)	32	20	22	25	23

TABLE XI. ESTIMATES OF %N DERIVED FROM FIXATION (%NDFA), AMOUNTS OF FIXED N, AND PERCENT FIXED N IN THE ROOT OF *G. SEPIUM* AND *L. LEUCOCEPHALA* IN THE ESTABLISHMENT YEAR, 1992/93 (EXPT. 2)

Species	Lf.	Brnch.	Trnk	Root	Whole plant	Lf.	Brnch.	Trnk.	Root	Whole plant	N in root
	(%Ndfa)					(Ndfa g N plant^{-1})					(%)
L. leuco.	61	64	52	41	55	5.8	0.7	3.1	2.0	11.3	19
G. sepium	59	54	38	32	47	9.5	2.7	1.6	3.1	17.4	22
Significance	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
$LSD_{0.05}$	-	-	-	-	-	-	1.5	-	-	-	-
CV(%)	25	20	30	35	27	26	50	56	31	42	52

3.2.4. N_2 fixation

On a whole-plant basis, %Ndfa values in *G. sepium* and *L. leucocephala* were not significantly different, at 47 and 55%, respectively (Table XI). Furthermore, %Ndfa values in individual plant parts were similar to that of the whole plant. This contrasted with the total amount of N fixed which was higher in *G. sepium* (17 g plant^{-1}) than in *L. leucocephala* (11 g plant^{-1}), although not significantly so due to the high CV. Among above-ground plant parts, leaves had the highest proportion of the fixed N (50-55%), and a substantial amount (18-22%) was present in roots.

3.3. Experiment 3

3.3.1. Total dry matter of prunings

The total DM of prunings (leaves and branches) as affected by pruning practice, tree age and species are shown in Table XII. Total pruning DM was higher ($P \leq 0.01$) in *G. sepium* and *S. siamea* than in *L. leucocephala.* Also, except in the first year (1993/94) after initial pruning, total DM values for *G. sepium* and *S. siamea* were comparable and significantly higher than for *L. leucocephala.* In all three species, there was a progressive increase in total DM with age to the second year (1994/95), but the rates of DM accumulation in pruned material was higher in *G. sepium* and *S. siamea* than in *L. leucocephala.* Although pruned and unpruned trees produced similar biomass yields in the first year, pruned trees produced more in the third year (1995/96). High biomass in unpruned trees in the second year after initial pruning was followed by a marked drop in the third year. Except in the second year, the 4-monthly pruning was superior to the 6-month interval in terms of total biomass yield (Table XII). The DM yields from prunings increased progressively with tree age. Among above-ground plant parts, the distribution of DM in branches was higher ($P \leq 0.01$) than that in leaves in both NFT species but, the opposite trend occurred with *S. siamea* (Table XII).

3.3.2. Total N yield and NUE

The total-N yields in the prunings were higher ($P \leq 0.01$) in *G. sepium* (58 g N plant^{-1}) followed by *L. leucocephala* then *S. siamea* (33 g N plant^{-1}) in the first year after initial pruning (Table XIII). But, in the second and third years the non-N_2 fixing *S. siamea* accumulated more ($P \leq 0.01$) N in prunings (104 and 95 g N plant^{-1}, respectively) than did *L. leucocephala* (46 and 41 g N plant^{-1}, respectively). Also, the rates of N accumulation with age were higher in *G. sepium* and *S. siamea* compared to *L. leucocephala.*

