

PLANT NUTRIENT EFFECTS ON GROWTH, NODULATION,
NITROGENASE AND NODULE COMPOSITION OF
LEUCAENA LEUCOCEPHALA (LAM) DE WIT

By

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CHAPTER I

INTRODUCTION

Among the tropical legumes, *Leucaena* probably offers the widest assortment of uses. *Leucaena* sp. produce nutritious forage, firewood, timber, rich organic fertilizer, and other uses that include revegetating tropical hillslopes, windbreaks, firebreaks, shade and ornamentation. Individual *Leucaena* trees have produced extraordinary yields of wood that are among the highest annual amounts recorded. The plant is responsible for high weight gains measured for cattle feeding with forage. However, it remains a neglected crop for utilization by many tropical countries. Varieties with exceptional size, growth vigor and other desirable characteristics have been developed only during the past two decades and their use is still limited and literature sparse (1, 2, 4, 9, 16, 22).

Leucaena is the common name for *Leucaena leucocephala* (Lam) de Wit. Some strains are many branched shrubs that average 5 m. (15 ft.) in height at maturity. Others are single trunked trees that grow as high as 20 m. (65 ft.). Originating in Central America, some of the varieties spread throughout the region thousands of years ago. Sometime during the past 250 years, this species reached the Philippines, Netherlands East Indies (now Indonesia), Papua New Guinea, Thailand and other countries of Southeast, West Africa and Australia. It is well adapted to humid tropical areas (3, 12, 15, 24, 29).

Leucaena is a genus of the family Leguminosae. As with most other legumes, they form a mutually beneficial partnership with soil bacteria of the genus Rhizobium. These bacteria penetrate young rootlets and multiply to form nodular swellings of the root tissues. The Rhizobium within nodules have the capability of absorbing large amounts of inert nitrogen gas from air, transforming it into biological active nitrogen compounds known as "Nitrogen Fixation". Leucaena usually has large prolific nodules and requires little or no fertilizer nitrogen because the active Rhizobium provide nitrogenous compounds in adequate amounts for normal growth. This permits Leucaena to thrive in some soil where nitrogen levels are inadequate to sustain the growth of most other crops. The nodules occur on rootlets developing in the aerated-surface soil layer. Leucaena also develops a taproot that penetrates to deep soil layers and utilize water and minerals below the root zone of many agricultural crops (31, 33).

Leucaena will grow vigorously in lowland areas. Although the plant can survive and grow aggressively in many marginal soils and environments, its exceptional yields occur only in fertile, well-drained soil where rainfall or irrigation is adequate. This is particularly true when the plant is intensively harvested for forage or green manure. Soil fertility is of less concern when Leucaena is used for reforestation or halting soil erosion (10, 18, 30).

Like all legumes and grasses, Leucaena requires a reasonable mineral balance in the soil, so that attention to nutrient inputs, particularly phosphorus, sulfur, calcium, molybdenum, and zinc, is very important. Even under favorable conditions, continual browsing or cutting and removing the wood or foliage will deplete a Leucaena plant of some vital

nutrients. Fertilizations is then required. There are a number of types of poor soils where *Leucaena* cannot survive easily, for example poor adaptation acid soils. Lime pelleting and the addition of a special *Rhizobium* strain as well as fertilizer containing molybdenum, phosphorus, sulfur and calcium are needed to get it well-established. The plant's main potential appears to be for those areas with nonacid soils. *Leucaena* also grows poorly in high-alumina soils and requires careful fertilization with phosphate and calcium if it is to survive and grow. Nevertheless, with fertilization good yields are possible in aluminous soils (16, 23, 25).

CHAPTER II

LITERATURE REVIEW

Plant

Leucaena is a genus of Central American shrubs and trees with about 10 species. Although all the species may have value throughout the tropics, Leucaena leucocephala has been recognized as outstanding. It has been recorded in the literature under several botanical names. The most universal common name is "Leucaena" but many countries use different local names (20). In Thailand we call it "Hauxin".

Hutton and Gray (14) reported that L. Leucocephala can be classified into these three types.

1. Hawaiian type: Short, bushy varieties to 5 m. (15 ft.) in height that flower when very young (4-6 months old). Its yield of wood and foliage is low.

2. Salvador type: tall, treelike plant to 20 m. (65 ft.) in height having large leaves, pods and seeds and branchless trunks. These cultivars now being planted as sources of timber, woodproduct and industrial fuel.

3. Peru type: tall plants to 15 m. (45 ft.) but with extensive branching even low down on the trunks. They produce little trunk, but extremely high qualities of foliage grow on branches.

Takashi and Ripperton (32) described the plant botanically as follows:

Leaves: lupinnate, 15 to 25 cm. long, rachesis pubescent, pinnate 4 to 8 pairs, 5 to 10 cm. long, leaflets 10 to 15 pairs, leaflets linear oblong acute, inequilateral, 7 to 15 mm. long and 3 to 4 mm. long.

Flowers: white, 100-180 flowers clustered in a globular head 2.5 to 3 cm. in diameter, solitary auxillary, long pedicelled, about 4 cm. in lengths.

Seed pods: thin, flat, strap-shaped, acuminate, 12 to 18 cm. long, 1.4 to 2 cm. wide, usually 15 to 60 per cluster, covered with fine hair when young, 15 to 25 seeds per pod.

Seeds: elliptic compressed, shiny brown, 3 to 4 mm. wide, 6 to 8 mm. long, and about 2 mm. thick.

Dijkman (6) pointed out that *Leucaena* is restricted to the tropics and subtropics and it withstands large differences in rainfall, sunlight, salinity, and land terrain as well as periodic inundation, fire, wind-storm, slight frost, and drought. And it grows best where annual rainfall is 600-1,700 mm. (25-65 inches) and in neutral or alkalic soils but *Leucaena* grows poorly in acidic soils.

Leucaena shows high resistance to pests and diseases. A common pest is the seed weevil which attacks the young pods and eats the developing seeds. Fungal diseases such as damping-off can occur in wet soils (5).

The Uses

Young *Leucaena* foliage is mainly used to feed cattle, water buffalo, and goats. It can be harvested and carried fresh to the animals dried into a leaf meal, or fermented to silage.

Owen (21) stated that in the lowland tropics large quantities of protein can be produced efficiently and economically from *Leucaena* grown on well-drained, fertile soils and harvested as hay or forage.

Mendoza et al. (17) showed that *Leucaena*'s protein is high nutritional quality. Amino acids are present in well-balanced proportion and it can also be a rich source of carotene and vitamins.

TABLE I
COMPOSITION (DRY WEIGHT BASIS) OF LEUCAENA (16)

Composition	Amount
1. Total Ash	11.0 %
2. Total Nitrogen	4.2 %
3. Crude Protein	25.9 %
4. Modified-acid-detergent fiber	20.4 %
5. Calcium	2.36%
6. Phosphorus	0.23%
7. Beta carotene	536.0 (mg/Kg)
8. Gross energy	20.1 (KJ/g)
9. Tannin	10.15 (mg/g)

The newly discovered arboreal *Leucaena* varieties grow rapidly, yielding wood of useful size for lumber and timber and the *Leucaena* wood has the potential to become a major source for pulp and paper, roundwood

(e.g., poles and posts), and construction materials.

Leucaena wood makes excellent firewood and charcoal. Large areas are already being planted to provide fuel for electric generators, factories and agriculture processing facilities. Leucaena helps to enrich soil and aid neighboring plants because its foliage rivals manure in nitrogen content, and the natural leaf-drop returns this to the soil beneath the shrubs (20).

Dijkman (6) proposed that Leucaena's ability to thrive on steep slopes, in marginal soils, and in areas with extended dry seasons makes it a prime candidate for restoring forest cover to watersheds, slopes, and grasslands that have been denuded through reforestation or fire.

Takahashi and Ripperton (32) obtained highly significant response to N on a soil of pH 4.5 to 6.5 deficient in Ca, P and K. However, N application was not considered to be economically justified. Ca and P applied together increased yield by 27.4%, applying these elements was considered worthwhile for forage production on acid to moderately acid soils with low levels of available Ca and P. The response to added K was not significant.

Nodulation

Trinick (34) indicated that Leucaena seedlings develop a taproot to reach water before the vulnerable young plant is caught by drought. Seedlings will usually have a taproot almost as long as the plant is tall. Even on adult plants, lateral roots are few and they usually grow downward at a sharp angle. But small laterals occur near the soil surface and develop the nitrogen-fixing Rhizobium nodules which are usually 1.5-2.5 mm (0.1-1.5 inches) in diameter and are frequently

multilobed. Functioning nodules are bright pink inside.

Norris (19) reported that the *Leucaena*-*Rhizobium* partnerships is capable of annually fixing more than 500 Kg nitrogen per ha (500 lb. per acre). This is equivalent to 2,500 Kg ammonium sulphate per ha per annum (2,500 lb. per acre per annum).

On an acid soil in Costa Rico, Esquilvel (8) obtained the greatest weight and number of nodules on *Leucaena* when a complete fertilizer plus lime and inoculum were applied. Mo and B, in particular, increased the weight and number of nodules. The effects of lime was to alter soil pH, thus allowing more efficient nodulation. However, nitrogen fixation occurs only if the correct *Rhizobium* strains are present in the soil. *Leucaena* plants that are not nodulating are usually stunted, unproductive, and frequently have pale green or yellow foliage low in protein. *Leucaena* is naturalized where bacteria are normally widespread. However, in areas where *Leucaena* has never been grown before, the seed must be inoculated with an appropriate *Rhizobium* strain just before it is sown. In nature, the fine roots hairs are also usually infected with a beneficial mycorrhiza fungus whose vast network of hyphae helps the plant obtain phosphorus and other minerals (13).

CHAPTER III

MATERIALS AND METHODS

Greenhouse experiments were performed to compare the effects of plant nutrients P, K, and Ca in factorial combination on top, root growth, nodulation, nitrogenase, and associated nodule enzyme activity levels.

