UNIVERSITY OF GHANA, LEGON.

ASSESSING THE GROWTH, NODULATION AND NITROGEN FIXING POTENTIAL OF SOME MULTIPURPOSE TREES AND SHRUBS

BY

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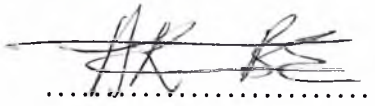
DEDICATION

I dedicate this work to Apostle Dr. Kwadwo Safo and my parents, Mr. and Mrs. A. K. Boakye.

DECLARATION

I hereby declare that except for references to the literature and to the work of other researchers, which have been duly cited herein, this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.



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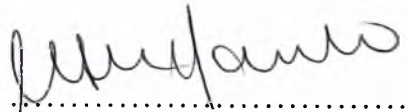

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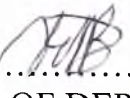

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ABSTRACT

This study was conducted to assess the growth, nodulation and nitrogen fixing potential of some native multipurpose trees and shrubs.

A total of fourteen species of indigenous leguminous trees and shrubs were initially screened for nodulation in three Ghanaian soils (Toje, Hatso and Alajo soil series), after 8 weeks of growth in nursery bags. The experiment was repeated with nine species of the tree/shrub legumes, this time under two levels of phosphorus (0 and 60 mg P/kg soil). The final study involved four of the tree/shrub legume species, which were assessed for both inoculation and phosphorus responses, and abilities to fix nitrogen as assessed by the ^{15}N Isotope dilution method.

Nodulation of the tree/shrub legumes by native rhizobia was highly variable, with five of the tree/shrub species, namely, *Albizia lebbek, Sesbania aculiata, Pithecelobium spp, Tephrosia spp* and *Acacia farresiana* being nodulated by native rhizobia in all three soils. In contrast, the following six tree legumes, *Acacia adianthifolia, Albizia zygia, Acacia mangium, Senna occidentalis, Cassia occidentalis* and *Tamarindus indica* did not form any nodule in any of the three soils. The presence of indigenous *Rhizobium* in all three soils, capable of nodulating *A. lebbek, Pithecelobium spp*, and *Tephrosia spp* was confirmed in a final study with four leguminous trees and shrubs; unioculated *Leucaena spp* however nodulated only in Toje soil. The number of rhizobia counted by the most probable number (MPN) method for the four legumes in the three soils ranged from 31/g soil to 1700/g soil. The populations of native *Rhizobium/g* soil capable of nodulating each of these four legumes were found to be highest in Alajo soil (mean 9.95 $x10^2$) while the least *Rhizobium* counts occurred in Hatso soil (mean 1.10 $x10^2$).

Rhizobium isolates obtained from *Tephrosia spp* were found to be most promiscuous, and except for *Leucaena spp*, isolates from *Tephrosia spp* nodulated the two other tree species (i. e. *A. lebbek,and Pithecelobium spp*), in contrast to isolates from *A. lebbek* and *Pithecelobium spp* which were found to be specific only for their respective host plants. Phosphorus application alone resulted in significantly improved nodulation (on average about 63%). *Sesbania specioca, S. Aculiata* and *A. farresiana* did not nodulate with the indigenous rhizobia without phosphorus in Toje soil but did nodulate after phosphorus application. Similarly, *S. rostrata* and *Leucaena spp* nodulated in Hatso and Alajo soils, only after phosphorus application. Despite the general response by the trees to phosphorus, some species, like *A. lebbek, S. aculiata* and *Tephrosia spp* did not respond significantly to phosphorus application on Alajo soil. Phosphorus application, however did not result in significant increase in both %N fixed (about 38%) and total N (about 44 mg) fixed. Also, with the exception of Tephrosia spp, P application did not result in significant increase in total dry matter yield of the tree legumes. Although inoculation resulted in more than double nodule numbers of the tree species, it did not result in significant increase in both total N fixed and %N fixed. It also did not significantly increase total dry matter yield except in the case of *Tephrosia spp*. Leucaena responded highest to inoculation with an increase of over 123% total N fixed. In general however, *Tephrosia spp* gave the highest Biological Nitrogen Fixation (BNF) followed by *Pithecelobium spp* and *A. lebbek* with the lowest being Leucaena in terms of both percent and total N fixed. These studies therefore identified *Tephrosia spp* as having high potential for both dry matter yield and nitrogen fixation. Because of the high numbers of native rhizobia present in the soils studied, *Tephrosia spp* stands a good chance of being used for nitrogen recycling.

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CHAPTER ONE

1.1 INTRODUCTION

Ghana is richly endowed with forest and savannas which provide timber for its wood industries, building materials for construction, fuel wood (which is the most important source of energy in rural areas) and minor forest products like oils, medicine, chewing- sticks etc. They provide sanctuary for wildlife, protect and enrich the soils, maintain and regulate stream flow and preserve the genetic diversity of the flora and fauna. These forests and savannas have to be protected and used rationally if they are to continue to provide the above functions. These renewal natural resources are, however, being depleted at an alarming rate through certain farming practices like shifting cultivation and bush fallow as well as other human related activities like indiscriminate and sometimes unplanned commercial logging of timber, excessive harvesting of fuel wood, bush fires, overgrazing etc.

