# SOIL FERTILITY EFFECTS GOVERNING GROWTH
## AND NODULE PARAMETERS AT ANTHESIS OF TWO WINGED BEAN CULTIVARS WITH A TYPIC EUTRUSTOX[1]

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ABSTRACT - Winged Bean cultivars, Tinge WB-21-8 and Siempre WB-12-11, were grown with complete factorial soil fertility experiments for P, K, Ca, Mg, and S combinations on a Typic Eutrustox (dark-red latosol) from Central Brazil. Nitrogenase ($C_2H_2$ reduction), growth, and associated nodule enzyme parameters and composition were determined at anthesis. Highly significant increases in nitrogenase activity levels resulted with applied P and K for both cultivars with significant increases for Ca additions. Growth, nodulation and cytosol enzyme activity levels of GOT, GDH, GS and GOGAT all increased significantly with applied P, with the exception of GDH to applied K. α KG was increased to highly significant levels with K treatment. Cytosol composition was increased significantly for each plant nutrient element contained in the treatment combinations. Highly significant inverse retroversion existed for K and Na. Na was negatively correlated with every parameter determined in these studies for both cultivars. Practical application of these data include the requirement for adequate available soil K and P for increased symbiotic N fixation and $NH_4^+$ incorporation into amino acid components of the host legume symbiont.

Index terms: *Psophocarpus tetragonolobus*, nitrogenase, symbiotic nitrogen fixation, tropical edible legume.

## EFEITOS DA FERTILIDADE DO SOLO NO CRESCIMENTO E PARÂMETROS NODULARES DURANTE A ANTESE EM DUAS CULTIVARES DE FEIJÃO-ALADO COM UM TYPIC EUTRUSTOX

RESUMO - Duas cultivares de feijão-alado, Tinge WB-21-8 e Siempre WB-12-11, foram cultivadas num Typic Eutrustox (Latossolo Vermelho-Escuro) do Brasil Central, recebendo tratamentos de P, K, Ca, Mg e S em combinações como um fatorial completo. Durante a antese, foram determinados a atividade da nitrogenase (redução de $C_2H_2$), o crescimento das plantas, os parâmetros enzimáticos dos nódulos, bem como a sua composição mineral. Aumentos altamente significativos na atividade da nitrogenase ocorreram com aplicação de P e K em ambas as cultivares, com aumento significativo para a adição de Ca. O crescimento das plantas, a nodulação e os níveis de atividade enzimática no citosol para GOT, GDH, GS e GOGAT aumentaram significativamente com aplicação de P, e com exceção do GDH, com aplicação de K. Ocorreu um aumento altamente significativo na concentração de αKG quando houve aplicação de K. A composição mineral do citosol do nódulo aumentou significativamente para cada nutriente contido nas combinações de tratamento de fertilidade de solo. Uma correlação negativa altamente significativa existiu entre K e Na. Em ambas cultivares, o Na se correlacionou negativamente com cada parâmetro estudado neste trabalho. A aplicação prática destes dados, indica a necessidade de níveis adequados de P e K no solo para se aumentar a fixação biológica de N e a incorporação de $NH_4^+$ em aminoácidos no macrosimbionte.

Termos para indexação: *Psophocarpus tetragonolobus*, nitrogenase, fixação simbiótica de nitrogênio, leguminosa tropical.

## INTRODUCTION

The great potential of the winged bean, (*Psophocarpus tetragonolobus* (L.) DC), is certainly enhanced by profuse nodulation with concomitant exceptionally high levels of nitrogen fixation (Harding et al. 1978, Hymowitz & Boyd 1977 and Masefield 1961). However, soil environmental factors govern the effectiveness of these eminent symbiotic microbial phenomena. Adaptation and improved productivity

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of this legume are usually dependent upon surmounting many edaphic soil limitations. Some natural restraints are apparent with the diversity among the Ultisol and Oxisol (Latosol) soil orders that comprise the vast humid tropical regions of the world. Fortunately, a number of intrinsic soil properties are diagnostic criteria for soil management and fertilization to attain improved productivity of specific sites. Few have been studied for winged bean culture with enhanced symbiotic nitrogen fixation.

The soil used in this study was a Typic Eutrustox (dark-red latosol) of Jaiba, Minas Gerais, Brazil. In common with other soils of the tropics that are highly weathered, acidic, clayey and subjected to intensive leaching, the humified soil organic matter component provides a principal repository for essential plant nutrients. Thus, the continuous, dynamic biosequences of immobilization and mineralization reactions within the soil epipedon are dominant for soil fertility and essential to sustained productivity. The humus fraction ameliorates the detrimental influences of hydrous oxides of iron and aluminum, high phosphorus fixation, low base cation levels, unfavorable physical structure and low moisture holding capacity. Costly erosion losses of humus and surface soil result from intense, torrential rainfall.

The objective of this research was to determine the influence of the principal soil basic cations, K, Ca, and Mg, applied in factorial combinations with P and S as soil amendments in a Typic Eutrustox. This paper reports results from a four-year experiment with two winged bean cultivars, WB-21-8 Tinge and WB-12-11 Siempre, including interactions with growth, nodulation, nitrogenase ($C_2H_2$ reduction), associated nodule cytosol enzyme activity levels and composition at anthesis. Treatment effects on seed pod and tuber yield during two-wear growth periods as a perennial will be reported in a separate paper.

## MATERIALS AND METHODS

Seed of the winged bean cultivars, WB-12-11 Siempre and WB-21-8 Tinge, both originally from Nigeria, were obtained from the Mayaquez Institute of Tropical Agriculture, Puerto Rico (Fig. 1). Individual plants were container grown in order to recover the entire root and nodule system. Each pot culture was inoculated at planting with 3 ml of *Rhizobium leguminosarum* cultured from nodules of *Strophostyles* sp. with liquid media containing more than $10^8$ viable cells ml$^{-1}$.