The soil used in these studies was the epipedon, 20 cm depth, of a dark red latosol (Typic Eustrtox, isohyperthermic, fine kaolinite) from Jaiba, Minas Gerais, Brazil (7, 26). The soil pH was 6.1, 3.3% organic matter, cation exchangeable capacity 25.4 meq/100g with exchangeable cations as meq/100g, Ca^{++} 13.8, Mg^{++} 2.5, K^+ 0.2, Na^+ 0.01, available P 7.5 ppm, Fe 680.0 ppm, Mn 208.0 ppm, Zn 1.0 ppm, $\text{SO}_4^{=}$ and Al^{+++} 1.0 ppm with sand 24.5%, silt 19.5%, and clay 56.0%. The soil class was clay soil.

In common with most heavy clayey tropical soils, an irreversible destruction of their natural granular structure results with soil displacement from the natural field site and the ensuring mixing and processing for the pot studies, massive, brick-like physical structure usually develops that is highly restrictive for plant growth. Dilution with sterile, sharp, coarse quartz sand to attain a porous, single grained structure is requisite for optimum root development and nodulation (27, 28). The sand dilution 4 sand + 1 soil, resulted in pot cultures of 11.2% clay with a desirable stabilized, porous, single grain structure.

Leucaena seed utilized in these experiments was the native variety from Thailand. Leucaena was planted on October 16, 1980 and harvested on June 3, 1981 for experiment I. There were 81 plants in 27 treatments for both experiments (3 replicates for each treatment). For experiment II Leucaena was planted in the same soil on June 19, 1981, and harvested on October 12, 1981. Each culture contained 1 Kg of soil sample diluted with white quartz sand, and planted with 1 Leucaena (Leucaena Leucocephala) seedling.

Sources of the nutrient elements for these two experiments levels and combinations are summarized as follows:

P	$\text{Ca}(\text{H}_2\text{PO}_4)_2$
K	KCl
Ca	CaCO_3
Trace	Phillips Hart Salt mixture

Series I: 6 P levels; 0, 100, 200, 300, 400, 500 ppm
2 K levels, 0, 400 ppm

Complete factorial with 3 replicated per treatment.

Series II: 4 K levels, 0, 200, 400, 600 ppm
2 P levels, 0, 200 ppm
2 Ca levels, 0, 6 me/100 g soil

Complete factorial with 3 replicates per treatment.

Series III: Repeat of Series I

Series IV: Repeat of Series II plus 100 ppm of P.H.
(Phillips Hart) trace element salt mixture
with composition as follows:

P.H. salt mixture composition:

Calcium carbonate	30.0	%
Calcium Phosphate .2 H ₂ O	7.5	%
Cobalt Chloride	0.005	%
Copper Sulfate	0.003	%
Dipotassium Diphosphate	32.2	%
Ferric Citrate	2.75	%
Manganese Sulfate	0.51	%
Magnesium Sulfate (hydrate)	10.2	%
Potassium Iodide	0.08	%
Sodium Chloride	16.7	%
Zinc Chloride	0.0025	%

At harvest the root-nodules were separated, washed free of soil, blotted with paper toweling to remove wash-water and placed in serum cap bottles for nitrogenase activity determinations (C₂H₂ reduction) (11). Approximately one hour was the time period from plant harvest until the initiation of acetylene incubations.

Acetylene reduction was determined using 0.1 atm C₂H₂. Ethylene production during incubation at 27° C was determined at 30 minute intervals with a Perkin Elmer GC 3920 with 1.83 X 3.2 mm Paropak N 80/100 column. The ethylene standard utilized for calibration and monitoring Gas Chromatography (GC) analysis was the Scott Ev. Tech. 1090 ppm±5% C₂H₄/N₂.

Nodules were picked from the roots and weighed immediately following the gas chromatography analysis. Nodule Cytosol determinations by the method of Vance, et al. (35) were slightly modified to separate the cell-free nodule extract. Aliquot of the fresh nodules were crushed

within glass tubes g/ml (1:10 ratio) in 0° to 5° C double distilled water. The filtered homogenate was subjected to ultrasonic 7.3 pulse frequency in an ice bath for 30 seconds using a PT 10 ST Williams Polytron (Brinkman Instruments, Inc.) and followed by refrigerated centrifugation at 12×10^3 g for 10 minutes. The clear, cell-free supernatant was aseptically transferred to sterile culture tubes and stored at 0°-5° C. Following enzyme and cytosol component analysis, the residual nodule extracts were lyophilized for storage preservation using a Unitrap Model 10-100 (Vitris Co.). The nodule cytosol components were determined using a Perkin-Elmer 373 Atomic Absorption Flame Spectrophotometer with K, Ca, Fe, and Mg in lanthanum Chloride (0.1 N HCl) solution and Na without the lanthanum addition. Nonconjugate and inorganic P were determined with the ascorbic acid oxidation method as phosphomolybdenum blue.

CHAPTER IV

RESULTS AND DISCUSSIONS

The results from the series I experiment are present in Table II to VII.

Highest top yield as dry weight was obtained at the pooled P_1 level with a pooled mean of 4.13 grams per plant. A quadratic response was apparent with increased P levels. Although reduced yields were apparent with K addition to the P levels, significantly higher yields with K resulted with P_0K_0 and P_5 treatments.

Percent of top growth as dry leaf weight increased with levels of P addition but generally were slightly less with PK combination. Highest leaf percentage 58.18 % was with the P_5 treatment and was significantly higher than the lowest P_1 treatment.

Increased root growth was quadratic when P levels were applied alone and with K treatment combination except P_5 treatment. Although the 4.82 grams of P_1 treatment significantly resulted in the highest root dry weight. The K effect resulted in root growth decreases when K treatment was combined with the P levels.

Fresh nodule weight increased in quadratic response to increased P levels with and without K treatment combination. The significantly highest fresh nodule weight 1.6172 grams resulted with P_2 treatment. The effects of K treatment addition with P levels gave slightly higher fresh nodule weight than P levels alone.

TABLE II
EFFECTS OF P LEVELS WITH AND WITHOUT K ON TOP DRY WEIGHT,
PERCENT LEAF DRY WEIGHT, ROOT DRY WEIGHT, FRESH NODULE
WEIGHT, NUMBER OF NODULE AND NITROGENASE
ACTIVITY LEVEL

Parameter	Treatment	0	P ₁	P ₂	P ₃	P ₄	P ₅	\bar{X}
Top Wt (g dry)	0	0.47	4.60	3.83	1.93	2.00	0.55	2.23
	K	0.60	3.67	1.75	1.60	1.50	2.30	1.91
	\bar{X}	0.53	4.13	2.79	1.17	1.75	1.45	
% Leaf	0	57.4	45.6	52.2	53.3	56.5	58.1	53.8
	K	50.0	49.0	50.2	55.3	52.1	58.1	52.4
	\bar{X}	53.3	47.3	51.2	55.7	55.9	55.1	
Rt Wt	0	0.63	4.82	2.97	2.52	1.38	0.43	2.13
	K	1.03	2.22	1.38	1.35	1.50	1.25	1.45
	\bar{X}	0.83	3.52	2.18	1.93	1.44	0.84	
Fresh Nod Wt (g fresh)	0	0.1945	1.0855	1.6172	0.7994	0.8912	0.2553	0.8072
	K	0.2172	1.3576	0.7573	0.8835	0.7006	1.0616	0.8295
	\bar{X}	0.2058	1.2211	1.1823	0.8414	0.7959	1.8085	
No. of Nod (Nodules /plant)	0	24	147	132	84	74	28	82
	K	27	178	96	88	44	106	90
	\bar{X}	36	163	114	86	59	67	
Nase (μ mole C ₂ H ₄ /culture hr.)	0	87.67	269.30	314.6	166.7	158.60	98.00	182.50
	K	45.00	381.30	232.6	304.0	233.3	382.6	263.17
	\bar{X}	66.30	325.30	273.6	235.3	196.0	240.30	

Treatment levels as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca(H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, No. = Number,
Nase = Nitrogenase.

TABLE III

EFFECTS OF P LEVELS WITH AND WITHOUT K ON TOP DRY WEIGHT, PERCENT LEAF DRY WEIGHT, ROOT DRY WEIGHT, FRESH NODULE WEIGHT, NUMBER OF NODULE AND NITROGENASE ACTIVITY LEVEL

Treatment	Top	% Leaf	Rt Wt	Nod Wt	# Nod	Nase
0	0.47 b	57.06 a	0.63 b	0.1943 b	23 b	87.67 b
P ₁	4.60 a	46.40 b	4.82 a	1.0853 ab	147 ab	269.33 ab
P ₂	3.83 ab	53.23 ab	2.97 ab	1.6170 a	131 ab	341.67 ab
P ₃	1.93 ab	58.90 ab	2.52 ab	0.7993 ab	84 ab	166.67 ab
P ₄	2.00 ab	55.60 ab	1.38 b	0.8913 ab	74 ab	158.67 ab
P ₅	0.55 b	57.40 a	0.43 b	0.2550 ab	28 b	98.00 ab
K	0.60 b	50.00 ab	1.03 b	0.2170 ab	27 b	381.33 a
P ₁ K	3.67 ab	49.27 ab	2.21 ab	1.3567 ab	178 a	45.00 b
P ₂ K	1.75 ab	50.77 ab	1.38 b	0.7570 ab	96 ab	232.67 ab
P ₃ K	1.60 ab	58.93 a	1.35 b	0.8833 ab	88 ab	304.00 ab
P ₄ K	1.50 ab	55.17 ab	1.50 b	0.7000 ab	44 b	233.33 ab
P ₅ K	2.37 ab	51.60 ab	1.25 b	1.0613 ab	106 ab	382.67 a

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca(H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Top Wt = g dry, Rt Wt = g dry, Nod Wt = g fresh, Nod No = Nodules/Culture, Nase = μ mole C₂H₄/Culture/hr.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, # = Number, Nase = Nitrogenase.