For instance, the dominant farming system in Ghana today is based on shifting cultivation and bush fallow practices where the soil had to be rested for many years for its fertility to be restored, after few years of cropping. These practices have led to increased deforestation. According to F.A.O. 1982, the tropics are

loosing more than 10 million hectares of forest cover annually and shifting cultivation is responsible for almost 70% of deforestation in tropical Africa. The situation is alarming in tropical Africa where deforestation exceeds the projected rate of tree planting by a ratio of 29% (FAO,1982). This problem has been aggravated by increase in land pressure due to increase in population which has let to shortening of the fallow period needed to restore soil fertility in the traditional farming systems. Continuous cultivation (in response to increasing population) without much fertilizer addition to soil has led to drastically reduced soil fertility and increased soil erosion in the humid tropics and the semi-arid areas (EL-Ashry, and Ram,1987). This is compounded by the fact that most soils in humid tropical Africa are sandy, highly weathered, low in organic matter content and susceptible to soil erosion and compaction (Lal, 1987). In drier areas, overgrazing and harvesting of trees for firewood have been major factors in reduced productivity, increased soil erosion and desertification (F.A.O 1982). In order to reverse the present trend, there is the need to reduce the rate of population growth, introduced settled agriculture, develop alternative sources of energy and embark on serious tree planting programmes.

Attempts to improve the productivity of traditional farming systems in Ghana, by introducing inorganic fertilizers have proved futile, mainly, because of the inability of poor farmers to afford the prices of these fertilizer as a results of the removal of subsidies by the government. The effective management of biological nitrogen fixing trees and other leguminous plants in our farming systems is a major key to restoring and maintaining soil fertility (Kang *et al.*, 1981a.).

In recent years, the potential of leguminous and actinorhizal trees for restoring and maintaining soil fertility has aroused considerable interest and international agencies (e.g. CSC, IITA, IAEA, NAS,ACIAR, IDRC etc) have recommended their increased use in agroforestry systems and for crop shade. The integration of trees, especially nitrogen fixing trees into agroforestry systems can make a major contribution to low input sustainable agriculture by restoring and maintaining soil fertility and in combating erosion and desertification as well as providing the needed firewood for domestic use. The major advantages of nitrogen fixing trees are their ability to establish in nitrogen deficient soils, and the benefits of the nitrogen fixed and extra organic matter to succeeding or associated crops. Sanginga *et al*, 1986 found that maize yields in soil in which

inoculated *Leucaena spp* had grown for 6 months, were increased from 1.5t/ha to 2.5t/ha with prunings removed and from 2.2 to 4t/ha with prunings returned to the soil. Ladha *et al.*(1989) indicated that, nitrogen fixed by *Sesbania rostrata* can significantly increase subsequent grain yield of lowland rice.

It has been assumed over the years that, all leguminous trees fix nitrogen and are therefore suitable for integration into agroforestry systems . Although nodules are important for nitrogen fixation in legumes, till now, many leguminous trees have not been examined for nodulation and the need to inoculate with rhizobia for enhanced nodule formation (Allen and Allen,1981). Recent preliminary studies (Ampadu and Danso personal communication) have shown that there was no nodulation in "Dawadawa" and *Tetrapleura tetraptera*. With the exception of some species such as *Leucaena leucocephela* which have been reported to fix large amounts of nitrogen in Hawaii (Guevarra, 1976) and in Nigeria (Kang *et al.*, 1981(a) our knowledge of nitrogen fixation in trees is still very limited, there is therefore the urgent need for more studies on nitrogen fixing trees especially in developing countries like Ghana. In this study, 14 species of

some common indigenous leguminous trees would be assessed for their growth, nodulation and nitrogen fixing ability on three (3) different soil types that occur in the coastal savanna zone.

The objectives of this study are:

To investigate the growth, nodulation and nitrogen fixing potential of some common indigenous multipurpose trees.

To assess the need for inoculation for improving nitrogen Fixation in various leguminous trees of potential use in agroforestry and forestry.

To select leguminous tree species with capacity for use in agroforestry and forestry.

To isolate rhizobia likely to be of use in the preparation of inoculants.

To test whether nodulation of indigenous nitrogen fixing trees (NFT's) can be improved by phosphorus fertilization.

1.1.2 Justification: Available literature shows that little work has been done on biological nitrogen fixation in trees. It is to fill this gab as well as towards improving and sustaining

soil fertility and productivity for food sufficiency in Ghana that this study is being proposed.

CHAPTER TWO

LITERATURE REVIEW.

2.1 NITROGEN FIXING TREES.

Nitrogen fixing trees fall in two main groups, the nodulated legumes and actinorhizal trees. The family leguminosae is divided into three sub-families, the papilionoideae (Pea-like flowers), the Mimosoideae (compound inflorescence with reduced petals) and the Caesalpinoideae (flowers usually with five petals, apparently radially symmetrical)(Polhill and Raven, 1981). The majority of trees used for agroforestry are nodulated members of the papilionoideae and the mimosoideae, but there are few species in the caesalpinoideae many of which do not form nodules but which have been used experimentally in agroforestry (Allen, *et al.*, 1981).

2.1.1 Nodulated Trees Legume For Agroforestry.

The most commonly used nodulated tree legumes in agroforestry include: *(a) Acacia spp (b) Leucaena spp (c) Faidherbia spp (d) Prosopis spp (e) Calliandra spp (f) Gliricidia spp and (g) Sesbania spp.*

2.1.1.1 *Acacia spp.* (Mimosoideae)

Acacia is the largest and most divers genus of legume trees, containing approximately 1200 species which are distributed throughout the tropics and subtropics (Brewbaker, 1987). Most species are found in the semi-arid tropics and they are generally resistant to drought, due at least in part, to having very deep root systems. Species of *Acacia* noted for their potential or actual use in agroforestry include; *A. Senegal*, *A. mearnsii*, *A. aneura*, *A. auriculiformis*, *A. holoserica*, *A. mangium* and *A. tortillis*. Turnbull, (1987) reported many additional species that occur naturally in Australia and South-east Asia which could be of widespread use in arid regions of the tropics.