All experiments utilized a randomized block design with 32 fertility treatments in three replications as complete $2^5$ factorial using P, K, Ca, Mg, and S combinations. Each cultivar experiment was repeated three times.

FIG. 1. Obvious contrasting characteristics of the two winged bean cultivars, Siempre WB-12-11 and Tinge WB-12-8, include seed color and size (above). Siempre seed pods are long, straight, 15 to 20 cm length, 16 to 24 seeds per pod. Tinge seeds pods are relatively short (8 to 12 cm length) with a more flattened shape, 8 to 14 seeds per pod. Both cultivars originated from Nigeria.

This paper reports plant response to soil fertility treatments at peak nitrogenase activity levels occurring at anthesis, approximately at 70-day age from seed emergence.

The soil used in these studies was the epipedon, 20 cm depth, of a dark-red latosol, (Typic Eutrustox, isohyperthermic, fine, kaolinitic), from Jaíba, Minas Gerais, Brazil. Complete analyses, soil properties, and mineralogical characterization have been previously reported. (Empresa de Pesquisa Agropecuária de Minas Gerais, MG, 1976, Purcino 1980). The soil pH was 6.1, 3.3% O.M., cation exchange capacity 25.4 mEq/100 g with exchangeable cations as mEq/100 g $Ca^{++}$ 2.5, $K^{+}$ 13.8, $Mg^{++}$ 2.5, $K^{++}$ 0.4, $Na^{+}$ 0.01, available P (Bray ext.) 7.5 ppm, Fe 680.0 ppm, Mn 208.0 ppm, Zn 1.0 ppm, $SO_4$ and $Al^{+++}$ $<$ 1.0 ppm with sand 24.5%, silt 19.5%, clay 56.0%.

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 with the ensuing mixing and processing for 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. Detailed studies for the determination of optimum soil-sand ratios for this soil have been reported previously (Purcino 1979 and 1980). In this study, the sand dilution 4 sand + 1 soil resulted in pot cultures of 11.2% clay with a desirable stabilized, porous, single grain structure.

The soil fertility treatments, sources and plant nutrient levels included: 50 ppm P ($NH_4H_2PO_4$), 50 ppm S ($Na_2SO_4$), 6 mEq. $C^{++}$ ($CaCO_3$), 2 mEq. $Mg^{++}$ ($MgCl_2 : 6H_20$), and 2 mEq. $K^+$ (KCl). The base cation ration was

$$\frac{K}{\sqrt{\frac{Ca + Mg}{2}}} = 1$$

with the base cations expressed as mEq/100 g soil. Ammonium acetate provided the $NH_4$ equivalent to all pot cultures that did not receive the phosphorus treatments as $NH_4H_2PO_4$ (26.95% P, 12.17% N). At harvest, the root-nodule systems were separated, washed free of soil, blotted with paper toweling to remove wash-water and placed in serum cap bottles for nitrogenase (E.C. 1.7.99.2) activity determinations ($C_2H_2$ reduction) (Trinick eta al. 1976). Approximately one hour was the time lapse from plant harvest until the initiation of acetylene incubations.

Acetylene reduction was determined using 0.1 atm $C_2H_2$ (lab. spec, purified grade, Linde Div., Union Carbide, Inc.). Ethylene production during incubation at 27°C was determined at 30 min. intervals with a Perkin Elmer GC 3920 with 1.83 x 3.2 mm Poropak N 80/100 column (Waters/Assoc.). The ethylene standard utilized for calibration and monitoring GC analyses was the Scott Ev. Tech. 1090 ppm 5% $C_2H_2/N_2$ (Supelco, Inc.).

Nodules were picked from the roots and weighed immediately following the gas chromatography analyses. Nodule cytosol determinations by the methods of Vance et al. (1979) were slightly modified to separate the cell-free nodule extract. Aliquots of the fresh nodules were crushed within glass tubes g:ml (1:10 ratio) in 0-5°C buffer, pH 7.41. The filtered homogenate was subjected to ultrasonic 7.3 pulse frequency in an ice bath for 30 sec. using a PT 10 ST Willems Polytron (Brinkman Instruments, Inc.) followed by refrigerated centrifugation at 12 x $10^3$ g for 10 min. The clear, cell-free supernatant was aseptically transferred to sterile culture tubes and stored at 0-5°C. Following enzyme and cytosol component analyses, the residual nodule extracts were lyophilized for storage preservation using a Unitrap Model 10-100 (Vitis Co.).

Enzyme activities determined in the nodule cytosol extract are expressed as International Units (U), and defined as the amount of enzyme which causes transformation of 1.0 $\mu$ mole of specific substrate per minute determined in 3.0 ml of reaction volume, 1 cm light path, at 27°C.

Enzyme determinations included glutamate-oxaloacetate transaminase (GOT) (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1.) (Bergmeyer & Bernt 1974), glutamate-pyruvate transaminase (GPT) (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2) (Bergmeyer & Bernt 1974a), glutamate dehydrogenase (GDH) L-glutamate: NAD $(P)^+$ oxidoreductase deaminating, EC 1.4.1.3) (Schmidt 1974), glutamine synthetase (GS) (L-glutamate: ammonia ligase, ADP-forming EC 6.3.1.2) (Shapiro & Stadtman 1970), glutamate synthase (GOGAT) (L-glutamate: $NAD(P)^+$ oxidoreductase (transaminating), EC 1.4.1.13.

Levels of the tricarboxylic acid intermediate, alpha-ketoglutarate ($\alpha$KG) (2-oxoglutarate), were determined with the method proposed by Bergmeyer & Bernt (Bergmeyer & Bernt 1974b), soluble protein was measured by the Folin phenol reagent as described by Lowry et al. (Lowry et al. 1951), and nonstructural carbohydrate components with the Smith techniques (Smith 1969).