TABLE IV
EFFECTS OF P LEVELS WITH AND WITHOUT K ON NODULE CYTOSOL
(% P, % K, % Ca, % Mg, % Na AND Fe (ppm))

Parameter	Treatment	0	P ₁	P ₂	P ₃	P ₄	P ₅	\bar{X}
% P	0	-	0.20	0.23	0.25	0.25	-	0.23
	K	-	0.23	0.22	0.18	0.24	0.25	0.22
	\bar{X}	-	0.21	0.22	0.21	0.24	0.25	
% K	0	-	2.61	2.22	2.30	2.88	-	2.50
	K	-	2.35	2.41	2.16	2.78	2.69	2.47
	\bar{X}	-	2.48	2.31	2.23	2.83	2.69	
% Ca	0	-	0.08	0.21	0.29	0.17	-	2.50
	K	-	0.11	0.15	0.02	0.04	0.01	0.07
	\bar{X}	-	0.09	0.18	0.15	0.10	0.01	
% Mg	0	-	0.55	0.64	0.89	0.68	-	0.19
	K	-	0.70	0.54	0.36	0.56	0.62	0.55
	\bar{X}	-	0.62	0.59	0.62	0.62	0.62	
% Na	0	-	0.06	0.27	0.43	0.21	-	0.24
	K	-	0.41	0.14	0.08	0.09	0.18	0.18
	\bar{X}	-	0.23	0.20	0.25	0.15	0.18	
Fe (ppm)	0	-	88.00	116.25	73.0	90.00	-	91.81
	K	-	49.75	69.75	80.5	41.25	49.75	58.20
	\bar{X}	-	68.87	93.00	76.75	65.62	49.75	

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca (H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

- means no data.

TABLE V
EFFECTS OF P LEVELS WITH AND WITHOUT K ON NODULE CYTOSOL
(% P, % K, % Ca, % Mg, % Na, AND Fe (ppm))

Treatment	% P	% K	% Ca	% Mg	% Na	Fe (ppm)
0	-	-	-	-	-	-
P ₁	0.20 ab	2.61 a	0.08 abc	0.55 bc	0.06 a	88.0 ab
P ₂	0.20 ab	2.22 a	0.21 ab	0.64 abc	0.27 a	116.25 a
P ₃	0.25 ab	2.30 a	0.29 a	0.89 a	0.43 a	73.0 abc
P ₄	0.25 ab	2.88 a	0.17 abc	0.68 abc	0.21 a	90.0 ab
P ₅	-	-	-	-	-	-
K	-	-	-	-	-	-
KP ₁	0.23 ab	2.35 a	0.11 abc	0.70 ab	0.41 a	49.75 c
KP ₂	0.22 ab	2.41 a	0.15 abc	0.54 bc	0.14 a	69.75 abc
KP ₃	0.18 b	2.16 a	0.02 bc	0.36 c	0.08 a	80.5 ab
KP ₄	0.24 ab	2.78 a	0.04 bc	0.56 c	0.09 a	41.25 bc
KP ₅	0.25 a	2.68 a	0.01 c	0.62 abc	0.18 a	49.75 bc

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0, P₅ = 4.0 as Ca(H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

- Means no data

TABLE VI
EFFECTS OF P LEVELS WITH AND WITHOUT K ON NODULE ORGANELLE
RESIDUE (% P, % K, % Ca, % Mg, % Na, and Fe (ppm))

Parameter	Treatment	0	P ₁	P ₂	P ₃	P ₄	P ₅	\bar{X}
% P	0	-	0.28	0.28	0.16	0.25	-	0.24
	K	-	0.23	0.24	0.28	0.21	-	0.24
	\bar{X}	-	0.25	0.26	0.22	0.23	-	
% K	0	-	1.38	0.94	0.42	1.14	-	0.97
	K	-	0.96	1.32	1.60	1.14	1.16	1.23
	\bar{X}	-	1.17	1.13	1.01	1.01	1.14	
% Ca	0	-	2.17	0.51	0.53	0.62	-	0.95
	K	-	0.56	0.58	0.63	0.44	0.54	0.55
	\bar{X}	-	1.36	0.54	0.58	0.53	0.54	
% Mg	0	-	0.53	0.48	0.40	0.49	-	0.47
	K	-	0.51	0.52	0.47	0.42	0.47	0.38
	\bar{X}	-	0.52	0.50	0.43	0.45	0.47	
% Na	0	-	0.07	0.13	0.15	0.10	-	0.11
	K	-	0.20	0.09	0.60	0.05	0.09	0.09
	\bar{X}	-	0.13	0.11	0.10	0.07	0.09	
Fe (ppm)	0	-	0.07	0.13	0.15	0.10	-	0.11
	K	-	0.20	0.09	0.06	0.05	0.09	0.09
	\bar{X}	-	0.13	0.11	0.10	0.07	0.09	

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca (H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

- Means no data

TABLE VII

EFFECTS OF P LEVELS WITH AND WITHOUT K ON NODULE ORGANELLE
RESIDUE (% P, % K, % Ca, % Mg, % Na, AND Fe (ppm))

Treatment	% P	% K	% Ca	% Mg	% Na	Fe(ppm)
0	-	-	-	-	-	-
P ₁	0.28 a	1.38 ab	2.17 a	0.53 a	0.07 a	0.07 ab
P ₂	0.28 a	0.94 b	0.51 b	0.48 a	1.13 a	0.13 ab
P ₃	0.16 b	0.42 c	0.53 b	0.40 a	0.15 a	0.15 b
P ₄	0.25 ab	1.44 b	0.62 b	0.49 a	0.10 a	0.10 ab
P ₅	-	-	-	-	-	-
K	-	-	-	-	-	-
KP ₁	0.23 c	0.96 c	0.56 b	0.51 a	0.20 a	0.20 ab
KP ₂	0.24 ab	1.32 ab	0.58 b	0.52 a	0.09 a	0.09 a
KP ₃	0.28 a	1.60 a	0.63 b	0.47 a	0.60 a	0.06 ab
KP ₄	0.21 ab	1.14 b	0.44 b	0.42 a	0.05 a	0.05 b
KP ₅	-	1.16 b	0.54 b	0.47 a	0.09 a	0.09 ab

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca(H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

- Means no data

Increased number of nodules resulted with the increased P levels with and without K treatment combination. The significantly highest number of nodules was 178 nodules per plant with the P₁K treatment. Increased number of nodule resulted with K treatment combined with P levels over P levels alone.

The acetylene reduction (C₂H₂ reduction) technique was employed in these studies to assay nitrogenase activity. A quadratic increase in nitrogenase activity levels occurred with increased P levels alone and with K treatment combination. The P₅K treatment resulted in 382.67 μmol/g as the significantly highest nitrogenase activity level. Higher nitrogenase enzyme activity (C₂H₂ reduction) resulted from the K with P levels combination than only P levels alone.

Effects of P levels with and without K treatment combination on percent of P, K, Ca, Mg, Na, and Fe (ppm) composition of nodule cytosol is presented in Table IV.

P levels slightly increased higher percent of P than P levels with K treatment combination. The P₃ and P₄ treatments with 0.25% of P were significantly higher in percent of P as compared to the content at PK₃ treatment of 0.18% P.

The effects of K treatment addition to P levels resulted in lower percent of K than with P levels alone. The nonsignificantly highest percent of K was 2.88% of the P₄ treatment compared to the lowest P₃K treatment.

Much higher percent of Ca occurred with no K treatment addition to P levels. The highest content was from the P₃ treatment of 0.29% and was a significantly higher percent of Ca than the lowest PK₅ treatment.

The P levels alone resulted in slightly higher percent Mg than P levels with K treatment combination. The P₃ treatment with 0.89% Mg was significantly higher than the lowest P₃K treatment.

The combination of K treatment with P levels resulted in lower percent Na than P levels alone. The P₃ treatment although nonsignificant had the highest percent Na with 0.43% as compared to the lowest P₁ treatment with 0.06%.

Higher Fe (ppm) occurred with increased P levels alone compared to K with P levels combination. The significantly highest P₂ treatment was 116.25 Fe (ppm) with the lowest P₄K treatment with 41.25 Fe (ppm).

The effects of P levels with and without K treatment combination on the percent of P, K, Ca, Mg, Na, and Fe (ppm) composition of the nodule organelle residue (no data on P₅ treatment) are shown in Table VI.

The P levels with and without K treatment combination, indicated near the same results for the percent composition of P. The highest P₁, P₂ and P₃K treatments with 0.28% P was significantly higher in percent P than the lowest P₃ treatment.

The K treatment addition to P levels resulted in higher percent K than P levels alone. The highest P₃K treatment 1.60% K was significantly greater than the lowest P₃ treatment with 0.42% K.

The effects of K treatment combined with P levels resulted in less percent Ca than P levels alone. The P₁ treatment resulted in significantly highest percent Ca with 2.17% compared to the lowest 0.44% Ca of KP₄ treatment.

Higher percent of Mg occurred in the absence of K treatment addition to P levels. The nonsignificant but highest percent K resulted from P₁ treatment of 0.53% as compared to the lowest 0.40% Mg of the P₃ treatment.

The combination of K treatment with P levels resulted in slightly lower percent Na than from the effects of P levels alone. The nonsignificantly highest 0.20% Na was from the P₁K treatment and the lowest 0.05% Na was from the P₄K treatment.

Slightly lowest Fe (ppm) resulted with no K treatment addition to P levels. Comparison of the highest P₂K treatment with 48.9 Fe (ppm) was significantly higher than the lowest from the P₃ treatment.

The results from the series II experiment are presented in Table VIII to XIII.

The top dry weight increased when this soil was fertilized at increased K levels with CaP and P addition. The significantly highest yield, 9.20 grams, resulted with the P₁ treatment. Without P, the effects of K levels alone and with Ca treatment combination depressed the top dry weight production except with the K₃ treatment.

The percentage of leaf dry weight apparently fluctuated among these treatments. There was no significant difference among the various combination levels.

Increased root dry weight was obtained with the K levels with and without P and CaP treatment combination. The P treatment resulted in the significantly highest root yield 5.42 grams. Decreased root dry weight occurred with the K levels and Ca addition except K₃ treatment.

K level treatment alone and with CaP and P combination significantly increased the number of nodules. The P treatment with 297 nodules was the highest number of nodules per plant. P was a first limiting factor for nodule numbers. Without exception, K levels with and without Ca treatment combination produced less number of nodule than corresponding treatments that included P.