2.1.1.2 Leucaena (Mimosoideae)

This genus of leguminous trees consist of 13 species of which *L. leucocephela, L. diversifolia* and *L. esculenta* have excellent agroforestry potential (Pound and Martinez-Cairo, 1983; Anon, 1984a). The strong, deep root system allows *Leucaena* to tolerate drought and, once established, it can survive in areas with only 600mm annual rainfall. One of the major limitations of *L. leucocephela* is its poor growth on acid soils although improvement of acidity tolerance has been achieved by crossing with acid-tolerant accessions of *L. diversifolia* (Hutton, 1990). *Leucaena* is nodulated by fast-growing rhizobia isolated for several tropical hosts but generally not by most temperate *Rhizobium* sepcies or by slow-growing rhizobia (Trinick, 1996; Lewin *et al.*, 1977)

2.1.1.3 *Faidherbia* (Mimosoideae)

The only species in this genus is *Faidherbia albida* and is found throughout the drier regions of sub-Saharan Africa (Wickens, 1969). Its major characteristic is the unusual shedding of its leaves during raining season. *F. albida* forms nodules with *Bradyrhizobium* strains but not with

the fast-growing *rhizobia* strains tested so far (Dreyfus and Dommergues, 1981).

2.1.1.4 *Prosopis* (Mimosoideae)

All the 44 species of this genus are found in semi-arid and arid regions throughout the tropics (Anon, 1979). Most of the species are of potential use in agroforestry because of their ability to withstand extreme drought, and the fact that their pods are an important animal fodder in many regions (Felker, 1979). *Prosopis spp* nodulated by fast-and slow-growing rhizobia (Jenkinson *et al.*, 1987).

2.1.1.6 *Calliandra* (Mimosoideae)

This genus has over hundred species which occur in Central and South America. *Calliandra* is widely used for fuel wood and animal fodder. It is best suited to growth in humid areas with annual rainfall of 2000-4000mm on soils with good drainage as waterlogging can rapidly kill the trees. *Calliandra* nodulates rapidly with indigenous *Rhizobium* strains in soils in

Java and is nodulated by both fast-and slow growing strains (Dreyfus and Dommergues,1981).

***Gliricidia spp* (papilionoideae)**

The widely known species of this genus of agricultural use is the *Gliricidia sepium*. The species can form nodules with both fast-and slow-growing rhizobia and was found to fix N_2 most effectively with fast-growing strains originally isolated for *Gliricidia* nodules (Akkasaeng *et al.*, 1986).

2.1.1.7 *Sesbania spp* (Papilionoideae).

The over 60 species of this genus are distributed throughout the tropics with representatives to all continents (Evans and Rotar, 1987; Evans, 1970) and vary from annual to short-lived perennial. Several species including *S. bispinosa, S. cannabina, S. sesban, S. grandiflora, S. specioca,* and *S. rostrata* have traditionally been investigated for use in agroforestry. A major advantage of *Sesbania* is that many species are both tolerant of waterlogging and of saline and alkaline conditions. The stem nodulating

species *S. rostrata* has excited intense interest due to its ability to grow and fix N_2 in waterlogged conditions and its fast growth rate.

Rhizobia nodulating roots of *Sesbania spp* are generally fast-growing strains, and often exhibit rapid growth, produce large (74mm) colonies in less than 24hours, although a few slow-growing isolates have been recorded (Odee, 1990).

2.1.2 Actinorhizal Trees For Agroforestry.

Species of *Casuarina* are the most important non-leguminous N_2-fixing trees in the lowland tropics. Four genera are now recognised within the *Casuarinaceae* and include; *Allocasuarina, Casuarina, Gymnostoma*, and *Ceuthostoma* which together contain about 90 species native to Australia, Malaysia and Polynesia (Diem and Dommergues 1990). The most widely-used species is *C. equisetifolia* which is tolerant of saline soils and of strong winds, and two other species quite widely-cultivated are *C. cunninghamiana* and *C. glauca* (Anon, 1984b)

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Marked differences in effectiveness of N_2 –fixation have been found both between different strains of Frankia (Reddell and Bowen, 1985) and between different accessions of *Casuarina* (Sanginga *et al.*, 1990a)

2.2 THE ROLE OF TREES IN AGROFORESTRY

Alley cropping, also referred to as hedgerow inter-cropping (Torquebiau, 1990) entails managing rows of woody plants (hedgerows) with annual crops planted in the alleys between the hedgerows.

The most distinctive component of an agroforestry practice is the multipurpose trees and shrubs (MPTS) (Nair, 1990). Multipurpose trees are all woody perennials that are purposefully grown to provide more than one significant contribution to the production and/or service functions of a land use system. They are so classified according to the attributes of the plant species as well as the plant's functional role in the agroforestry practice under consideration (Huxley, 1984).

Soil improvements under alley cropping may result from the service function as well as the protective role of MPTS as influenced by management and environmental factors. Nair (1990) has enumerated some

points which support the soil improving capabilities of trees and shrubs under natural ecosystems. Even though there are reports of investigations conducted by Kang and Wilson (1987) Sanchez (1987), Juo (1989) and Avery *et al.* (1990) on alley cropping's role in soil improvements, the results are not quite conclusive. However, Nair (1990) expresses the hope that, given the substantial volume of scientific information on the soil improving attributes of trees, it is a matter of management to realize the significant contribution that tree-based systems like alley cropping can make to soil fertility and overall soil productivity.