TABLE XII. DRY MATTER PRODUCTION OF *L. LEUCOCEPHALA*, *G. SEPIUM*, AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE AND AGE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
						(kg plant^{-1})						
Leaves												
L. leuco.	1.21	0.94	0.60	0.92	0.63	0.73	1.25	0.87	0.94	0.91	0.08	0.65
G. sepium	1.71	1.13	1.09	1.31	1.62	2.45	3.17	2.41	2.14	2.04	0.44	1.54
S. siamia	1.29	1.49	0.68	1.15	3.06	3.58	2.89	3.18	5.95	3.37	0.26	3.19
Mean	1.41	1.19	0.79		1.77	2.25	2.44		3.01	2.10	0.26	
$LSD_{0.05}$[a]		0.12				0.15				0.14		
Branches												
L. leuco.	1.44	1.53	1.76	1.58	0.80	1.30	3.35	1.82	2.62	2.70	0.61	1.98
G. sepium	1.34	1.51	1.90	1.58	1.06	3.38	5.67	3.37	3.98	4.16	0.98	3.04
S. siamia	0.78	0.94	1.34	1.02	1.55	2.80	3.49	2.61	2.64	3.74	0.15	2.17
Mean	1.19	1.33	1.67		1.14	2.49	4.17		3.08	3.53	0.58	
$LSD_{0.05}$[a]		0.22				0.18				0.49		
Whole pruning												
L. leuco.	2.65	2.47	2.37	2.50	1.43	2.03	4.60	2.69	3.56	3.61	0.70	2.62
G. sepium	3.05	2.64	2.99	2.90	2.68	5.82	8.84	5.78	6.12	6.20	1.42	4.58
S. siamea	2.08	2.43	2.02	2.18	4.60	6.39	6.38	5.79	8.60	7.10	0.41	5.37
Mean	2.59	2.51	2.46		2.91	4.75	6.61		6.09	5.00	0.84	
$LSD_{0.05}$[a]		0.33				0.32				1.57		

[a]For comparing means of species or pruning practices.

TABLE XIII. NITROGEN YIELDS OF *L. LEUCOCEPHALA* , *G. SEPIUM*, AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE AND AGE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
						(g N $plant^{-1}$)						
Leaves												
L. leuco.	44	29	22	32	23	26	42	30	33	34	3	23
G. sepium	59	39	38	45	53	88	102	81	69	73	15	52
S. siamea	33	34	14	27	76	96	81	84	152	91	7	83
Mean	45	34	24		51	70	75		85	66	8	
$LSD_{0.05}$ [a]		5.2				6.1				35		
Branches												
L. leuco.	11	12	15	13	6	13	27	15	22	25	6	17
G. sepium	14	12	14	13	10	37	50	32	32	35	11	26
S. siamea	6	5	7	6	12	27	19	19	15	19	0.8	12
Mean	10	10	12		9	26	32		23	26	5.9	
$LSD_{0.05}$ [a]		2.3				2.8				9.1		
Whole pruning												
L. leuco.	56	41	36	44	29	39	69	46	55	58	9	41
G. sepium	73	51	51	58	64	125	152	113	101	108	25	78
S. siamea	39	39	21	33	88	123	100	104	168	110	8	95
Mean	56	44	36		60	96	107		108	92	14	
$LSD_{0.05}$ [a]		7.0				9.0				36		

[a]For comparing means of species or pruning practices.

The effects of pruning on total-N yields of all three species followed trends similar to those of DM production, i.e. pruned trees accumulated more N than did unpruned trees, except in the second year after initial pruning. The 4-monthly interval was superior to the 6-monthly, except in the second year. Irrespective of species and pruning regime, accumulation of N in leaves was higher ($P \leq 0.01$) than in branches, with *G. sepium* and *S. siamea* accumulating more N than did *L. leucocephala*. Furthermore, among pruning treatments, leaf-N accumulation over the 3-year period on trees pruned at 4 monthly intervals (100-182 g N $plant^{-1}$ was higher or comparable to these harvested twice yearly (89-200 g N $plant^{-1}$), whereas unpruned trees accumulated less leaf N (66-154 g N $plant^{-1}$).

Non-fixing *S. siamea* more efficiently utilized soil mineral N for biomass production than did the NFTs in years 1 and 2 (Table XIV). However, the NUE of *L. leucocephala* was significantly higher than that of *S. siamea* in year 3. Generally, NUE remained fairly constant with tree age in all three species, with generally higher values ($P \leq 0.01$) in unpruned trees at least in the first and second years, but with no marked effect of pruning frequency.

3.3.3. Atom %^{15}N excess

Senna siamea had the highest ^{15}N enrichments in its prunings (0.279-0.351%), followed by *L. leucocephala*, whereas ^{15}N enrichment was significantly lower ($P \leq 0.01$) in *G. sepium* (Table XV). The unpruned trees in all three species had the lowest ^{15}N enrichments in leaves and branches and, except in the second year, the enrichments of 6-monthly prunings were less than those cut every 4 months. Irrespective of the species and pruning treatment, enrichment declined with age, except in a few instances.