The nodule cytosol components were determined using a Perkin-Elmer 373 Atomic Absorption Flame Spectrophotometer with K, Ca, and Mg in Lanthanum chloride (0.1 HCl) solution and Na without the Lanthanum addition. Nonconjugate and inorganic phosphorus were determined with the ascorbic acid oxidation method as phosphomolydenum blue.

## RESULTS AND DISCUSSION

Summaries of experimental results with the WB-21-8 Tinge cultivar are presented in Table 1,3 and 4. Results with the WB-12-11 Siempre cultivar are presented in Tables 2, 5 and 6. The composite, comparative correlations for both cultivars are presented in Table 7 and 8.

Most parameters determined for both winged bean cultivars were significantly influenced by the main effects of the applied plant nutrient elements. Using analysis of variance, the null hypothesis (no nutrient main effect) was rejected for $P \leq .05$ levels of statistical significance. The $\Delta$ effect was defined as the difference between the mean of 144 pot cultures for each cultivar treated with a specified nutrient element and the mean of the other 144 pot cultures of each cultivar that did not teceive that nutrient. Thus, the five possible $\Delta$ effects of the mais treatment for each parameter can be represented as: $\Delta$ effect: $(\overline{P}, \overline{S}, \overline{Ca}, \overline{Mg}, \text{ or } \overline{K})_1 - (\overline{P}, \overline{S}, \overline{Ca}, \overline{Mg}, \text{ or } \overline{K})_0$.

The marked similarity between the two winged bean cultivars for nitrogenase activity levels at anthesis with applied soil fertility treatment is illustrated with Tables 1 and 2. Although the magnitude in $C_2H_2$ reduction levels per g fresh nodule was significantly different between cultivars for most treatments, the overall response of both to applied plant nutrients was similar. Highly significant responses resulted with P and K additions, with a significant effect for Ca fertilization. However, the P x K interaction indicated that the K effect was highest for both cultivars providing phosphorus wasn't included. Available soil phophorus is well

**TABLE 1. Effects of soil fertility treatment combinations on nodule nitrogenase activity levels ($C_2H_2$ reduction) at anthesis, WB-21-8 Tinge Winged Bean, dark-red latosol (Typic Eutrustox) Jaíba, Minas Gerais, Brazil.**

| Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ | Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ | Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ | Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ |
|---|---|---|---|---|---|---|---|
| O | 7.9 | Mg | 11.6 | S | 13.0 | MgS | 8.7 |
| K | 69.2 | KMg | 64.9 | KS | 61.6 | KMgS | 61.8 |
| Ca | 21.4 | CaMg | 18.5 | CaS | 20.6 | CaMgS | 20.0 |
| KCa | 57.8 | KCaMg | 73.2 | KCaS | 70.2 | KCaMgS | 46.7 |
| P | 43.0 | PMg | 40.7 | PS | 40.4 | PMgS | 43.2 |
| PK | 80.0 | PKMg | 77.0 | PKS | 65.5 | PKMgS | 74.5 |
| PCa | 49.4 | PCaMg | 52.6 | PCaS | 52.1 | PCaMgS | 62.8 |
| PKCa | 79.4 | PKCaMg | 77.3 | PKCaS | 84.3 | PKCaMgS | 76.3 |

Principal Composite Effects of Applied Plant Nutrients*

| | P | S | Ca | Mg | K |
|---|---|---|---|---|---|
| With | 62.4 | 50.1 | 53.9 | 50.59 | 69.96 |
| Without | 39.2 | 51.5 | 47.7 | 50.98 | 31.61 |
| Δ Effect | 23.2** | -1.4 | 6.2* | -.39 | 38.35** |

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* Each figure is the pooled mean of all treatment combinations with or without the specified nutrient element, $P \leq .05$* and .01**.

**TABLE 2. Effects of soil fertility treatment combinations on nodule nitrogenase activity levels ($C_2H_2$ reduction) at anthesis, WB-12-11 Siempre Winged Bean, dark-red latosol (Typic Eutrustox) Jaíba, Minas Gerais, Brazil.**

| Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ | Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ | Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ | Trt | $\mu$ moles $C_2H_4$ $g^{-1}$ fresh nod. $hr^{-1}$ |
|---|---|---|---|---|---|---|---|
| O | 4.7 | Mg | 9.9 | S | 14.0 | MgS | 8.4 |
| K | 84.2 | KMg | 78.2 | KS | 72.7 | KMgS | 72.0 |
| Ca | 20.2 | CaMg | 17.9 | CaS | 20.0 | CaMgS | 16.5 |
| KCa | 83.3 | KCaMg | 84.4 | KCaS | 82.0 | KCaMgS | 51.5 |
| P | 45.1 | PMg | 41.9 | PS | 40.2 | PMgS | 42.9 |
| PK | 86.7 | PKMg | 86.6 | PKS | 72.7 | PKMgS | 79.0 |
| PCa | 52.0 | PCaMg | 54.2 | PCaS | 53.0 | PCaMgS | 66.6 |
| PKCa | 84.2 | PKCaMg | 78.3 | PKCaS | 88.2 | PKCaMgS | 78.5 |

Principal Composite Effects of Applied Plant Nutrients*

| | P | S | Ca | Mg | K |
|---|---|---|---|---|---|
| With | 65.3 | 54.43 | 58.64 | 54.48 | 78.92 |
| Without | 45.6 | 56.53 | 52.45 | 56.44 | 32.04 |
| Δ Effect | 19.7** | -2.10 | 6.19* | -1.96 | 46.88** |

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* Each figure is the pooled mean of all treatment combinations with or without the specified plant nutrient element, $P \leq .05$* and .01**.