Characteristics that make MPT desirable for soil improvement under alley cropping have not been adequately elucidated. However, some guidelines are offered by Young (1989a) and Kang *et al.*, (1984). These properties include high biomass production (below and above ground), nitrogen fixation and mycorrhiza association, a well-developed rooting system and rapid growth and ability to coppice. The other properties are fast or moderate rate of litter decay to suite either nutrient release or soil erosion control, high nutrient content in the biomass and absences of toxic substances in foliage or root exudates.

Young (1989a) has provided a list of 32genera and 55 species which have been identified to be beneficial for the maintenance or improvement of soil fertility from several sources. Included in the lists are species such as *Albizia lebbeck, Gliricidia sepium, Leucaena leucocephela, Cajanus cajan, Dactyladenia barterii, Alchornea cordiflora*, among others which have been used in hedgerow intercropping trials in the tropics. The possible contributions of the biological nitrogen fixing species among the list should be cherished, especially in most tropical countries where inorganic nitrogen fertilizers are expensive.

Amara (1987a, 1987b) has provided a list of potential trees for use in agroforestry systems. Of importance are *Albizia lebbek, C. siamea, E. cyclocarpum, G. sepium and L. leucocephela.*

2.3 ATTRIBUTES OF *LEUCAENA* AS A MULTI-PURPOSE TREE

Leucaena leucocephela seems to have taken the lead among other nitrogen fixing trees (NFTs) in agroforestry systems. (Brewbaker, 1975; Young, 1989a). It is considered to be the most widely used MPT in scientific

agroforestry (Young, 1989a). *Leucaena* is an arborescent legume belonging to the mimosaceae family. Native to Mexico, leucaena is now pantropic. It is said to thrive better on moderately free draining, alkaline to neutral and light to heary soils. (RAPA, 1987; F/FRED, 1994). There is no unanimity on its annual rainfall requirements and dry season tolerance. However, it is generally accepted that water logging conditions do not favour its growth (GAPA, 1987; F/FRED, 1994; Young, 1989a)

The extensive root system of leucaena partly accounts for its soil improving properties. Dijkman (1950) associated leucaena with deeply penetrating tap root and lateral roots enabling them to exploit phosphorus and other minerals for the plants use (Brewbaker, 1975). The level of nitrogen in the leaves reported as 2.5 – 4.0% (Agboola, 1982; Buck, 1986) makes leucaena a good nitrogen and protein source for crops and livestock respectively. The high biomass production of leucaena is one of its attractive characteristics as a MPT. Young (1989a) quotes 10,000 – 25,000 kg/ha/yr as the biomass production by leucaena. This figure compares favourably with the value of 20,000 kg/ha/yr which is the average estimate of evergreen rainforest biomass production (Nair, 1990). In savannah ecosystems, biomass production rates of 10,000 kg/ha/yr and 5,000

kg/ha/yr have been reported for moist and dry savannah, respectively (Young, 1989a).

Agboola (1982), working in a bimodal moist sub – humid area in Nigeria, reported a leaf biomass production of 2470kgDM/ha/yr for leucaena under alley cropping. Figures for *Cajanus cajan* and *Tephrosia candida* under the same conditions were 4100 and 3070 kg MD/ha/yr respectively (Agboola, 1982). While biomass production on the whole is an indicator of good MPT, the plant parts that go into the soil to maintain organic matter status, that is, leaves and roots are of paramount importance in alley cropping. Young (1989a) has provided some estimates of the amounts of above ground dry matter that need to be added to the soil to maintain soil organic matter content in three climatic zones of the tropics. While these figures did not include root biomass which can make immense contribution of organic inputs to the soil, they included woody components which may be harvested and taken out of the soil.

Another interesting attribute of leucaena is the pattern of roots distribution. Earlier reports on the root system of leuceana seemed to have assumed the

"commonality" of all trees as deep rooted. Working on a Sandy loam soil in Tanzania, Johnssen et al. (1988) found that, the pattern of root distribution in leucaena was similar to that of maize. Other workers like Ong (1991) and Noordwijk *et al.* (1990) have reported the superficial nature of roots in leucaena and other MPTs.

The high nutritive value of green and dry forage of leucaena is widely acclaimed (Brewbaker, 1975; RAPA, 1987). However, it has been found that when non-runinants are fed more than 5% (dry weight) of their ration, thyroid problems and other side effects are noticed (Brewbaker, 1975)

2.4 NITROGEN CONTRIBUTION BY SOME MULTIPURPOSE TREES (MPTs) TO CROPS

Prunings of leguminous hedgerows have been shown to increase yields of various crops. This is partly attributable to their N contribution (Guevarra, 1976; Kang *et al.*, 1984; Widyanatha, 1984; Yamoah, *et al.*, 1986; Kang, *et al.*, 1989; Sangakkara, 1989). For instance, trials conducted at IITA in Ibadan in Nigeria have shown that prunings of the leguminous species

Cassia siamea, Gliricidia sepium and *Leucaena leucocephala* can increase crop yields and improve soil properties (Yamoah *et al.*, 1986a; Kang and Wilson, 1987). The effective N contribution by the hedgerows to the associated crops in alley cropping can be calculated as the difference between N content of alley cropped plants and those grown in the control treatment (with no hedgerows). This method of estimation, however, ignores the priming effect that can occur after the application of green manures (Herridge and Bergersen, 1988). Most precise data on contribution from the hedgerow prunings to the associated crops can be obtained by using N-labelled plant material. A wide range of estimated N contribution values from hedgerow prunings to associated crops have been reported. Mulongoy (1986) has reported of an N contribution of 38-43kgN/ha with the application of 3-4t/ha of *Sesbania rostrata* prunings. Kang (1988)) estimated effective N contribution from *L. leucocephela* and *G. sepium* prunings to alley cropped maize to be about 40kg N/ha. On the other hand, Mulongoy and Van der Meersch (1988) reported a lower N contribution of 4.4 – 23.8kgN/ha from *L. leucocephela* prunings to the associated maize crop. The N contribution in Mulongoy and Van der Meersch (1988) represents less than 30% of the N yield of the prunings.