TABLE VIII

EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP
ON TOP DRY WEIGHT, % LEAF DRY WEIGHT, ROOT DRY
WEIGHT, FRESH NODULE WEIGHT, NUMBER OF
NODULE AND NITROGENASE ACTIVITY

Parameter	Treatment	0	K ₁	K ₂	K ₃	\bar{X}
Top (g dry)	0	0.47	0.20	0.46	0.64	0.44
	P	9.20	4.11	3.38	4.37	5.26
	Ca	0.31	0.08	0.11	0.05	0.13
	CaP	5.05	2.50	4.35	3.40	3.82
% Leaf (dry Wt)	0	57.4	45.0	60.8	57.8	55.2
	P	47.8	43.3	47.3	45.0	45.8
	Ca	67.7	50.0	57.1	20.0	48.7
	CaP	46.5	48.0	47.5	45.0	46.6
Rt Wt (g dry)	0	0.63	0.53	0.48	0.80	0.61
	P	5.42	4.57	2.85	3.62	4.11
	Ca	0.40	0.30	0.33	0.23	0.32
	CaP	7.17	2.03	2.98	2.85	3.76
Fresh Nod Wt (g fresh)	0	0.1945	0.0591	0.1198	0.2529	0.1566
	P	3.0777	1.7401	1.4903	1.8170	2.0313
	Ca	0.1419	0.0386	0.0428	0.0628	0.0733
	CaP	2.1307	0.9196	1.5103	1.2798	1.4601
No of Nod (Nodules/plant)	0	24	14	28	32	24
	P	297	158	174	223	213
	Ca	18	14	8	5	11
	CaP	214	186	273	235	227
Nase (μ mole) C ₂ H ₄ culture/hr)	0	87.67	14.67	72.33	53.0	56.92
	P	620.67	341.33	244.0	260.0	366.5
	Ca	53.67	7.67	10.33	5.67	19.34
	CaP	701.33	361.33	446.67	378.67	442.75

Treatment level as g/Kg soil; K₁ = 0.4, K₂ = 0.8, K₃ = 1.2 as KCl,

P = 1.0 g/Kg soil as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, No =
Number, Nase = Nitrogenase.

TABLE IX

EFFECT OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP
ON TOP DRY WEIGHT, % LEAF DRY WEIGHT, ROOT DRY
WEIGHT, FRESH NODULE WEIGHT, NUMBER OF
NODULE AND NITROGENASE ACTIVITY

Treatment	Top Wt	% Leaf	Rt Wt	Nod Wt	# Nod	Nase
K ₁	0.19 de	45.00 a	0.52 d	0.0591 e	14 c	14.67 c
K ₂	0.47 de	60.80 a	0.48 d	0.1198 e	28 c	72.33 c
K ₃	0.63 de	57.80 a	0.80 d	0.2529 e	32 c	53.00 c
P	9.20 a	47.80 a	5.41 a	3.0777 a	297 a	602.66 a
PK ₁	4.11 bc	43.30 a	4.56 ab	1.7401 b	158 b	341.33 abc
PK ₂	3.38 bc	47.30 a	2.85 bc	1.4903 bcd	174 b	244.00 bc
PK ₃	4.37 bc	45.00 a	3.61 bc	1.8170 bc	223 ab	260.00 bc
Ca	0.31 de	67.70 a	0.40 d	0.1419 e	18 c	53.67 c
CaK ₁	0.08 e	50.00 a	0.30 d	0.0386 e	14 c	7.67 c
CaK ₂	0.11 e	57.10 a	0.33 d	0.0428 e	8 c	10.33 c
CaK ₃	0.05 e	20.00 a	0.21 d	0.0628 e	5 c	5.66 c
CaP	5.05 b	41.50 a	3.60 bc	2.1307 b	214 ab	701.33 a
CaPK ₁	2.50 cd	48.00 a	2.03 cd	0.9196 d	186 b	361.33 abc
CaPK ₂	4.35 bc	47.50 a	2.96 bc	1.5103 cd	273 a	446.66 ab
CaPK ₃	3.40 bc	45.00 a	2.83 bc	1.2798 cd	235 ab	378.66 abc

Treatment level as g/Kg soil, K₁ = 0.4, K₂ = 0.8, K₃ = 1.2, as KCl, P = 1.0 as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan' Multiple Range analysis at the 0.05 level.

Top Wt = g dry, Rt Wt = g dry, Nod Wt = g fresh, Nod No = Nodules/
Culture, Nase = μ mole C₂H₄/culture/hr.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, # = Number,
Nase = Nitrogenase.

TABLE X
EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP ON NODULE
CYTOSOL (% P, % K, % Ca, % Na AND Fe (ppm))

Parameter	Treatment	0	K ₁	K ₂	K ₃	\bar{X}
% P	P	0.24	0.22	0.23	0.19	0.22
	CaP	0.27	0.21	0.19	0.22	0.22
	\bar{X}	0.25	0.21	0.21	0.20	
% K	P	1.43	2.94	2.80	2.39	2.39
	CaP	1.89	2.11	2.41	2.56	2.24
	\bar{X}	1.66	2.52	2.60	2.47	
% Ca	P	0.16	-	0.15	0.19	0.16
	CaP	0.32	0.31	0.33	0.33	0.32
	\bar{X}	0.24	0.31	0.24	0.26	
% Mg	P	0.68	0.67	0.65	0.64	0.66
	CaP	0.59	0.52	0.59	0.57	0.56
	\bar{X}	0.63	0.59	0.62	0.60	
% Na	P	0.24	0.14	0.16	0.24	0.19
	CaP	0.33	0.19	0.14	0.17	0.20
	\bar{X}	0.28	0.16	0.15	0.20	
Fe (ppm)	P	94.50	37.00	106.75	66.25	76.12
	CaP	64.50	76.75	93.50	54.75	72.37
	\bar{X}	79.50	56.87	100.12	60.50	

Treatment level as g/Kg soil; K₁ = 0.4, K₂ = 0.8, K₃ = 1.2, as KCl,

P = 1.0 g/Kg soil as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

- means no data.

TABLE XI
EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP ON NODULE
CYTOSOL (% P, % K, % Ca, % Mg, % Na, AND Fe (ppm))

Treatment	% P	% K	% Ca	% Mg	% Na	Fe (ppm)
P	0.24 ab	1.43 c	0.16 ab	0.68 a	0.24 a	94.5 a
PK ₁	0.22 ab	2.94 a	-	0.67 a	0.14 a	37.0 a
PK ₂	0.23 ab	2.80 a	0.15 ab	0.65 a	0.16 a	106.75 a
PK ₃	0.19 ab	2.39 ab	0.19 ab	0.64 a	0.24 a	66.25 a
CaP	0.27 a	1.89 bc	0.32 a	0.59 a	0.33 a	64.5 a
CaPK ₁	0.21 ab	2.11 abc	0.31 a	0.52 a	0.19 a	76.75 a
CaPK ₂	0.19 b	2.41 ab	0.33 a	0.59 a	0.14 a	93.5 a
CaPK ₂	0.22 ab	2.56 ab	0.33 a	0.57 a	0.17 a	54.75 a

Treatment level as g/Kg soil, K₁ = 0.4, K₂ = 0.8, K₃ = 1.2, as KCl,

P = 1.0 as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

- Means no data

TABLE XII
EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP ON NODULE
ORGANELLE RESIDUE (% P, % K, % Ca, % Mg, % Na, Fe (ppm))

Parameter	Treatment	0	K ₁	K ₂	K ₃	\bar{X}
% P	P	0.28	0.20	0.24	0.19	0.22
	CaP	0.25	0.23	0.18	0.24	0.22
	\bar{X}	0.26	0.21	0.21	0.21	
% K	P	0.63	1.16	1.42	1.14	1.08
	CaP	0.77	1.23	1.26	1.30	1.14
	\bar{X}	0.70	1.19	1.34	0.63	
% Ca	P	0.73	0.56	0.58	0.56	0.60
	CaP	0.63	0.85	0.78	0.76	0.75
	\bar{X}	0.68	0.70	0.68	0.66	
% Mg	P	0.56	0.51	0.51	0.49	0.51
	CaP	0.44	0.50	0.59	0.51	0.48
	\bar{X}	0.50	0.50	0.50	0.50	
% Na	P	0.22	0.07	0.08	0.11	0.12
	CaP	0.15	0.12	0.07	0.09	0.10
	\bar{X}	0.18	0.09	0.07	0.10	
Fe (ppm)	P	323.75	278.50	375.50	528.75	376.62
	CaP	318.50	733.67	420.50	341.50	453.54
	\bar{X}	321.12	506.08	398.00	435.12	

Treatment level as g/Kg soil; K₁ = 0.4, K₂ = 0.8, K₃ = 1.2 as KCl,

P = 10 g/Kg soil as Ca (H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

TABLE XIII

EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP ON NODULE
ORGANELLE RESIDUE (% P, % K, % Ca, % Mg, % Na, Fe (ppm))

Treatment	% P	% K	% Ca	% Mg	% Na	Fe(ppm)
P	0.28 a	0.63 b	0.73 abc	0.56 a	0.22 a	323.75 a
PK ₁	0.20 a	1.16 ab	0.56 d	0.51 abc	0.07 b	278.50 a
PK ₂	0.24 a	1.42 a	0.58 cd	0.51 abc	0.08 b	378.50 a
PK ₃	0.19 a	1.14 ab	0.56 d	0.49 bc	0.11 ab	528.75 a
CaP	0.25 a	0.77 ab	0.63 cd	0.44 c	0.15 ab	318.50 a
CaPK ₁	0.23 a	1.23 ab	0.85 a	0.50 ab	0.12 b	733.67 a
CaPK ₂	0.18 a	1.26 ab	0.78 ab	0.59 bc	0.07 b	420.50 a
CaPK ₃	0.24 a	1.30 ab	0.76 ab	0.51 ab	0.09 b	341.50 a

Treatment level as g/Kg soil, K₁ = 0.4, K₂ = 0.8, K₃ = 1.2 as KCl,

P = 1.0 as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range Analysis at the 0.05 level.

The results from K levels with CaP and P treatment combination resulted in large increases with fresh nodule weight. There was significant difference between the highest treatment (P_1) and the check (no treatment). The K levels with and without Ca combination without P, resulted in lower fresh nodule weight.