**TABLE 3.** Principal composite effects of applied plant nutrients on growth nodulation, and nodule enzyme activity levels at anthesis, WB-21-8 Tinge Winged Bean, dark-red latosol (Typic Eutrustox) Jaiba, Minas Gerais, Brazil.

| | P | S | Ca | Mg | K |
|---|---|---|---|---|---|
| Top growth, dry g/plant | | | | | |
| With | 1.23 | 1.05 | 1.10 | 1.02 | 1.09 |
| Without | 0.82 | 1.01 | 0.98 | 1.04 | 0.99 |
| Δ Effect | 0.41** | 0.04 | 0.12 | -0.02 | 0.10 |
| Nodule fresh wt. g/plant | | | | | |
| With | 1.22 | 1.02 | 1.07 | 0.98 | 1.28 |
| Without | 0.71 | 0.92 | 0.96 | 0.95 | 0.65 |
| Δ Effect | 0.51** | 0.10 | 0.11 | 0.03 | 0.63* |
| GOT U/g nodule | | | | | |
| With | 50.2 | 40.9 | 42.5 | 41.5 | 47.0 |
| Without | 29.1 | 38.3 | 36.7 | 37.7 | 32.3 |
| Δ Effect | 21.1** | 2.6 | 5.8 | 13.8* | 14.7 |
| GDH U/g nodule | | | | | |
| With | 3.33 | 2.84 | 2.83 | 2.82 | 2.81 |
| Without | 2.27 | 2.76 | 2.77 | 2.79 | 2.79 |
| Δ Effect | 1.06* | 0.08 | 0.06 | 0.03 | 0.02 |
| GS U/g nodule | | | | | |
| With | 55.4 | 43.3 | 46.2 | 42.4 | 49.5 |
| Without | 29.9 | 41.9 | 39.1 | 42.9 | 35.7 |
| Δ Effect | 25.5** | 1.4 | 7.1* | 0.5 | 13.8** |
| GOGAT U/g nodule | | | | | |
| With | 4.04 | 3.33 | 3.40 | 3.26 | 3.56 |
| Without | 2.04 | 2.74 | 2.70 | 2.82 | 2.52 |
| Δ Effect | 2.00** | 0.59 | 0.70 | 0.44 | 1.04* |

Each figure is the pooled mean of all treatment combinations with or without the specified nutrient element.
P≤ 05* and .01**

**TABLE 4.** Principal composite effects of applied plant nutrients on nodule components at anthesis, WB-21-8 Tinge Winged Bean, dark-red latosol (Typic Eutrustox) Jaíba, Minas Gerais, Brazil.

| | P | S | Ca | Mg | K |
|---|---|---|---|---|---|
| | | CHO, Nonstructural nodule carbohydrate mg/g nodule | | | |
| With | 3.60 | 3.62 | 3.60 | 3.51 | 3.8 |
| Without | 3.40 | 3.41 | 3.41 | 3.52 | 3.2 |
| Δ Effect | 0.20 | 0.21 | 0.19 | - 0.01 | 0.6 |
| | | α Ketoglutarate μ moles/g nodule | | | |
| With | 0.59 | 0.63 | 0.59 | 0.61 | 0.72 |
| Without | 0.59 | 0.55 | 0.58 | 0.56 | 0.46 |
| Δ Effect | 0.00 | 0.08 | 0.01 | 0.05 | 0.26** |
| | | Cytosol Phosphorus μg/g nodule | | | |
| With | 342.2 | 243.0 | 241.9 | 262.9 | 264.5 |
| Without | 174.2 | 273.4 | 274.4 | 253.5 | 251.9 |
| Δ Effect | 168.0** | - 30.4 | - 32.4 | 9.4 | 12.6 |
| | | Cytosol Potassium μg/g nodule | | | |
| With | 2597 | 2461 | 2556 | 2485 | 3620 |
| Without | 2497 | 2633 | 2539 | 2609 | 1475 |
| Δ Effect | 100 | - 172 | 17 | - 124 | 2145** |
| | | Cytosol Calcium μg/g nodule | | | |
| With | 247.6 | 235.9 | 315.1 | 237.1 | 249.4 |
| Without | 261.9 | 273.6 | 194.4 | 272.4 | 260.1 |
| Δ Effect | - 14.3 | - 37.7 | 120.7** | - 35.2 | - 10.7 |
| | | Cytosol Magnesium μg/g nodule | | | |
| With | 316.9 | 291.9 | 322.0 | 384.6 | 315.2 |
| Without | 277.4 | 302.5 | 272.4 | 209.7 | 279.1 |
| Δ Effect | 39.5 | - 10.6 | 49.6* | 174.9** | 36.1 |
| | | Cytosol Sodium μg/g nodule | | | |
| With | 331.2 | 372.5 | 367.6 | 366.3 | 163.3 |
| Without | 381.1 | 339.8 | 344.7 | 346.0 | 548.9 |
| Δ Effect | - 49.9* | 32.7 | 22.9 | 20.3 | - 385.6** |

Each figure is the pooled mean of all treatment combinations with or without the specified nutrient element. $P \leq$ .05* and .01**.