This low efficiency in the crop's use of N from prunings probably results from the lack of synchronization between N release from the prunings and plant N demand, volatilization loss of N from prunings, and leaching loss (Mulongoy and Akobundum , 1990). Danso *et al.* (1996) indicated that, although N content from prunings of *Gliricidia sepium, Senna siamea* and *Gmelina arberea* were high, their N contribution to associate crop were low. They indicated that, the amount of nitrogen contributed to rice by the prunings of *Gliricidia sepium, Senna siamea* and *Gmelina arborea* was 20.4, 1.6 and 2.7kg N, respectively. This, on the average is equivalent to 25%, 7% and 8% of the crop's N met by the prunings. For cowpea grown in rotation with rice, Danso *et al.* reported that *Gliricidia sepium, Senna siamea* and *Gmelina arborea* contributed 2.3, 0.6 and 1.8kg N, respectively, corresponding to 12%, 5% and 10% of the crop's N obtained from prunings.

In most alley cropping systems, more than 70% of N in prunings is unaccounted for (Sanginga *et al.*, 1993). Some of the possible causes suggested are losses due to volatilization and leaching, denitrification, N retained in the soil organic matter or recovered by hedgerow trees and by

weeds. Research results have shown that N yield is highly correlated with pruning biomass yield. There are a number of management factors which affect the N yield of the hedgerow prunings. These factors include height and frequency of prunings (Duguma *et al.* 1988), Plant density and inter-hedgerow spacing (Kang *et al*, 1989) and Timing of pruning during cropping season.

Duguma *et al.* (1988) showed that low pruning height and high pruning frequency of *Leucaena leucocephela, Gliricidia sepium* and *Sesbania grandiflora*, gave lower N yield, where as high pruning height and low pruning frequency gave higher N yield and also increased wood production.

2.5 SOME NITROGEN FIXATION MEASUREMENTS IN NITROGEN FIXING TREES.

Several studies done using MPTs have shown that on the average, well nodulated woody legumes can fix nitrogen amounting to 134-274 kg N/ha/yr in the field using ^{15}N methodology. This represents an average of

45% of their total N content (Mulongoy, 1986; Herridge and Bergersen, 1988; Mulongoy *et al.*, 1988; Sanginga *et al.*, 1989)

2.5.1 *LEUCAENA spp*

Sanginga *et al.* (1989a) reported an amount of 133kgN/ha fixed by *Leucaena leucocephela* grown for 6 months in the field in Nigeria; this represents only 39% of the N in the plant, indicating that more than half of the N in leucaena was of soil origin. A similar % Ndfa was reported during the first year's growth of Leucaena in Hawaii Van *et al.* 1990. Greenhouse studies conducted at the IAEA laboratories in Seibersdorf using ^{15}N gave similar results. For a 36 weeks period, two genotypes of leucaena derived 43 and 36% of their N from fixation representing less than half of their total N (Sanginga *et al.* 1990b and 1990c). Zaharah *et al.* (1986) on the other hand reported a higher proportion (78%) and amount (231kgN/ha) of N fixed in six months in three provenances of leucaena grown in the field in Malaysia, while the average % Ndfa in 11 Isolines of *L. leucocephela* in one study of Sanginga et al. (1990) was 65%. Several factors could account for the large differences in reported proportions and

amounts of N fixed in leucaena, of important is the great differences in soil status. Luyindala and Haque (personal communication) found that % Ndfa dropped from 65 to 34 by raising the N rate from 20 to 100kg /ha. Also, genotypic differences in ability to fix N_2 could account for some of the differences observed. For 11 *L. leucocephela* Isoline studied, % Ndfa ranged from 37 to 74% (Sanginga *et al.*, 1990d) an indication of the large genotypic variation that can exist in the N_2 fixation abilities of leucaena Isolines.

2.5.2 *CASUARINAS spp*

Casuarinas have been identified for their high N_2 – fixing capability. Gauthier *et al.* (1985) under simulated field conditions measured only 53% Ndfa by isotope dilution (ID) method in *C. equisetifolia* grown in a very sandy soil low in N; by making some assumptions, the total N_2 fixed was estimated as 40 to 60 kgN/ha/yr. Similar results were also obtained by Sougoufara *et al.* (1990), who measured a maximum contribution of 59% Ndfa to the first year's growth of *C. equisetifolia* in this sandy soil. An almost equal amount of N was fixed during the second year of *C.*

equisetifolia growth as in the first year (Sougoufara *et al.*,1990). However, % Ndfa in the second year declined from 64.8 to 52.7%. Sougoufara et al. (1990) and Sanginga *et al.* (1990e), estimated similar amounts of N_2 fixed by *C. equisetifolia* using either the ID or total nitrogen difference (TND) method, although estimates by the TND method were less precise than the Isotope-derived ones.

Large differences have been reported for the N_2-fixing abilities of different *Casuarina* genotypes. Sougoufara *et al.* (1990) found an almost five fold difference in N_2 fixed by two genotypes of *C. equisetifolia,* while Sanginga *et al.* (1990e) found that 18 weeks after planting, *C. equisetifolia* fixed more N_2 than *C. cunninghamiana* (means 63% and 43% respectively). Large differences however, were also found in the abilities of the different *C. equisetifolia* and *C. cunninghamiana* provenances to fixed N_2. In the case of *C. equisetifolia*, the % Ndfa ranged from 25 to 75% (equivalent to 4 to 29 mgN/plant), and from 14 to 76% (equivalent to 2 to 25mgN/plant) for *C. cuninghamiana.* Thus, even though *C. cunninghamiana* was generally a poorer N_2 fixer some provenances were capable of achieving close to the maximum N_2 fixed by the *C. equisetifolia* provenances.