3.3.4. N_2 fixation

The prunings from *G. sepium* contained a higher ($P \leq 0.01$) proportion of fixed N (%Ndfa 48-65) than *L. leucocephala* (41-60) except in year 2 (Table XVI). A similar trend existed in leaves and branches although the differences in %Ndfa were not significant. In neither species was there marked change in %Ndfa with age except in the second year. Unpruned trees derived a higher proportion of total N from fixation (53-76%) except in the second year. The %Ndfa was not influenced by pruning interval.

Irrespective of age, *G. sepium* had higher amounts of fixed N in prunings, than did *L. leucocephala* (Table XVII). Nitrogen derived from fixation was appreciably higher in leaves than in branches. Also, BNF capacity in both species increased up to the second year and declined thereafter. Pruned trees in both species produced significantly higher amounts of fixed N, and there was no consistent pattern in the amounts of fixed N in unpruned trees except in year 2. There was no consistent trend in terms of superiority of any one pruning regime.

3.4. Experiment 4

3.4.1. Growth of coconut

The application of prunings from the tree legumes significantly increased girth and canopy size of the coconut saplings (Table XVIII). A similar trend was observed also in plant height, although the differences were significant only with the *Leucaena* prunings. In general the performance of the coconut saplings supplied with *L. leucocephala* and *G. sepium* prunings was better than when applied with *S. simea* prunings, which was better than that of the untreated control, albeit not necessarily significantly.

TABLE XIV. NITROGEN USE EFFICIENCY OF *L. LEUCOCEPHALA, G. SEPIUM* AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE AND AGE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
					(g DM g^{-1} plant N)							
L. leuco.	48	60	66	58	46	52	67	55	64	61	75	67
G. sepium	43	52	59	50	44	47	59	50	60	60	56	59
S. siamea	54	62	104	73	54	53	64	57	56	66	58	60
Mean	48	58	76		48	51	63		60	62	63	
$LSD_{0.05}$ [a]		10.2				2.4				3.7		

[a]For comparing means of species or pruning practice.

TABLE XV. ATOM % ^{15}N EXCESS IN HARVESTED PLANT MATERIALS OF *L. LEUCOCEPHALA, G. SEPIUM,* AND *S. SIAMEA* AS AFFECTED BY PRUNING PRACTICE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
						(% ^{15}N a.e.)						
Leaves												
L. leuco.	0.223	0.211	0.127	0.190	0.145	0.168	0.080	0.131	0.207	0.160	0.025	0.130
G. sepium	0.214	0.200	0.130	0.181	0.108	0.166	0.133	0.136	0.151	0.171	0.024	0.277
S. siamea	0.394	0.334	0.316	0.351	0.364	0.444	0.312	0.374	0.387	0.330	0.114	0.277
Mean	0.278	0.245	0.191		0.206	0.259	0.174		0.245	0.221	0.054	
$LSD_{0.05}$ [a]		0.027				0.045				0.020		
Branches												
L. leuco.	0.239	0.244	0.221	0.235	0.153	0.171	0.196	0.173	0.214	0.156	0.041	0.137
G. sepium	0.187	0.208	0.148	0.182	0.100	0.156	0.148	0.134	0.134	0.141	0.031	0.101
S. siamea	0.360	0.327	0.363	0.350	0.358	0.364	0.363	0.362	0.362	0.337	0.138	0.279
Mean	0.262	0.260	0.244		0.205	0.230	0.235		0.237	0.211	0.071	
$LSD_{0.05}$ [a]		0.037				0.041				0.020		
Whole pruning materials												
L. leuco.	0.226	0.222	0.165	0.204	0.148	0.167	0.126	0.147	0.211	0.161	0.032	0.134
G. sepium	0.210	0.203	0.134	0.182	0.107	0.163	0.136	0.136	0.147	0.167	0.024	0.112
S. siamea	0.389	0.333	0.330	0.351	0.365	0.429	0.322	0.373	0.383	0.333	0.120	0.279
Mean	0.275	0.253	0.210		0.207	0.253	0.194		0.247	0.220	0.059	
$LSD_{0.05}$ [a]		0.027				0.038				0.016		

[a]For comparing means of species or pruning practices.