**TABLE 5. Principal composite effects of applied plant nutrients on growth, nodulation, and nodule enzyme activity levels at anthesis, WB-12-11 Siempre Winged Bean dark-red latosol (Typic Eutrustox) Jaíba, Minas Gerais, Brazil.**

| | P | S | Ca | Mg | K |
|---|---|---|---|---|---|
| Top growth, dry g/plant | | | | | |
| With | 1.18 | 0.99 | 1.04 | 0.95 | 1.02 |
| Without | 0.75 | 0.94 | 0.89 | 0.97 | 0.90 |
| Δ Effect | 0.43** | 0.05 | 0.16* | 0.02 | 0.12* |
| Nodule fresh wt. g/plant | | | | | |
| With | 1.09 | 0.99 | 1.05 | 0.97 | 1.32 |
| Without | 0.79 | 0.88 | 0.83 | 0.91 | 0.57 |
| Δ Effect | 0.30* | 0.11 | 0.22* | 0.06 | 0.75** |
| GOT U/g nodule | | | | | |
| With | 55.1 | 45.96 | 47.2 | 45.77 | 51.50 |
| Without | 33.1 | 42.21 | 41.1 | 42.21 | 36.66 |
| Δ Effect | 22.0** | 3.75 | 6.1 | 3.31 | 14.84** |
| GDH U/g nodule | | | | | |
| With | 2.94 | 2.72 | 2.63 | 2.60 | 2.54 |
| Without | 2.18 | 2.41 | 2.51 | 2.53 | 2.59 |
| Δ Effect | .76** | 0.31* | 0.12 | 0.07 | - 0.05 |
| GS U/g nodule | | | | | |
| With | 50.75 | 38.83 | 45.51 | 37.47 | 44.42 |
| Without | 26.67 | 38.59 | 34.1.1 | 39.90 | 33.00 |
| Δ Effect | 24.08** | 0.24 | 9.40* | - 2.43 | 11.42** |
| GOGAT U/g nodule | | | | | |
| With | 4.17 | 3.60 | 3.55 | 3.44 | 3.72 |
| Without | 2.18 | 2.80 | 2.82 | 2.93 | 2.64 |
| Δ Effect | 1.99** | 0.80 | 0.73 | 0.51 | 1.08* |

Each figure is the pooled mean of all treatment combinations with or without the specified nutrient element.

P≤ .05* and .01**.

**TABLE 6. Principal composite effects of applied plant nutrients on nodule components at anthesis, WB-12-11 Siempre Winged Bean, dark-red latosol (Typic Eutrustox) Jaiba, Minas Gerais, Brazil.**

| | P | S | Ca | Mg | K |
|---|---|---|---|---|---|
| | | | Nonstructural nodule carbohydrate mg/g nodule | | |
| With | 3.68 | 3.81 | 3.83 | 3.64 | 4.02 |
| Without | 3.66 | 3.54 | 3.52 | 3.70 | 3.32 |
| Δ Effect | 0.02 | 0.27 | 0.31* | -0.06 | 0.70* |
| | | | α Ketoglutarate μ moles/g nodule | | |
| With | 0.66 | 0.72 | 0.68 | 0.69 | 0.82 |
| Without | 0.68 | 0.62 | 0.66 | 0.64 | 0.52 |
| Δ Effect | - 0.02 | 0.10 | 0.02 | 0.05 | 0.30** |
| | | | Cytosol Phosphorus μg/g nodule | | |
| With | 316.7 | 228.6 | 225.3 | 248.2 | 250.5 |
| Without | 168.3 | 256.4 | 259.0 | 237.1 | 234.5 |
| Δ Effect | 148.4** | - 27.8 | - 33.7 | 11.1 | 16.0 |
| | | | Cytosol Potassium μg/nodule | | |
| With | 2593.8 | 2503.7 | 2615.2 | 2515.5 | 3614.0 |
| Without | 2523.9 | 2614.0 | 2504.8 | 2600.4 | 1503.6 |
| Δ Effect | 69.9 | - 110.3 | 110.4 | - 84.9 | 2110.4** |
| | | | Cytosol Calcium μg/nodule | | |
| With | 230.1 | 219.6 | 290.8 | 214.4 | 232.2 |
| Without | 241.8 | 252.3 | 183.4 | 256.6 | 239.7 |
| Δ Effect | - 11.7 | - 32.7 | 107.4** | - 42.2 | - 7.5 |
| | | | Cytosol Magnesium μg/g nodule | | |
| With | 288.1 | 267.2 | 292.4 | 353.4 | 289.6 |
| Without | 253.9 | 274.7 | 250.4 | 192.0 | 252.3 |
| Δ Effect | 34.2 | 7.5 | 42.0* | 161.4** | 37.3 |
| | | | Cytosol Sodium μg/g nodule | | |
| With | 315.0 | 363.5 | 358.6 | 353.0 | 169.3 |
| Without | 384.9 | 336.4 | 341.7 | 341.7 | 530.6 |
| Δ Effect | - 69.9* | 27.1 | 16.9 | 5.9 | 361.3** |

Each figure is the pooled mean of all treatment combinations with or without the specified nutrient element.
$P \leq$ .05* and .01**

**TABLE 7. Correlation coefficients with nodule enzyme components at anthesis, WB-21-8 Tinge and WB-12-11 Siempre Winged Bean cultivars.**

| WB-12-11 SIEMPRE CULTIVAR | WB-21-8 Tinge cultivar | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Top Wt. | Nod Wt. | Nase | GOT | GDH | GS | GOGAT | α KG | CHO |
| Top Wt. | | .164 | - .463** | - .704** | .365* | 650** | - .378* | - .738** | - .393* |
| Nod. Wt. | .065 | | .225 | - .366* | 381* | 230 | - .127 | - .106 | 034 |
| Nase | - .170 | 211 | | .379* | - .196 | - .342* | 252 | 597** | 463** |
| GOT | - .685** | - .132 | .170 | | - .383* | - .565** | .541** | .730** | .437** |
| GDH | .376* | .115 | - .117 | -.389* | | .521** | - .261 | - .420** | - .242 |
| GS | .663** | .091 | - .145 | - .578** | .536** | | - .495** | - .646** | - .237 |
| GOGAT | - .329* | - .088 | .067 | .557** | - .241 | - .463** | | .537** | .392 * |
| α KG | - .547** | - .003 | .594** | .573** | - .345* | - .458** | .391* | | .456** |
| CHO | - .375* | - .052 | - .198 | .469** | - .243 | .424** | .345* | .524** | |

Correlations were with top wt., dry g/plant; nod wt. g fresh wt./plant; nase nitrogenase μ moles $C_2H_4$/g nodule $hr^{-1}$; GOT, GDH, GS and GOGAT U/g fresh nodule; α KG μ moles/g fresh nodule; CHO nonstructural carbohydrate mg/g fresh nodule; P≤ .05* and .01**.