Nitrogenase activity levels as reduction of acetylene (C_2H_2) represents an estimation of the amount of N biologically fixed that is available for incorporation into plant amino acids. The levels of nitrogenase activity was increased with increased K levels only when combined with P, both with and without Ca combination. The CaP treatment was significantly higher in nitrogenase activity levels than the check (no treatment). Reduced nitrogenase levels resulted with both K and Ca treatments in the absence of P combination.

Effects of P treatment addition to Ca and K treatments on the percent of P, K, Ca, Mg, Na, and Fe (ppm) composition of the nodule cytosol extract are shown in Table X.

Although the same pooled mean results for % P with K levels for all P and CaP treatment combination but % P apparently decreased with K levels. The significantly highest percent P was 0.27 of the CaP treatment as compared to the lowest 0.19 of % P of $CaPK_2$ treatment.

K levels with P combination showed higher percent K than K levels with CaP combination. The highest PK_1 treatment as 2.94 % K was significantly higher in percent K than the lowest P treatment.

The combination of K levels with P treatment produced lower percent of Ca than the K levels with CaP combination. The highest $CaPK_2$ and $CaPK_3$ treatments of 0.33 % Ca were nonsignificantly higher compared to the lowest composition of the PK_2 treatment.

The effects of Ca addition to P and K levels combination resulted in less percent Mg than P and K levels combination. The nonsignificantly highest content of 0.68 % Mg was from the P treatment with the lowest 0.521 Mg for the CaPK₁ treatment.

The addition of Ca to K levels with P combination resulted in higher percent Na than only K levels with P combination. The highest percent of Na with 0.33% was nonsignificantly higher than the lowest of 0.14% from PK₁ and CaPK₂ treatments.

The Ca addition to K levels with P combination resulted in less Fe (ppm) than no Ca treatment combinations. The nonsignificantly highest PK₂ treatment was 106.75 Fe (ppm) as compared to the lowest from the PK₁ treatment.

The effects of P and CaP treatment addition to K levels on composition of nodule residue percent of P, K, Ca, Mg, Na, and Fe (ppm) are shown in Table XII.

Pooled mean of CaP and P with K levels combination resulted with the same percent of P. The highest CaP treatment with 0.28% P was nonsignificantly higher than the lowest from the CaPK₂ treatment.

Higher percent K occurred with the Ca treatment addition to the K levels in all P combination without Ca addition. The highest PK₂ treatment with 1.42 % K was significantly higher in percent K than the lowest of the P₁ treatment.

Ca addition to K levels treatment than included P treatment combination resulted in higher percent Ca than without Ca treatment addition. The significantly highest CaPK₁ treatment with 0.85 % Ca yielded higher percent Ca than the lowest of the P₁ treatment.

The effects of K levels with P treatment combination without Ca addition resulted in higher percent Mg than with the Ca combination. The P₁ treatment was significantly high compared to the lowest from the CaP treatment.

Ca effects with K levels including all P treatment combination resulted in lower percent Na than without Ca. The highest P₁ treatment with 0.22 % Na was significantly higher in percent Na than the lowest 0.07 % Na with the CaPK₂ treatment.

The K levels and CaP combination resulted in higher Fe (ppm) than the K levels with P treatment combination. Nonsignificantly highest of 733.67 Fe (ppm) was from the CaPK₁ treatment with the lowest Fe (ppm) content from the PK₁ treatment.

The results from the series III experiment are presented in Table XIV to XVII.

Higher top dry weight occurred when P levels with and without K treatment combination were applied. The PK₁ treatment gave the significantly highest top dry weight yield of 8.16 grams. Slightly lower top dry weight resulted from K addition with P levels.

The percentage of leaf dry weight apparently fluctuated with increased P levels alone and with K treatment combination. However, higher percent of leaf dry weight occurred with the PK₁ treatment. With that exception, lower leaf dry weight resulted with PK combination compared to P levels without K.

Increased root dry weight resulted with P levels with and without K combination. A yield of 2.98 grams was the significantly highest root dry weight with P₂ treatment. P levels with K treatment combination resulted in lower dry weight than P level treatment without K.

TABLE XIV

EFFECTS OF P LEVELS WITH AND WITHOUT K ON TOP DRY WEIGHT, PERCENT LEAF DRY WEIGHT, ROOT DRY WEIGHT, FRESH NODULE WEIGHT, NUMBER OF NODULE AND NITROGENASE ACTIVITY LEVEL

Parameter	Treatment	0	P ₁	P ₂	P ₃	P ₄	P ₅	\bar{X}
Top Wt	0	0.26	2.22	5.47	4.33	1.77	2.19	2.71
	K	0.78	8.16	1.95	3.69	2.76	1.45	2.67
	\bar{X}	0.52	5.19	3.71	4.01	2.26	1.82	
% Leaf	0	46.15	66.2	59.7	60.7	73.4	71.2	62.9
	K	51.2	17.5	70.3	64.2	72.4	17.2	58.8
	\bar{X}	48.1	41.8	65.0	62.4	72.9	74.2	
Rt Wt	0	0.25	1.30	2.98	2.28	0.95	1.50	1.54
	K	0.58	1.33	1.07	1.86	1.20	0.73	1.12
	\bar{X}	0.41	1.31	2.02	2.07	1.07	1.11	
Fresh Nod Wt (g fresh)	0	0.0914	0.7141	1.2518	1.1526	0.6838	1.0314	0.8214
	K	0.3029	0.7910	0.7991	1.0734	1.0052	0.6906	0.7770
	\bar{X}	0.1971	0.7525	1.0254	1.1148	0.8445	0.8610	
No of Nod (Nodules/plant)	0	24	124	200	169	118	170	134
	K	54	175	136	113	132	102	118
	\bar{X}	39	149	168	141	125	136	
Nase/ μ mole C ₂ H ₄ /culture/hr.	0	14.3	89.00	144.30	143.00	156.00	152.60	116.50
	K	48	353.00	158.00	145.30	147.6	130.00	124.70
	\bar{X}	31.10	104.30	151.10	144.10	151.80	141.30	

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca(H₂PO₄)₂, K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, No = Number, Nase = Nitrogenase.

TABLE XV

EFFECTS OF P LEVELS WITH AND WITHOUT K ON TOP DRY WEIGHT, PERCENT LEAF DRY WEIGHT, ROOT DRY WEIGHT, FRESH NODULE WEIGHT, NUMBER OF NODULE AND NITROGENASE ACTIVITY LEVEL

Treatment	Top Wt	% Leaf	Rt Wt	Nod Wt	# Nod	Nase
0	0.25 d	46.15 a	0.25 d	0.0910 c	23 e	14 b
P ₁	2.21 bcd	66.20 a	1.30 bcd	0.7136 ab	124 bc	89 b
P ₂	5.46 a	59.70 a	2.93 a	1.2516 a	200 a	144 ab
P ₃	4.33 ab	60.70 a	2.28 ab	1.1560 a	169 abc	143 ab
P ₄	1.76 cd	73.40 a	0.95 cd	0.6836 ab	118 bcd	156 ab
P ₅	2.20 bcd	71.20 a	1.50 bcd	1.0313 a	170 abc	152 ab
K	0.78 d	51.20 a	0.58 cd	0.3026 bc	54 de	48 b
P ₁ K	8.16 a	17.50 a	1.33 bcd	0.7910 ab	175 ab	353 a
P ₂ K	1.95 bcd	70.30 a	1.06 bcd	0.7990 ab	136 abc	158 ab
P ₃ K	3.68 abc	64.20 a	1.86 abc	1.0730 a	112 bcd	145 ab
P ₄ K	2.76 bcd	72.40 a	1.20 bcd	1.0050 a	131 abc	147 ab
P ₅ K	1.45 cd	17.20 a	0.73 cd	0.06906 ab	102 cd	130 ab

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca(H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Top Wt = g dry, Rt Wt = g/dry, Nod Wt = g fresh, Nod No = Nodules/Culture, Nase = μ mole, C₂H₄/Culture/hr.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, # = Number, Nase = Nitrogenase.

Quadratic response in fresh nodule weight was apparent with increased P levels with and without K combination. The significantly highest fresh nodule weight 1.2518 grams was from the P₂ treatment. The effect of K addition to P levels resulted in lower fresh nodule weight than with P levels alone.

The number of nodules increased with P levels with and without K combination. The effect of K treatment combined with P levels resulted in less number of nodule than P levels alone. The significantly highest number of nodules, 200 nodules per plant, occurred with the P₂ treatment.

The P levels with and without K treatment addition resulted in higher nitrogenase activity levels than the check, no treatment. The PK₁ treatment resulted in significantly highest nitrogenase enzyme activity with 353 mole/gram. P levels alone resulted in higher nitrogenase acitivity levels than K addition.

Effects of P levels with and without K treatment combination on percent of P, K, Ca, Mg, Na, and Fe (ppm) composition of total nodule are presented in Table XVI.

Slightly increased percent of P resulted from increased P levels with and without K treatment combination. The P₄ treatment, 0.4 % P was the highest percent of P. K addition to P levels resulted in higher percent of P than with P levels alone.

Percent K decreased with increasing P levels with and without K. But with K addition to P levels resulted higher % K than P levels alone. The check with 4.60 % K was the significantly highest % K.