TABLE XVI. PERCENT N DERIVED FROM FIXATION IN HARVESTED PLANT MATERIALS OF *L. LEUCOCEPHALA* AND *G. SEPIUM* AS AFFECTED BY PRUNING PRACTICE (EXPT. 3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
						(%Ndfa))						
Leaves												
L. leuco.	43	36	59	46	59	62	73	65	47	53	77	59
G. sepium	45	41	58	48	70	62	54	62	61	49	81	64
Mean	44	38	58		64	62	64		56	51	79	
$LSD_{0.05}$ [a]				NS				NS				NS
[b]	7.7				NS				5.1			
Branches												
L. leuco.	32	25	34	31	56	53	41	50	40	54	65	53
G. sepium	47	37	56	47	71	57	56	61	63	58	79	67
Mean	40	31	45		64	55	48		52	56	72	
$LSD_{0.05}$ [a]				NS				NS				11
[b]	NS				NS				14			
Whole pruning materials												
L. leuco.	42	32	49	41	59	61	59	60	45	53	73	57
G. sepium	46	40	58	48	70	61	55	62	62	51	80	65
Mean	44	36	53		65	61	57		53	52	76	
$LSD_{0.05}$ [a]				6.8				NS				4.1
[b]	8.3				NS				5.0			

[a]For comparing means of species. [b]For comparing means of pruning practices.

TABLE XVII. AMOUNT OF N FIXED BY *L. LEUCOCEPHALA* AND *G. SEPIUM* AS AFFECTED BY PRUNING PRACTICE (EXPT.3)

Species	Year 1 (1993/94)				Year 2 (1994/95)				Year 3 (1995/96)			
	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean	4 mthly	6 mthly	unprnd	Mean
	(g N $plant^{-1}$)											
Leaves												
L. leuco.	19.8	10.5	12.8	14.4	13.5	16.0	29.9	24.7	15.2	17.6	2.3	11.7
G. sepium	27.7	16.0	21.4	21.7	37.6	54.3	54.7	55.4	42.5	34.1	12.1	29.5
Mean	23.7	13.2	17.1		25.6	35.2	42.3		28.8	25.8	7.2	
$LSD_{0.05}$ [a]				4.5				10.9				4.8
[b]	3.7				NS				5.9			
Branches												
L. leuco.	3.9	2.7	5.4	4.0	3.4	7.2	16.2	8.9	8.5	14.1	4.3	9.0
G. sepium	7.0	4.8	7.8	6.5	7.2	20.9	26.9	18.3	19.5	20.7	8.5	16.2
Mean	5.4	3.7	6.6		5.3	14.1	21.6		14.0	17.4	6.4	
$LSD_{0.05}$ [a]				2.6				5.9				3.2
[b]	3.2				4.8				3.9			
Whole pruning materials												
L. leuco.	24.0	13.1	17.8	18.6	17.0	23.8	46.1	28.9	24.4	31.3	6.7	20.8
G. sepium	34.6	20.8	29.4	28.3	44.9	76.3	81.6	67.6	62.4	54.4	20.5	45.8
Mean	29.3	17.0	23.6		31.0	50.0	63.9		43.4	42.9	13.6	
$LSD_{0.05}$ [a]				5.5				14.5				6.8
[b]	6.8				17.8				8.4			

[a]For comparing means of species
[b]For comparing means of pruning practices.

3.4.2. *Availability of N from prunings*

The coconut saplings obtained about 36% of their N from *L. leucocephala* and 40% from *G. sepium* prunings (Table XVIII). The fraction of N taken up from *Senna* green manure was significantly lower.

4. CONCLUSIONS

The selection of a suitable reference species is a critical consideration in estimating BNF by trees, when using the ^{15}N-dilution method. In this work, biomass production of the non-nodulating tree legume *S. siamea* was similar to those of the NFTs, and had higher ^{15}N enrichments In the light of these observations, *S. siamea* appeared to be a good choice as a reference for estimating BNF in *G. sepium* and *L. leucocephala* for Sri Lankan conditions.

4.2. Experiment 1

In view of the widespread use of *G. sepium* as a component of agroforestry systems in many ecological niches, it is important to select genotypes/provenances with superior BNF ability. An important finding arising from this study is the significant variation in BNF ability among *G. sepium* genotypes, provenance OFI 14/84 from Guatemala being superior to others. Also, it is noteworthy that the local land-race demonstrated considerable capacity for fixing atmospheric N_2. The lower ^{15}N enrichment in plant tissues of provenance 14/84 indicates superior ability to fix N_2. This study shows that the selection of superior genotypes with high biomass yield and N_2-fixation ability is a valid strategy for increasing the N contribution from fixation in agroforestry systems. Although %Ndfa values for the whole plant did not differ appreciably among *G. sepium* genotypes, (52-64%), the amounts of fixed N varied significantly (18-62 g N plant^{-1}) as a result of differences in biomass production, suggesting that BNF depends heavily on the supply of photosynthates. Furthermore, results of this study suggest that exclusion of roots in calculating BNF would not seriously underestimate (a mean of 9%) the amounts of N_2 fixed by *G. sepium*, at least during the first year.