2.5.3 *GLIRICIDIA spp*

Gliricidia spp has been reported as having great potential in Agroforestry after *Leucaena spp*. However, results obtained using the acetylene reduction assay suggested that *Gliricidia* is a poor N_2 – fixer (Duhoux *et al.*, 1990). This finding is in contradiction to results of Awonaike et al. (1990) who found that N_2 fixed in *Gliricidia sepium* was comparable to, or slightly higher than most values reported for *L. leucocephela*, which is regarded as a high N_2 fixer. The average % Ndfa for five plant genotypes and five *Rhizobium* strains studies was reported as 60% or 3.95gN/plant (Awonaike *et al.*,1990). Thus on average, these five genotypes obtained more than half of their total N from fixation. The results indicated high variability in N_2 fixation, a significant proportion of which was due to *Rhizobium* strain and plant genotype interaction. No one *Rhizobium* strain was ideal for all plant genotypes, and depending on the infecting *Rhizobium* strain, N_2 fixed in particular plant genotype was either high or low. By adding small amounts of ^{15}N at frequent intervals, Sanginga, Zapata, Danso and Bowen (unpublished) measured 72%Ndfa in *G. sepium*, a higher proportion than even that of Awonaike *et al.* (1990). Liya, Odu,

Agboola and Mulongoy (unpublished) using the ID method also estimated 85% Ndfa in *G. sepium* whiles the TND method gave 72% Ndfa.

2.5.4 *Acacia spp*

The N_2 fixation data available for many of the *Acacia spp* indicate that, *Acacia* often has a low potential for N_2 fixation (Dommergues, 1987; Sanginga *et al.*, 1990a). In a study using N-15 methodology to assess N_2-fixation potential of legumes in the Sonoran desert, Shearer *et al.*(1983) concluded that *A. greggii* for example, did not fix any N_2. Sanginga *et al.* (1990d) reported that average % Ndfa in 11 provenances of *A. albida* was 20% compared to 65% in 11 isolines of *L. leucocephela*. In view of the low N_2 fixation in *A. albida*, Sanginga *et al.* (1990d) found that, errors attributable to the reference crop were of greater significance than for leucaena, which is in agreement with the suggestion by Hardarson *et al.* (1988) that errors involved in N_2 fixation measurements are more serious where fixation is low.

2.5.5 *Sesbania spp*

Sesbania, particularly the stem nodulating species have been of great value as organic manure in rice farming systems, where they are capable of fixing virtually all their N (Pareek *et al.*, 1990). Both the ID and TND methods have been used to assess N_2 fixed in Various types of Sesbania. Ndoye and Dreyfus (1988) estimated 83 to 109kgN/ha fixed within 6 months in the stem nodulating *S. rostrata*, compared to only 7 to 18kgN/ha in the root nodulating *S. sesban*. Higher values of N_2 fixed than those of Ndoye and Dreyfus (1988) were measure by Pareek *et al.* (1990), who found that *S. rostrata* and and *S. aculeata* derived 80% of their N in one season, and 94% in another, with the TND approach giving significantly lower estimates of N_2 fixed, ranging form 59 to 88%.

2.5.6 ALDER spp

Alders are noted for their large nodule sizes, which may sometimes reach the size of a tennis ball (Alexander, 1961), and are important component of many natural ecosystems in temperate climate. Cote` and Camire (1984) in

their study estimated % Ndfa to be 68% (equivalent to 53kgN/ha). From leaf samples collected from *A. glutinosa*, Domenach and Kurdali (1989) measured 87% Ndfa, after taking into account translocation of natural N reserves and isotopic discrimination. Domenach *et al.* (1987) used both natural ^{15}N abundance and ^{15}N fertilizer addition and found that both approaches gave similar estimates, with the % Ndfa in alders of different origin ranging from 40 to 80%.

2.6 FACTORS INFLUENCING N-15 ESTIMATES OF BNF IN TREES.

The measurement of biological Nitrogen fixation in plants (BNF) is a crucial step towards efforts aimed at increasing the contribution of atmospheric nitrogen to plant nutrition and soil fertility. Although there are many methods for measuring BNF in plants, none is perfect in terms of measuring N-fixed accurately. The advantages and limitations of each method have been discussed in several reviews (Bremner, 1977; Burris, 1974; Danso, 1985; Dardy *et al.*, 1973; and Knowles, 1981). However, of the several methods available the ^{15}N methodologies are the most reliable

for measuring N_2 fixed in plants and hence, are often the methods of choice (Duhoux and Dommerques, 1985; Duque *et al.*, 1985; Hardarson et al., 1985b; Leg and Sloger, 1975; Rennie, 1982; 1984; Sanginga *et al.*, 1985; West and Wedin, 1985). These ^{15}N methodologies are particularly useful as they can at a single harvest measure the integrated amounts of N_2 assimilated by both greenhouse and field-grown plants in addition to measuring the N contributed from soil or fertilizer sources (Danso, 1985; Fried *et al.*, 1983; Vase and Victoria, 1986). There is therefore an increased use in the ^{15}N methodologies for measuring N_2 fixed in various crops and cropping systems (Chalk, 1985; Ledgard *et al.*, 1985; Weaver, 1986).