The response of P levels with and without K treatment combination resulted in higher percent Ca than the check. The P₂ treatment with 1.26 % Ca was the highest percent of Ca. The P levels with K combination

TABLE XVI

EFFECTS OF P LEVELS WITH AND WITHOUT K ON TOTAL NODULE COMPOSITION
OF % P, % K, % Ca, % Mg % Na AND Fe (ppm)

Parameter	Treatment	0	P ₁	P ₂	P ₃	P ₄	P ₅	\bar{X}
% P	0	0.40	0.40	0.35	0.45	0.46	0.44	0.41
	K	0.38	0.42	0.43	0.41	0.42	0.42	0.49
	\bar{X}	0.39	0.41	0.39	0.43	0.44	0.43	
% K	0	4.60	2.21	1.67	1.40	2.30	2.36	2.42
	K	2.97	2.14	2.34	3.19	2.72	3.28	2.77
	\bar{X}	3.78	2.17	2.00	2.29	2.51	2.82	
% Ca	0	1.00	1.16	1.26	1.08	1.06	1.04	1.10
	K	1.04	1.09	1.14	1.24	1.17	1.11	1.13
	\bar{X}	1.02	1.12	1.20	1.20	1.16	1.11	
% Mg	0	1.00	1.24	1.38	1.34	1.19	1.29	1.24
	K	1.04	1.19	1.17	1.08	1.17	1.03	1.11
	\bar{X}	1.02	1.21	1.27	1.21	1.18	1.16	
% Na	0	0.20	0.58	0.97	0.89	0.62	0.88	0.69
	K	0.33	0.77	0.56	0.23	0.45	0.27	0.43
	\bar{X}	0.26	0.67	0.76	0.56	0.53	0.57	
Fe (ppm)	0	756.00	374.40	420.90	339.10	349.10	229.70	423.20
	K	363.50	437.80	454.10	390.00	395.50	410.10	408.50
	\bar{X}	559.70	406.10	437.50	364.00	372.30	354.90	

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca(H₂PO₄)₂, K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

TABLE XVII

EFFECTS OF P LEVELS WITH AND WITHOUT K ON TOTAL NODULE COMPOSITION
OF % P, % K, % Ca, % Mg, % Na AND Fe (ppm)

Treatment	% P	% K	% Ca	% Mg	% Na	Fe(ppm)
0	0.40 ab	4.60 a	1.00 a	1.00 b	0.20 b	756 a
P ₁	0.40 ab	2.21 de	1.16 a	1.24 ab	0.58 ab	374.4 b
P ₂	0.35 b	1.67 ef	1.26 a	1.38 a	0.97 a	420.9 b
P ₃	0.45 ab	1.40 f	1.08 a	1.34 ab	0.89 ab	339.1 b
P ₄	0.46 a	2.30 de	1.06 a	1.19 ab	0.62 ab	349.1 b
P ₅	0.44 a	2.36 cde	1.04 a	1.29 ab	0.88 ab	299.7 b
K	0.38 ab	2.97 bcd	1.04 a	1.04 ab	0.33 ab	363.5 b
KP ₁	0.42 a	2.14 de	1.09 a	1.19 ab	0.77 ab	437.8 b
KP ₂	0.43 a	2.34 cde	1.14 a	1.17 ab	0.56 ab	454.1 b
KP ₃	0.41 ab	3.19 bc	1.24 a	1.08 ab	0.23 b	390 b
KP ₄	0.42 a	2.72 bcd	1.17 a	1.17 ab	0.45 ab	395.5 b
KP ₅	0.42 a	3.28 b	1.11 a	1.03 b	0.27 b	410.1 b

Treatment level as g/Kg soil; P₁ = 0.5, P₂ = 1.0, P₃ = 2.0, P₄ = 3.0,

P₅ = 4.0 as Ca(H₂PO₄)₂; K = 0.8 g/Kg soil as KCl.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Quadratic response in fresh nodule weight was apparent with increased P levels with and without K combination. The significantly highest fresh nodule weight 1.2518 grams was from the P₂ treatment. The effect of K addition to P levels resulted in lower fresh nodule weight than with P levels alone.

The number of nodules increased with P levels with and without K combination. The effect of K treatment combined with P levels resulted in less number of nodule than P levels alone. The significantly highest number of nodules, 200 nodules per plant, occurred with the P₂ treatment.

The P levels with and without K treatment addition resulted in higher nitrogenase activity levels than the check, no treatment. The PK₁ treatment resulted in significantly highest nitrogenase enzyme activity with 353 μ mole/gram. P levels alone resulted in higher nitrogenase acitivity levels than K addition.

Effects of P levels with and without K treatment combination on percent of P, K, Ca, Mg, Na, and Fe (ppm) composition of total nodule are presented in Table XVI.

Slightly increased percent of P resulted from increased P levels with and without K treatment combination. The P₄ treatment, 0.4 % P was the highest percent of P. K addition to P levels resulted in higher percent of P than with P levels alone.

Percent K decreased with increasing P levels with and without K. But with K addition to P levels resulted higher % K than P levels alone. The check with 4.60 % K was the significantly highest % K.

The response of P levels with and without K treatment combination resulted in higher percent Ca than the check. The P₂ treatment with 1.26 % Ca was the highest percent of Ca. The P levels with K combination

TABLE XVIII

EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca AND CaP
ON TOP DRY WEIGHT, % LEAF DRY WEIGHT, ROOT DRY
WEIGHT, FRESH NODULE WEIGHT, NUMBER OF
NODULE AND NITROGENASE ACTIVITY

Parameter	Treatment	0	K ₁	K ₂	K ₃	\bar{X}
Top Wt (g dry)	0	0.64	2.93	1.73	2.53	1.95
	P	2.23	2.76	1.22	1.96	2.04
	Ca	3.22	2.06	1.56	1.90	2.18
	CaP	1.43	1.86	2.28	2.36	1.93
% Leaf dry Wt	0	75.00	64.84	66.47	71.14	69.36
	P	69.95	65.21	67.21	68.87	67.81
	Ca	65.83	61.16	67.94	68.42	65.83
	CaP	65.03	68.81	75.87	57.62	66.83
Rt Wt (g dry)	0	0.45	1.70	1.06	1.04	1.15
	P	1.20	1.70	0.83	1.06	1.19
	Ca	2.00	1.13	0.83	1.40	1.34
	CaP	0.70	0.90	1.20	1.23	1.01
Fresh Nod (g fresh)	0	0.0256	1.0633	0.7085	0.9316	0.7399
	P	0.8440	1.1224	0.980	0.8285	0.9437
	Ca	1.1106	0.7539	0.6788	0.6887	0.8080
	CaP	0.3720	0.7276	0.9275	0.7766	0.7009
No of Nod (Nodules/ plant)	0	32	145	120	136	108.25
	P	116	256	111	101	146.00
	Ca	177	86	120	106	122.25
	CaP	53	119	127	123	105.50
(Nase (μ mole C ₂ H ₄ /culture/ hr.)	0	41.33	48.00	167.00	178.00	108.58
	P	132.00	177.67	114.67	191.00	152.33
	Ca	179.00	143.67	123.00	148.00	148.41
	CaP	93.33	146.33	167.33	171.00	144.49

Treatment level as g/Kg soil; K₁ = 0.4, K₂ = 0.8, K₃ = 1.2, as KCl,

P = 1.0 as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, No = Number,
Nase = Nitrogenase.

TABLE XIX

EFFECT OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP
ON TOP DRY WEIGHT, % LEAF DRY WEIGHT, ROOT DRY
WEIGHT, FRESH NODULE WEIGHT, NUMBER OF
NODULE AND NITROGENASE ACTIVITY

Treatment	Top Wt	% Leaf	Rt Wt	Nod Wt	# Nod	Nase
0	0.64 b	75.00 a	0.45 e	0.2563 c	32 e	41 c
K ₁	2.93 a	64.84 a	1.70 abc	1.0633 a	145 bc	202 a
K ₂	1.73 ab	66.47 a	1.06 bcde	0.7085 abc	120 bcd	167 ab
K ₃	2.33 ab	71.14 a	1.04 bcde	0.9316 ab	136 bcd	178 ab
P	2.23 ab	69.95 a	1.20abcde	0.8440 abc	116 bcd	132 ab
PK ₁	2.76 a	65.21 a	1.70 abc	1.1224 a	256 a	171 ab
PK ₂	1.35 ab	67.21 a	0.83 cde	0.9800 abc	111 bcde	114 abc
PK ₃	1.96 ab	68.87 a	1.06 bcde	0.8285 abc	101 bcde	191 a
Ca	3.21 a	65.83 a	2.00 a	1.1106 a	177 b	179 ab
CaK ₁	2.06 ab	61.16 a	1.13abcde	0.7539 abc	86 cde	143 ab
CaK ₂	1.56 ab	67.94 a	0.83 cde	0.6788 abc	120 bcd	123 abc
CaK ₃	1.90 ab	68.42 a	1.40 abcd	0.6887 abc	106 bcde	148 ab
CaP	1.76 ab	65.03 a	0.70 de	0.3720 bc	53 de	93 bc
CaPK ₁	1.86 ab	68.81 a	0.90 bcde	0.7276 abc	119 bcd	146 ab
CaPK ₂	2.28 ab	75.87 a	1.20abcde	0.9275 ab	127 bcd	167 ab
CaPK ₃	2.36 ab	57.62 a	1.23abcde	0.7766 abc	123 bcd	171 ab

Treatment level as g/Kg soil, K₁ = 0.4, K₂ = 0.8, K₃ = 1.2, as KCl,
P = 1.0 as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

Means followed by the same letter are not significantly different
according to Duncan's Multiple Range Analysis at the 0.05 level.

Top Wt = g dry, Rt Wt = g/dry, Nod Wt = g fresh, Nod No = Nodules/
Culture, Nase = μ mole C₂H₄/culture/hr.