TABLE XVIII. LEAF %N, ^{15}N ENRICHMENTS AND GROWTH COMPONENTS OF COCONUT SAPLINGS AND THE RECOVERY OF N FROM PRUNINGS (EXPT. 4)

Pruning source	Leaf N (%)	^{15}N (% excess)	Height (cm)	Girth (cm)	Canopy (cm)	%N from prunings
L. leucocephala	1.74	0.086	143	82.5	205	36
G. sepium	1.66	0.083	121	79.1	184	40
S. siamea	1.74	0.070	114	71.2	151	15
Control	1.63	0.019	91.8	52.4	124	
Significance	NS	*	*	*	***	*
$LSD_{0.05}$	-	0.04	34.3	10.1	40.1	17
CV (%)	12	41	19	9	16	36

4.2. Experiment 2

The comparison of *G. sepium* (12/86) and *L. leucocephala* (K 636) under coconut over a 1-year period revealed the former to be superior in terms of total DM production, N yield and amount of N fixed. A notable feature was the high NUE achieved by *Gliricidia* and *Leucaena* (75-78 g DM g^{-1} N). In these species, %Ndfa values did not differ markedly, ranging between 47 and 55% ,whereas fixed N in *G. sepium* (17 g N $plant^{-1}$) was higher than in *L. leucocephala* (11 g), although not significantly so due to a high CV. In Experiment 1 the same provenance of *G. sepium* grown in full sunlight produced similar amounts of fixed N_2, showing that BNF capacity of *G. sepium* was not adversely affected by the 30% shade under the coconut-tree canopy. In this study, roots accounted for substantial proportions of total DM (27-32%), total N (25-32%) and fixed N (19-22 g $plant^{-1}$) in both NFT species, suggesting that exclusion of roots would lead to gross underestimation of BNF in *G. sepium* and *L. leucocephala* grown under such conditions.

4.3. Experiment 3

Currently, there is a serious lack of information and understanding of the BNF capacity of NFT species, as influenced by tree management and age. This issue was addressed by estimating the BNF potential of *G. sepium* and *L. leucocephala* grown under coconut and subjected to three pruning frequencies. *Gliricidia sepium* was found to be the superior N_2 fixer, as shown by higher amounts of fixed N in prunings (28-68 g N $plant^{-1}$), irrespective of tree age. Also, amounts of fixed in both species increased up to the second year after initial pruning, which resulted in declines in ^{15}N enrichment. The total DM in its prunings was significantly higher in *G. sepium* than in *L. leucocephala,* which correlates with its high N_2-fixing capacity. Pruning increased biomass production and N yield except in the second year after initial pruning, 4-monthly pruning being superior to 6-monthly. In contrast, unpruned trees produced higher total DM and N yields in the second year of growth after initial pruning. The amount of fixation by NFTs with frequent pruning may have been due to less soil N being available as a result of greater demand for N for regrowth. In contrast, there was a remarkable drop in the N_2 fixation by trees not subjected to pruning, particularly in the third year, probably as a result of more soil N being made available via root and nodule senescence and due to absence of regrowth. These results suggest that pruning would have a positive effect on BNF capacity in trees in the long term.

4.4. Experiment 4

Increased growth of coconut saplings by the application of *G. sepium* and *L. leucocephala* as a mulch is a reflection of rapid decomposition and availability of nutrients; 35-40% of sapling N was obtained from the green manure. In the light of these results, it would appear that *G. sepium* could be recommended as a component in agroforestry systems associated with coconut. However, the amounts of fixation and contribution of atmospheric N by regularly pruned *Gliricidia* and its transfer to coconut palms have to be investigated in longer-term studies. Also, long-term effects of *G. sepium* on maintaining and restoring soil fertility of marginal soils under coconut, its impact on the soil-plant environment and implications on socio-economic considerations also warrant study, in order to promote its widespread use in the development of sustainable farming systems in the tropics.

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