The increased use of ^{15}N techniques in most N_2-fixation measurement has revealed some practical problems. As suggested by Rose and Victoria (1986), a major problem with the use of ^{15}N techniques has been a general lack of understanding of some of the underlying concepts. It is therefore likely that with better understanding of the concepts, and adequate precautions to reduce some of the avoidable errors, many of the limitations associated with the ^{15}N techniques could be reduced. N_2-fixation measurements in trees are more problematic than for annual legumes, and

Danso *et al.*, (1992) have suggested some reasons for it. Among the factors likely to affect and limit the use of ^{15}N methodologies in measuring BNF in trees include the following: (a) Selection of appropriate reference plant (b) ^{15}N Labelling (c) ^{15}N application rates.

2.6.1 Selection of appropriate reference plant:

The selection of appropriate reference plant for a particular N_2 fixing plant appears to be the greatest problem encountered with the ^{15}N methodology. Fried *et al.* (1983) suggested the criteria for selecting reference crops for estimating the amounts and proportions of N_2 fixed by the ^{15}N methodology. It is important that, serious attempts are made to ensure that, the reference plant is a non-N_2 fixer, with similar and N uptake pattern as the nitrogen fixing tree (NFT), and that, both are obtaining their N from a similar soil horizon. However, the higher the N_2 fixed, the less stringent are the requirements for all the criteria to be met rigidly (Hardarson *et al.*, 1988). According to Danso *et al.* (1986), Philips *et al.* (1986), the need for the precise quantification of N_2 fixation may not be compelling in many agronomic trials, e.g., in comparing some treatment effects, or ranking

varieties or *Rhizobium* strains for N_2 fixing effectiveness. To achieve such objectives, a reference crop is often not necessary, as the results can be obtained using the relative ^{15}N enrichments of the different trees; the lower the ^{15}N enrichment, the higher the N_2 fixation.

The problem of selecting a suitable reference crop is more serious in trees than in seasonal crops, as the ^{15}N uptake patterns need to be matched over the different seasons and not over one or only a few seasons.

Where the ^{15}N is added once, only at the beginning, it is likely that because the ^{15}N /^{14}N ratio in soil declines less rapidly with time (Pareek *et al.*, 1990), the errors due to mismatched reference and fixing plants are likely to become smaller with time (Fried *et al.*, 1983; Witty, 1983). Sanginga *et al.* (1990) and Pareek *et al.* (1990) have emphasised on the criteria for the selection of reference plants for NFTs. The results of Sanginga *et al.* (1990) clearly show that highly erroneous values can be obtained unless efforts are made to select suitable reference crops.

2.6.2 ^{15}N Labelling.

The problem associated with the ^{15}N labelling include lack of knowledge on what N rate to apply to the fixing and reference plants, the time and frequency of application. Although for most N_2 fixation studies, ^{15}N labelled fertilizers have been used mainly as tracers, the N they contain can affect growth and N_2 fixation and hence the N rate to be used in fixation studies must not be over looked. In many studies, N rates may have been used without prior knowledge of how they would affect N_2 fixation under the local conditions. For the use of the ^{15}N techniques to estimate N_2 fixation, a zero ^{15}N fertilizer rate which would have been ideal is only possible with the ^{15}N natural abundance method. As a rule, the ^{15}N applied must be high enough to ensure satisfactory ^{15}N detection in plants, without reducing N_2 fixation. Low N rates will therefore necessitate the use of highly enriched ^{15}N fertilizers (West and Wedin, 1985). However, cost consideration may not always make this approach the best (rennie, 1986; Danso *et al.*, 1987). Increasing the quantity of ^{15}N fertilizer (if it does not significantly inhibit N_2 fixation) with a corresponding decrease in ^{15}N enrichment is thus economical and more advisable (Danso *et al.*, 1988). It

is generally advisable to apply high versus low rates of N to the reference and fixing crops respectively, to estimate N_2 fixed. This approach overcomes the limitation of poor growth of the reference crop when soil N is inadequate, while avoiding the suppression of N_2 fixation by high inorganic N (Fried and Broeshart, 1975).

2.6.3 ^{15}N Application Rates:

A single application of ^{15}N labelled fertilizer satisfactory for N_2 fixation measurements over more than one season, for instance, in perennial crops, may be very expensive and or inhibitory to N_2 fixation. Besides, the greatest problem with this approach, even with annuals is the sharp decline in ^{15}N /^{14}N ratio of soil N, often necessitating the rigorous selection of reference crop. To overcome these problems, equal-sized, multiple additions of small amounts of ^{15}N fertilizer during various times of the growth cycle were first used by Vallis *et al.* (1967), to estimate N_2 fixed. Since then, several studies using this approach have been reported (Boddey *et al.*, 1983a and b; Danso *et al.*, 1988; Edmeades and Goh, 1987; Vallis *et at* ., 1977). Boddey *et al.* (1984) found that frequent additions of small

doses of ^{15}N fertilizer ensured high ^{15}N fertilizer enrichments in soil, and resulted in small declines in ^{15}N /^{14}N ratio, compared to the single application. Frequent ^{15}N additions may therefore assist in minimizing errors due to mismatch between reference and fixing crop. Rennie (1985;1986) has, however, criticised the use of repeated ^{15}N additions on plots. However, the arguments and evidence he adduced in support of his claim appear unconvincing.