**TABLE 8. Correlations coefficients with nodule cytosol analyses at anthesis, WB-21-8 Tinge and WB-12-11 Siempre Winged Bean cultivars.**

| WB-21-8 SIEMPRE CULTIVAR | WB-21-8 Tinge cultivar | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Top Wt. | Nod Wt. | Nase | K | Na | Ca | Mg | P |
| Top Wt. | | .164 | - .463** | .160 | - .063 | .660** | 679** | 505** |
| Nod Wt. | .065 | | .225 | - .182 | - .235 | .208 | 293 | .496** |
| Nase | - .170 | 211 | | .320* | - .109 | .340* | .350* | .137 |
| K | .108 | .372* | .190 | | - .577** | .077 | - .029 | .043 |
| Na | - .043 | - .058 | - .031 | - .501** | | - .219 | - .184 | - .355* |
| Ca | .683** | .047 | - .115 | - .019 | - .281 | | .745** | .570** |
| Mg | .701** | .096 | - .128 | .012 | - .193 | .470** | | .776** |
| P | .536** | .103 | - .081 | .002 | - .253 | .517** | .663** | |

Correlations were with top wt., dry g/plant; Nod wt. g fresh wt/plant; nase nitrogenase μ moles $C_2H_4$/g nodule $hr^{-1}$; all nutrient elements as μg/g fresh nodule; P≤ .05* and .01**.

recognized as a limitation for symbiotic nitrogen fixation with edible beans (*Phaseolus vulgaris* L.) and pulses within the tropics (Carvalho et al. 1971, Graham & Rosas 1979).

The principal composite effects of applied plant nutrients on growth, nodulation and nodule components are presented in Tables 3 and 4 (Tinge) and Tables 5 and 6 (Siempre). Top growth of both cultivars was increased with high significance for phosphorus application. The Siempre cultivar also responded significantly to K and Ca applications.

Effects of applied phosphorus and potassium were highly significant for increased nodule mass per plant for Tinge, Table 3. Siempre, Table 5, was slightly less responsive to applied P but significantly increased nodule mass for Ca and K fertilization. Analogous to nitrogenase activity levels, nodule mass response to K usually diminished when P was a component of the applied plant nutrient combination treatments. Previous studies have reported the relationship of nodule mass and nitrogenase activity levels as influenced by available plant nutrients, particularly adequate soil potassium (Andrew & Robins 1969, Duke et al. 1980, Mengel et al. 1974 e Purcino 1979, 1980).

A significant response to applied P for enzyme activity levels associated with nitrogenase was apparent for both cultivars. The higher enzyme activity levels (except GDH) that resulted with applied K were not favorably influenced by P in the, treatment combinations. S significantly increased GDH activity levels in Siempre nodules and Tinge GOT activity levels were also significantly increased with Mg application. Apparently, Ca fertilization was required for increased GS activity levels with both cultivars.

Similar studies with nodule cytoplasm of twelve herbaceous legume species (Boland et al. 1978) and alfalfa (Duke et al. 1980) have proposed these fixed N ammonia assimilation pathways as apparently universal in legume nodule metabolism. Optimum soil environmental conditions were necessary, including soil fertility factors, for attaining the maximum enzyme activity levels. Other than nitrogenase, however, these enzyme pathways function within essential cell metabolism nonspecific for symbiotic N fixation (Mishustin et al. 1973). Thus, it may be difficult to evaluate their role strictly for N fixation.

Significance for increased nodule content of nonstructural cellular carbohydrates (CHO) was apparent with Ca and K for Siempre only. However, αKG levels increased with applied K for both cultivars. This was of particular interest because of the key substrate role of αKG for enzyme pathways involved with biosynthesis of nitrogenase fixed nitrogen as ammonia to glutamate and subsequent transferases to other amino acid components (Figure 2). Other studies have demonstrated that bacteroids, "symbiotic cells" of *Thizobium japonicum* did not utilize glucose, fructose, or sucrose, but readily oxidized citric acid cycle intermediates: αKG, succinate, fumarate, malate, and oxalacetate (Mishustin et al. 1973, Purcino 1980 e Tuzimura & Meguro 1960).

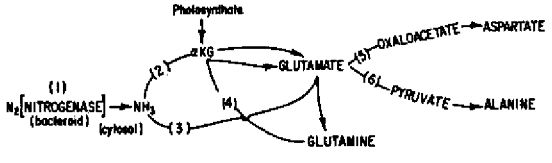

FIG. 2. A schematic composite of enzymatic pathways apparent for symbiotic nitrogen fixation-assimilation within legume nodules.