Abbreviations are Wt = Weight, Rt = Root, Nod = Nodule, # = Number,
Nase = Nitrogenase

TABLE XX
EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP ON TOTAL NODULE
COMPOSITION OF % P, % K, % Ca, % Mg, % Na AND Fe (ppm)

Parameter	Treatment	0	K ₁	K ₂	K ₃	\bar{x}
% P	0	0.43	0.45	0.41	0.47	0.44
	P	0.44	0.43	0.44	0.45	0.44
	Ca	0.45	0.37	0.44	0.40	0.41
	CaP	0.45	0.43	0.41	0.43	0.43
% K	0	3.19	3.18	3.50	3.28	3.28
	P	3.17	2.04	3.19	3.74	3.03
	Ca	2.94	3.61	3.40	2.55	3.12
	CaP	3.51	3.08	2.16	3.68	3.10
% Ca	0	0.85	0.91	1.01	0.98	0.93
	P	0.84	1.22	1.15	1.13	1.08
	Ca	1.19	1.21	1.29	1.22	1.22
	CaP	1.31	1.27	1.44	1.33	1.33
% Mg	0	1.00	1.10	1.02	1.12	1.06
	P	1.06	1.23	1.10	1.03	1.10
	Ca	1.11	1.01	1.12	1.05	1.07
	CaP	1.07	1.09	1.08	1.00	1.06
% Na	0	0.14	0.45	0.23	0.45	0.31
	P	0.23	0.77	0.33	0.18	0.37
	Ca	0.47	0.20	0.24	0.35	0.31
	CaP	0.21	0.35	0.28	0.18	0.25
Fe (ppm)	0	471.44	236.79	369.20	380.33	364.44
	P	367.02	384.12	420.20	364.39	383.93
	Ca	371.39	406.22	401.50	344.75	380.84
	CaP	411.26	333.87	317.08	331.20	348.35

Treatment level as g/Kg soil; K₁ = 0.4, K₂ = 0.8, K₃ = 1.2, as KCl,
P = 1.0 g/Kg soil as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

TABLE XXI

EFFECTS OF K LEVELS WITH AND WITHOUT P, Ca, AND CaP ON TOTAL NODULE
COMPOSITION OF % P, % K, % Ca, % Mg, % Na, AND Fe (ppm)

Treatment	% P	% K	% Ca	% Mg	% Na	Fe(ppm)
0	0.43 ab	3.19 a	0.85 a	1.00 a	0.14 a	471.44 a
K ₁	0.45 ab	3.18 a	0.91 a	1.10 a	0.45 a	236.79 b
K ₂	0.41 ab	3.50 a	1.01 a	1.02 a	0.23 a	369.20 b
K ₃	0.47 ab	3.28 a	0.98 a	1.12 a	0.45 a	380.33 b
P	0.44 a	3.17 a	0.84 a	1.06 a	0.23 a	367.02 b
PK ₁	0.43 ab	2.04 a	1.22 a	1.23 a	0.77 a	384.12 b
PK ₂	0.44 ab	3.19 a	1.15 a	1.10 a	0.33 a	420.20 b
PK ₃	0.45 ab	3.74 a	1.13 a	1.03 a	0.18 a	364.39 b
Ca	0.45 ab	2.94 a	1.19 a	1.11 a	0.47 a	371.39 b
CaK ₁	0.37 ab	3.61 a	1.21 a	1.01 a	0.20 a	406.22 b
CaK ₂	0.44 ab	3.40 a	1.29 a	1.12 a	0.24 a	401.5 b
CaK ₃	0.40 ab	2.55 a	1.22 a	1.05 a	0.35 a	344.75 b
CaP	0.45 b	3.51 a	1.31 a	1.07 a	0.21 a	411.24 b
CaPK ₁	0.43 ab	3.08 a	1.27 a	1.09 a	0.35 a	333.87 b
CaPK ₂	0.41 ab	2.16 a	1.44 a	1.08 a	0.28 a	317.08 b
CaPK ₃	0.43 ab	3.68 a	1.33 a	1.00 a	0.18 a	331.20 b

Treatment level as g/Kg soil, K₁ = 0.4, K₂ = 0.8, K₃ = 1.2, as KCl, P = 1.0 as Ca(H₂PO₄)₂, Ca = 3.0 g/Kg soil as CaCO₃.

Figures are means of three reps.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

with P, Ca, and CaP treatment combinations.

Increased fresh nodule weight resulted from fertilizing K levels alone and with P, Ca, and CaP treatment combinations. The PK₁ treatment yielded the significantly highest fresh nodule weight with 1.1224 grams. P addition to K levels resulted in higher fresh nodule weight compared to K levels alone.

The K levels with and without P, Ca, and CaP combinations resulted in higher number of nodule than the check (no treatment). The PK₁ treatment significantly yielded the highest number of nodule as 256 nodules per plant. Increased K levels without P resulted in higher number of nodule.

Nitrogenase activity levels increased with increasing K levels with and without Ca, P, and CaP combinations. The highest nitrogenase activity level was 191 μ mole/gram. The effect of P, Ca, and CaP with K level combinations produced higher nitrogenase activity levels than K treatment.

The effects of K levels with and without P, Ca, and CaP treatment combinations, on the percent of P, K, Ca, Mg, Na, and Fe (ppm) composition of total nodule are presented in Table XX.

The K levels with and without P, CaP treatment combinations resulted in slightly higher percent of P than the check (no treatment) except Ca with K levels combination. The K₃ treatment was the highest percent with 0.47 % P.

Lower percent of K resulted when the K levels were combined with P, Ca, and CaP treatments. However, the highest percentage of K, 3.74 % K resulted with the PK₃ treatment. The K levels alone resulted in higher percent than with P, Ca, and CaP combination and the check.

The percentage of Ca increased with increasing K levels with and without P, Ca, and CaP combinations. The 1.44 % Ca was the highest level from CaPK₂ treatment. The K levels alone resulted in lower Ca than with P, Ca, and CaP combinations.

The response of K levels with and without P, Ca, and CaP combinations resulted in higher % Mg than no treatment. The PK₁ treatment with 1.23 % Mg was the highest percentage of Mg. K levels combined with P resulted in a higher percent of Mg than K levels with and without Ca and CaP combinations.

Higher percent of Na occurred with increased K levels with and without P, Ca, and CaP combinations. The highest percent of Na was the PK₁ treatment with 0.77 % Na. The P with K levels combinations resulted in higher percent of Na than K levels alone and with Ca and CaP combinations.

The K levels with and without P, Ca, and CaP combinations resulted in lower Fe (ppm) than the check (no treatment). However, the P effect combined with K levels produced higher Fe (ppm) than K levels with and without Ca and CaP combinations.

Tables XXII to XXVI presents the correlation coefficients with results of these studies. These data indicate that nitrogenase activity levels (μ mole C₂H₄ g⁻¹ nod hr.⁻¹) were positively related to nodule weight and nodule number, as well as to plant growth and development except for the percent leaf component for all series. These may provide an indication that maximization of plant growth and nitrogen fixation of *Leucaena* requires P, K, and Ca fertilization with this dark red latosol soil.

Results shown in Table XXIV indicated that Fe was negatively related

TABLE XXII
CORRELATION COEFFICIENTS FOR PLANT GROWTH PARAMETERS,
NITROGENASE AND NODULATION OF LEUCAENA

Treatment	Series I					
	Top	Leaf	Root	Nod No	Nod Wt	Nase
Top		-0.39966*	0.8329**	0.89745**	0.89683*	0.66819**
Leaf	0.02532		-0.38880*	-0.37579*	-0.24636	-0.23615
Root	0.91320**	0.03256		0.63629**	0.64533**	0.47677*
Nod No	0.88056**	0.08220	0.82171**		0.92743**	0.71865**
Nod Wt	0.98220**	0.06465	0.92682**	0.87219**		0.72747**
Nase	0.79898**	0.12111	0.80599**	0.73565**	0.82295**	
	Series II					

*,** = Significant at P = 0.05 and 0.001 respectively

Nase = Nitrogenase

Nod = Nodule

Wt = Weight

No = Number

TABLE XXIII
CORRELATION COEFFICIENTS FOR PLANT GROWTH PARAMETERS,
NITROGENASE AND NODULATION OF LEUCAENA

Treatment	Series III					
	Top	Leaf	Rt Wt	Nod Wt	# Nod	Nase
Top		-0.18394	0.95788**	0.74117**	0.88739**	0.35107*
Leaf	-0.21741		-0.18706	-0.04660	-0.36230	-0.06362
Root	0.90804**	-0.10002		0.76591**	0.83708**	0.34903*
Nod No	0.57390**	-0.04873	0.65172**		0.80724**	0.41384*
Nod Wt	0.88139**	-0.02732	0.88074**	0.75969**		0.38359*
Nase	0.65067**	-0.11675	0.60340**	0.45972*	0.63729**	

Series IV

*,** = Significant at P = 0.05 and 0.001 respectively

Nase = Nitrogenase

Nod = Nodule

Wt = Weight

No = Number

TABLE XXIV
 CORRELATION COEFFICIENTS FOR NODULE CYTOSOL EXTRACT
 AND NODULE ORGANELLE RESIDUE OF LEUCAENA₁

	Nodule Cytosol Extract					
	P	K	Ca	Mg	Na	Fe
P		0.60963*	0.10851	0.63634*	0.16729	-0.16809
K	0.75130*		-0.34917	0.13220	-0.55051	-0.11094
Ca	0.20716	0.28429		0.66541*	0.67501*	0.21878
Mg	0.00527	0.25427	0.38544		0.76231*	-0.11857
Na	-0.53837*	-0.42055	-0.06294	0.49671		-0.24543
Fe	0.28259	0.25058	0.22606	0.41454	-0.25153	
	Nodule Organelle Residue					

*,** = Significant at P = 0.05 and 0.001 respectively

1 = Series I

TABLE XXV
 CORRELATION COEFFICIENTS FOR NODULE CYTOSOL EXTRACT
 AND NODULE ORGANELLE RESIDUE OF LEUCAENA₁

	Nodule Cytosol Extract					
	P	K	Ca	Mg	Na	Fe
P		-0.08381	-0.02538	-0.12759	0.07571	0.25295
K	0.01474		-0.26158	-0.24205	-0.55491*	0.10379
Ca	0.15130	0.13374		-0.28064	0.33959	-0.11114
Mg	0.25968	0.07927	0.42036		0.18838	-0.30722
Na	0.34347	-0.74010	0.09518	0.31980		-0.11536
Fe	-0.19467	0.32514	-0.05431	0.14756	-0.19051	
	Nodule Organelle Residue					

*,** = Significant at P = 0.05 and 0.001 respectively

1 = Series II

TABLE XXVI
CORRELATION COEFFICIENTS OF TOTAL COMPOSITION
NODULE OF LEUCAENA

	Series III					
	P	K	Ca	Mg	Na	Fe
P		0.09683	-0.31431	-0.15172	-0.04857	-0.08344
K	0.19888		-0.08709	-0.74454*	-0.81378**	-0.57550*
Ca	-0.50258	-0.19683		0.31509	-0.00667	0.13415
Mg	0.25469	-0.64020*	0.02636		0.87428**	-0.23557
Na	0.27859	-0.71585**	-0.16344	0.81517**		-0.32084
Fe	-0.36494	-0.01206	0.79958**	-0.05738	-0.23015	
	Series IV					

*,** = Significant at P = 0.05 and 0.001 respectively

to P, K, Mg. However, P was positively correlated among the elements. For K, Ca, Mg, and Na content showed positive correlation except K with Ca and Na within nodule cytosol extract.