1. Nitrogenase (Nase (EC. 1.7.99.2)
2. Glutamate dehydrogenase (GDH) - (L-glutamate: NAD(P)$^+$ oxidoreductase deaminating EC 1.4.1.3)
3. Glutamine synthetase (GS) L-glutamate: ammonia ligase (ADP), EC 6.3.1.2)
4. Glutamate Synthetase (GOGAT) (L-glutamate: NAD(P)$^+$ oxidoreductase (transaminating), EC 1.4.1.13)
5. Glutamate-oxaloacetate transaminase (GOT) (L-apartate: 2-oxoglutarate aminotransfrase, EC 2.6.1.1)
6. Glutamate-pyruvate transaminase (GPT) L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2)

αKG alpha ketoglutarate (2-oxoglutarate)

In general, the elemental composition of nodule cytosol was increased for the applied plant nutrient element with both cultivars. Mg favorably influenced increased cytosol Ca although an inverse influence was apparent among many of cytosol components. A highly significant retroversion influence was apparent with the monovalent cations K and Na. This inverse relationship was consistent for all differential applied K treatment combinations for both cultivars. The significant negative influence of Na to P content occurred in these studies but with less consistence than the K x Na inversion interactions.

The complex interrelationships among and between the parameters determined for both cultivars were evaluated with all possible correlations and presented in Tables 7 and 8. In general, the negative or positive correlations were similar for both Tinge and Siempre in these studies. Contrasts were apparent for several parameters including nitrogenase activity levels (Table 7). For Tinge, significant correlations included nitrogenase (Nase) x GOT, negative Nase x GS, Nase x αKG, Nase x CHO (nonstructural nodule carbohydrate), and negative Nase x dry top weight. A highly significant nitrogenase correlation with αKG, was apparent for Siempre.

Significant correlations of particular interest that assay interrelations for enzyme pathways proposed within nodule cytosol (Figure 2), that were prevalent for both cultivars include: top wt. x GDH, GOGAT x CHO, negative top wt. x GOGAT, negative top wt. x CHO, negative GOT x GDH, and negative GDH x αKG. Highly significant correlations include top wt x GS, negative top wt. x GOT, negative top wt. x αKG, negative GOT x GS, negative GS x αKG, GOT x GOGAT, GOT x αKG, GDH x GS, GOGAT x α KG, and αKG x CHO.

The principal soil base cation plant nutrients were apparently similar in effect for both cultivars (Table 8). Highly significant correlations included: top wt. x Ca, top wt. x Mg, top wt. x P, negative K x Na, Ca x Mg, Ca x P, and Mg x P. Significant correlation was apparent with nodule wt. x P and Nase x K for Tinge, and with nodule wt. x K for Siempre.

The correlations, as shown in Tables 7 and 8, were useful for initial estimates that are necessary for independent variable contributions with multiple regression. The predictive equations of models with the most reliable response surfaces were attained with stepwise regression. The independent variables were evaluated in various combination and sequence, and then tested by backward elimination with each computed as the last contributing variable with the model. The nonsignificant and overfitting parameters were progressively excluded to yield the following:

$$\text{Tinge Nase} = 69.51\,\alpha KG + 2.78\,\text{nod wt} + .035\,K + .052\,GOT, \quad (1)$$
$$R^2 = .664,\ CV = 32.7\%$$

$$\text{Tinge GOT} = 19.53\alpha KG + 0.12\,\text{Nase} + .018\,K + .033\,GS + 8.73\,GOGAT \quad (2)$$
$$R^2 = .804,\ CV = 18.5\%$$

$$\text{Siempre Nase} = 30.14\,\alpha KG + 2.30\,\text{nod wt} + .025\,K + .019\,GOT \quad (3)$$
$$R^2 = .776,\ CV = 26.8\%$$

$$\text{Siempre GOT} = 31.42\,\alpha\,KG + .056\,\text{Nase} + .035\,K + .111\,GS + 7.15\,GOGAT \quad (4)$$
$$R^2 = .823,\ CV = 18.9\%$$

The nitrogenase (Nase) equations represent the "forward" reaction equation for best fit surface. The best estimates for terminal reaction with cognate parameters as highly significant independent variables were determined for the dominant transaminase GOT activity levels (Figure 2) as a "backward" criteria.

Multiple regression equations for both cultivars were similar. An interpretation of these data should recognize an apparent influential role of nodule cytosol $\alpha$KG and favorable K levels as essential for the entire enzyme sequence schematically briefed in Figure 2.

Both dependent nitrogenase (Nase) multiple regression equations (1) and (3) were highly significant with $R^2$ . 664 (Tinge) and $R^2$ .776 (Siempre). Cytosol $\alpha$Kg was the dominant component factor followed by fresh nodule wt, cytosol K and nodule GOT activity levels. The dependant GOT multiple regressions (2) and (4) were highly significant with $R^2$ .804 (Tinge) and $R^2$ .823 (Siempre). Cytosol $\alpha$KG was the principal independent factor followed by nitrogenase activity level(Nase, $C_2H_2$ reduction), cytosol K, GS and GOGAT activity levels.

Two nodule parameters, Lowry soluble protein and alanine aminotransferase (GPT), were nonsignificant within all statistical analyses and were excluded in this report in an effort to simplify the data summaries. Cytosol GPT activity levels were low, $\leq 1.0$ U/g nodule, with no statistical association to treatment. Other research reports (Duke et al. 1980, Shapiro & Stadtman 1970), have emphasized the incapacity of soluble protein levels to discern active (taut) and nonactivated (relaxed) enzyme fractions for specific activity/mg protein determinations.

Practical attributes from these studies corroborate the requirement for adequate available soil potassium and phosphorus for high levels of nitrogenase activity. Effect of soil calcium levels may be influenced by the physiology of different winged bean cultivars. High levels of sodium apparently are inhibitory to metabolic functions of K and result in depressed nitrogenase activity. Enzyme activity levels and nutrient components within nodule cytosol are indicative for an associated nitrogenase activity and provide diagnostic criteria for improved symbiotic nitrogen fixation.

## REFERENCES

ANDREW, C.S. & ROBINS, M.F. The effect of potassium on the growth and chemical composition of some tropical and temperate pasture legumes. II. Potassium, calcium, magnesium, sodium, nitrogen, phosphorus and chlorine. **Aust. J. Agric. Res., 20:** 1009-21, 1969.