Results with nodule organelle residue analyses indicated that P was positively correlated with all the elements except Na. The element, Na was negatively related to all the elements except Mg. The remainder of the elements indicated positive correlation to each other.

Results from series II are presented in Table XXV, correlations among the groups of elements were negatively related to each other except for Na with P, Ca, Mg, and Fe with P, K for nodule cytosol extract.

A positive correlation occurred among the groups of the elements except Fe with P, Ca, and Na for nodule residue.

Results from series III and IV are presented in Table XXVI, analysis of total nodule indicated negative correlation among these groups of elements except P with K, and Mg with Na, Ca for series III. Within series IV, negative correlation occurred except P with K, Mg, Na, and Ca with Mg, Fe.

CHAPTER V

SUMMARY AND CONCLUSIONS

These greenhouse experiments were conducted with a Dark Red Lactosol (Typic Eutruxox) from Brazil. The objective was to determine the effects of soil fertility treatments on the growth, development, nodulation, and nodule characteristics. The Leucaena' variety used was a native variety from Thailand. The soil fertility treatments consisted of P, K, Ca at various levels within a completely randomized design. Each treatment was replicated three times. The fertility nutrient sources for Leucaena were P as $\text{Ca}(\text{H}_2\text{PO}_4)_2$, K as KCl and Ca as CaCO_3 .

Seed of Leucaena were inoculated with of Rhizobium Leguminosarum and the pot cultures consisted of 1 Kg soil each. All series produced increased shoot growth when the soil was fertilized with P. However, all nutrients element effects on root growth were favorable for Leucaena. Nodule fresh weight responded to P, Ca, K fertilization but only P and K increased the number of nodules. These data indicate that Ca is required for nodule growth, but apparently has less influence on nodule setting.

Nitrogenase activity was determined as reduction of acetylene to ethylene expressed as μ mole C_2H_4 produced/g fresh nodule/hr. P had beneficial effect on this enzyme activity but K combined with P had increasing effect for these plants. However, when activity was expressed as reduction of ethylene per pot culture, in terms of μ moles C_2H_4 /pot culture/hr., a beneficial effects was noted for P and K, as well as a

negative one for only Ca.

Correlation between nitrogenase plant growth and development and nodulation, indicated that a practical way to increase nitrogen fixation with these plants was to fertilize this Dark Red Latosol with P, K, and Ca in order to obtain plants with larger shoot and root, as well as increase nodule fresh weight and number.

In the cytosol extract of nodule the % of P, K, Ca, Mg, Na, and Fe (ppm) was closely related to the treatment levels. However, higher levels of P, K, and Ca influenced % P, K, Ca, Mg, Na, and Fe (ppm) as compared to the check (no treatment) in total nodule composition.

LITERATURE CITED

1. Benge, M.D., and H. Curran. 1976. Bayani (giant ipil-ipil *Leucaena Leucocephala*): A source of fertilizer, feed and energy for the Philippines. USAID Agriculture Development Series. United States Agency for International Development, Manila.
2. Blunt, C.G. 1976. Preliminary cattle grazing trails on irrigated *Leucaena leucocephala* and pangola grass in the Ord Valley, N.W. Australia. Proceedings of the Australian Society of Animal Production 11:10 P.
3. Brewbaker, J.L., D.L. 1975. "Hawaiian Giant" koa haole, Hawaii Agriculture Experiment Station Miscellaneous Publication 125. University of Hawaii, College of Tropical Agriculture, Honolulu. 4 pp.
4. Brewbaker, J.L. 1975. Giant Ipil-ipil: Promising source of fertilizer, feed, and energy for the Philippines. USAID Seminar June 20, 1975. United States Agency for International Development, Manila.
5. Brewbaker, J.L., D.L. Plucknett, and V. Gonzalez. 1972. Varietal variation and yield trials of *Leucaena leucocephala* (koa haole) in Hawaii Agricultural Experiment Station Research bulletin No. 166 University of Hawaii, College of Tropical Agriculture, Honolulu. 29 pp.
6. Dijkman, M.J. 1950. *Leucaena*-a promising soil-erosion-control plant. *Economic Botany* 4:337-49.
7. Epamig, Embrapa, Duralminas. 1976. Levantamento de reconhecimento com detalhes dos solos do Distrito Agroindustrial de Jaiba Minas Gerais. *Boletim Tecnico* No. 54.
8. Esquilvel, S., C. 1965. Factors affecting legume nodulation in the tropics. *Turrialba* 15:252-3.
9. Gray, S.G. 1968. A review of research on *Leucaena leucocephala*. *Tropical Grasslands* 2:19-30.
10. Gray, S.G. 1967. Inheritance of growth habit and quantitative characters in intervarietal cross in *Leucaena leucocephala* (Lam). de Wit. *Aust. J. Agric. Res.* 18:63-70.

11. Hardy, R.W.F., R.D. Holten, E.K. Jackson, and R.C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation laboratory and field evaluation. *Plant Physiology* 43:1185-1207.
12. Hill, G.D. 1971. *Leucaena leucocephala* for pastures in the tropics. *Herbage Abstracts* 41:111-19.
13. Hutton, E.M., and W.M. Beattie. 1976. Field characteristics in three boxed lines of the legume *Leucaena leucocephala*. *Tropical Grasslands* 10:187-94.
14. Hutton, E.M., and S.G. Gray. 1959. Problems in adapting *Leucaena glauca* as a forage in the Australian tropics. *Empire Journal of Experimental Agriculture* 27:187-196.
15. Kinch, D.M., and J.C. Ripperton. 1962. Kao hoale production and processing. Hawaii Agricultural Experiment Station Bulletin No. 129. University of Hawaii, College of Tropical Agriculture, Honolulu. p. 57.
16. *Leucaena*, preliminary forage and tree crop for the tropics. 1977. p. 118 NTIS PB 268-124 National Academy Sciences, Washington, D.C.
17. Mendoza, R.C., T.P. Altamarino, and E.Q. Javier, Herbage, crude protein and digestible dry matter yield of ipil-ipil (*Leucaena latisliqua*, C.V. Peru) in hedge rows. Unpublished paper. Department of Horticulture, Pastures Division, University of the Philippines at Los Banos College, Laguna, Philippines.
18. Metzner, J.K. 1976. Lamtoronisasi: An experiment in soil conservation. *The Bulletin of Indonesian Studies*. (March 1976).
19. Norris, D.O. 1976. The intelligent use of inoculants and pelleting for tropical legumes. *Trop. Grasslands*, 1:107-21.
20. Oakes, A.J. 1968. *Leucaena leucocephala*: Description, culture, utilization, *Advancing Frontiers of Plant Sciences* (New Delhi, India) 20:1-114.
21. Owen, L.N. 1968. Hair loss and other toxic effects of *Leucaena glauca*. *Vet. Rec.* 70, 456-7.
22. Parfitt, R.I. 1976. Shifting Cultivation-How it affects the soil environment. *Harves* 3:63-66 (Published by Department of Primary Industries, Konedobu, Papua New Guinea).
23. Pendleton, R.L. 1963. Cogonals and reforestation with *Leucaena glauca*. *Lingna Science Journal* 12:555-60.
24. Pendleton, R.L. 1934. Philippine experience in reforestation with ipil-ipil (*Leucaena Glauca*). *Lingnan Science Journal* 13:211-24.

25. Plucknett, D.L. 1970. Productivity of tropical pastures in Hawaii, p. A38-A49. In Proceedings of the XI International Grassland Congress. University of Queensland Press, Brisbane, Australia.
26. Purcino, A.A.C. 1980. Nitrogenase nodule enzyme and carbohydrate components of Copada, (Cratylia florilunda, Benth) related to soil fertility effects on regrowth vigor and nodulation with an oxisol of Brazil. Ph.D. Thesis, Oklahoma State University, Stillwater, 74078.
27. Purcino, H.M.A. 1979. Effects of soil fertility treatments on growth and nodule parameters of Winged Bean (Psophocarpus tetragonolobus (L) DC) with a dark red latasol (Typic Eutrustox) from Jaiba, Minas Gerais, Brazil. M.S. Thesis, Oklahoma State University, Stillwater, 74078.
28. Purcino, A.A.C., H.M.A. Purcino and J.Q. Lynd. 1978. Soil Fertility response of a Typic Eutrustox from Jaiba, Minas, Gerais, Brazil. Agronomy Abstracts, p. 43.
29. Savory, R., and D. Thomas. 1977. The establishment and management of leucaena for cutting and browsing, In Pasture Handbook for Malawi, F.A.O., Rome.
30. Semple, A.T., and R.L. Pendleton. 1950. Woody legumes for the poor soil of humid equatorial lowlands. Indian Farming 11:223-25.
31. Shaw, N.H. 1967-68. Weed Control in Leucaena leucocephala. A Rep. Div. trop. Past. CSIRO (Aust.), p 11-13.
32. Takahashi, M., and J.C. Ripperton. 1949. Kao haole (Leucaena glauca). Its establishment, culture, and utilization as a forage crop. Bulletin Number 100. University of Hawaii, Agricultural Experiment Station, Honolulu.
33. Trinick, M.J. 1968. Nodulation of tropical legumes: Specificity in the Rhizobium symbiosis of Leucaena leucocephala. Experimental Agriculture: 4:243-53.
34. Trinick, M.J., M.J. Dilworth, and M. Grounds. 1976. Factors affecting the reduction of acetylene by root nodules of Lupines species. New Phytol. 77:359-70.
35. Vance, E.P., G.H. Heichel, D.K. Barnes, J.W. Bryan, and L.E. Johnson. 1979. Nitrogen fixation, nodule development, and vegetative regrowth of alfalfa (Medicago sativa L.) following harvest. Plant Physiol. 64:1-8.

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