BERGMEYER, H.U. & BERNT, E. Glutamate-oxaloacetate transaminase, UV assay, manual method. In: BERGMEYER, H.U. **Methods of enzymatic analysis.** 2.ed. Verlag Chemie Weinheim, Academic Press, Inc., 1974a. v.2. p.728.33.

BERGMEYER, H.U. & BERNT, E. Glutamate-pyruvate transaminase, UV assay, manual method. In: BERGMEYER, H.U. **Methods of enzymatic analysis.** 2.ed. Verlag Chemie Weinheim Academic Press, Inc., 1974b. v.2. p.752-8.

BERGMEYER, H.U. & BERNT, E. 2-oxoglutarate, UV spectrophotometric determination. In: BERGMEYER, H.U. **Methods of enzymatic analysis.** 2.ed. Verlag Chemie Weinheim Academic Press, Inc., 1974c. v.3. p.1577-80.

BOLAND, M.J.; FORDYCE, A.M. & GREENWOOD, R.M. Enzymes of nitrogen metabolism in legume nodules: a comparative study. **Aust. J. Plant Physiol. 5:**553-9, 1978.

CARVALHO, M.M. de.; FRANÇA, G.E.; BAHIA FILHO, A.F.C. & MOZZER, O.L. Ensaio exploratório de fertilização de seis leguminosas tropicais em um latossolo vermelho escuro, fase mata. **Pesq. agropec. bras., Sér. Agron., 6**(Único):285-90, 1971.

DUKE, S.H.; COLLINS, M. & SOBERALSKE, R.M. Effects of potassium fertilization on nitrogen fixation and nodule enzymes of nitrogen metabolism in alfalfa. **Crop Sci., 20:**213-9, 1980.

EMPRESA DE PESQUISA AGROPECUÁRIA DE MINAS GERAIS, Belo Horizonte, MG. **Levantamento de reconhecimento com detalhes dos solos do Distrito Agroindustrial de Jaíba-Minas Gerais.** EPAMIG/EMBRAPA/Ruralminas, 1976. (Boletim Técnico, 54).

GRAHAM, P.H. & ROSAS, J.C. Phosphorus fertilization and symbiotic nitrogen fixation in common bean. **Agron, J.,** 71:925-6, 1979.

HARDING, J.; LUGO-LOPEZ, M.A. & PEREZ-ESCOLAR, R. Promiscuous root nodulation of winged beans on a oxisol in Puerto Rico. **Trop. Agric.** Trinidad, 55:315-24, 1978.

HYMOWITZ, T. & BOYD, J. Origin, ethnobotany and agricultural potential of the winged bean (*Psophocarpus tetragonolobus*). **Econ. Bot.,** 31: 180-8, 1977.

LOWRY, O.N.; NIRA, J.; ROSEMBROUGH, A.; FARR, A.L. & RANDALL, R.J. Protein measurement with the Folin-phenol reagent. **J. Biol. Chem.,** 193:265-75, 1951.

MASEFIELD, G.B. Root nodulation and agricultural potential of the leguminous genus *Phophocarpus*. **Trop. Agric.** Trinidad, 38:225-9, 1961.

MENGEL, K.; HAGHPARAST, M.R. & KOCH, K. The effect of potassium on the fixation of molecular nitrogen by root nodules of *Vicia faba*. **Plant Physiol.,** 54:535-8, 1974.

MISHUSTIN, E.N.; KALNISKAYA, T.A. & SHEMATHANOVA, N.M. Fixation of molecular nitrogen by microorganisms. **Investiga. Akad. Nauk. Ser. Biologiya,** 6:779-95, 1973.

PURCINO, A.A.C. **Nitrogenase, nodule enzymes and carbohydrate components of Copada,** *(Cratylia floribunda,* **Benth)** **related to soil fertility effects on regrowth vigor and nodulation with an oxisol of Brazil.** Oklahoma State University, Stillwater, 1980. Tese Doutorado.

PURCINO, H.M.A. **Effect of soil fertility tratments on growth and nodule parameters of Winged Bean (***Psophocarpus tetragonolobus* **(L) DC) With a dark-red latosol (typic Eutrustox) from Jaíba, Minas Gerais, Brazil.** Oklahoma State University, Stillwater, 1979. Tese Mestrado.

SCHMIDT, E. Glutamate dehydrogenase. UV assay. In: BERGMEYER, H.U. **Methods of enzymatic analysis.** 2.ed. Verlag Chemie Weinheim, Academic Press, Inc., 1974. p.650-6. v.2.

SHAPIRO, B.M. & STADTMAN, E.R. Glutamine synthetase (*Escherichia coli*). In: TABOR, H.& TABOR, C.W. **Methods in enzymology.** New York, Academic Press, Inc., 1970. v.17 p.910-22.

SMITH, D. **Removing and analyzing total nonstructural carbohydrates from plant tissue.** s.l., Wis. Agric. Exp. Stn. Res., 1969. 11p. (Rep. 41).

TRINICK, M.J.; DILLWORTH, M.J. & GROUNDS, M. Factors affecting the reduction of acetylene by root nodules of *Lupinus species*. **New Phytol.,** 77:359-70, 1976.

TUZIMURA, K. & MEGURO, H. Respiration substrate of Rhizobium in the nodules. **J. Biochem.,** 47:391-7, 1960.

VANCE, C.P.; HEICHEL, G.H.; BARNES, D.K.; BRYAN, J.W. & JOHNSON, L.E. Nitrogen fixation, nodule development, and vegetative regrowth of alfalfa (*Medicago sativa*, L.) following harvest. **Plant Physiol.,** 64:1-8, 1979.