2.6.4 Sampling of plant material :

The ^{15}N enrichments in various plants parts (e.g. seed, herbage, crowns and roots) frequently differ (Butler, 1987; Butler and Ladd, 1985; Fried *et al.*, 1983; Jensin, 1986; Ladd, 1981; Rennie *et al.*, 1978). Measurements of nitrogen fixed based on only one plant part e.g. seed or leaf (Ruschel *et al.*, 1982) may therefore not adequately represent N_2 fixed in the whole plant (Ladd, 1981, Phillips *et al.*, 1983a). The sampling of plant material would not pose much problem in the case of annuals, but may be extremely difficult in trees, because of massive size of trees, which makes it difficult to completely sample the whole tree. There is also the difficulty in

harvesting roots which in the case of NFTs may contain more than half of the N in the whole plant (Sanginga *et al.*, 1990b). Significant N reserves occur in the roots of trees, and in NFTs, N reserves may account for a significant proportion of the N used for regrowth. Domenach and Kurdali (1989) reported that, the reserves of N in roots of *Alnus glutinosa* accounted for 10% of the N in leaves at the end of the growing period.

Part of the sampling problems is also due to the heterogeneity in ^{15}N enrichments in different plant parts (Sanginga *et al.*, 1990b; Shearer *et al.*, 1983). Leaves generally gives estimates of % Ndfa closest to that for the whole tree, and thus where %Ndfa is the most desired estimate, it seems appropriate to sample the leaves if the whole tree cannot be harvested.

Nitrogen cycling from litter decomposition influences the ^{15}N enrichment in the rhizosphere (Jordan, 1985). With trees, litter fall and decomposition can be significant, and can consequently cause large changes in the ^{15}N /^{14}N ratios of soil under the fixing and non-fixing crops. With the ^{15}N enrichment in a fixing crop being usually lower than that in a reference

crop, the expected net effect would be a lower ^{15}N /^{14}N ratio in soil under the fixing crop, and therefore a higher than real estimate of N_2 fixed.

2.7 INFLUENCE OF PHOSPHORUS ON NODULATION AND NITROGEN FIXATION

Phosphorus is a macronutrient element and it is an essential constituent of all living organisms. Apart from nitrogen, phosphorus has been reported as the most important limiting nutrient in Ghanaian soils (Nye, 1972). Most soils in Northern Ghana where most of Ghanaian cereals are produced are highly deficient in phosphorus (Nyamekye, 1987). Phosphorus fertilizers are therefore used to increase yield. This inherent low P level, according to Acquaye and Oteng (1982), is due to the nature of parent materials which are mainly sandstone and/or sesquioxide.

Several workers (Gate and Wilson, 1974; Jakobsen, 1985;Olofintoye, 1986; Pereira and Bliss, 1987) have reported that a high phosphorus level is needed for maximum nodulation and nitrogen fixation in legumes, but the amount required for optimum nodulation and N_2 fixation differs widely

between genotypes (Pereira and Bliss, 1987; Saleem and Kaufmann, 1986). Danso *et al.* (1992) have also reported the effect of phosphorus on nodulation, root proliferation and its overall beneficial effect on legumes. Sanginga, (1985) reported that, low level of phosphorus is among the main chemical constraints for establishing legume trees on tropical soils and that, the availability of phosphorus is closely associated with high nodulation and nitrogen fixation in tree legumes (Andrew, 1982; Sanginga, 1985). Benge (1992) also reported that, acute phosphorus deficiency in most tropical soils is among the main nutritional constraints to the successful establishment of *Leucaena spp* on some selected soils of Nigeria. Sanginga *et al.*, (1985) further indicated that leucaena plants require 80kgp/ha for good establishment even when they are inoculated with effective rhizobial strains.

Diem and Gauthier (1982), obtained increased nitrogen fixation of *Casuarina equisetifolia* by adding phosphate fertilizer to a phosphorus – deficient soil. Reddel *et al.* (1988) similarly found a 245% increase in wood volume of inoculated *C. cunninghamians* (field studies at Gypie, Australia) with the addition of phosphate to soil. Based on the results of

another study conducted in Australia, Reddel *et al.* (1986) concluded that low soil phosphorus status was a frequent limitation to nodulation of naturally occurring *Casuarina* and *Allocasuarina* species. Singleton *et al.* (1985) reported an increase in nodule dry weight and nitrogenous activity with increasing phosphorus supply. It has been suggested that the requirements of phosphorus for nodulation and maximum nodule activity are much greater than for host plant growth (de Mooy and Pesek, 1966). Independent of the source of phosphorus used, leaf area and seed production were found to be highest at 400mgP/kg (Bataglia and Mascarenhas, 1977). Offei (1990), applying P at varied rates of 0, 40,80 and 120kgP/ha in *L leucocephela* observed a significant increase in nodule and total P with increasing levels of P.

Genetic variation in the ability of the host legume as well as the rhizobia to grow in soils with marginal P content has been reported. By examining 23 provenances of *Gliricidia sepium* and 11 isoline of *L. leucocephela* for growth in low P soils, 2.3-fold and 2.1-fold differences in growth, respectively, occurred between the *L. leucocephela* and *G. sepium* genotypes (Beck and Munns, 1984). Also, by assessing P nutrition of 23

strains of *Rhizobium* and 17 Strains of *Bradyrhizobium*, Beck and Munns, (1984) observed that, most strains tested were able to grow at P levels as low as 0.06micomolar, but significant strain and species variation was found in rhizobial growth response to low phosphorus. Smart *et al.*, (1984) however, attributed this genotypic differences in low phosphorus tolerance of *(brady)rhizohia* to their ability to switch on efficient P uptake systems. The ability of *Bradyrhizobium* strains to acquire, store and utilize phosphorus under varying levels of soil P (luxury, sufficience and insufficiency), and their transfer to subsequent generations have also been examined (Smart *et al.*, 1984; Cassman *et al.*, 1981). Many workers have reported a close interaction between phosphorus and nitrogen. For instance, Hamissah *et al.* (1980) have reported that a combination 107kgN/ha and 36kgP/ha produced the greatest economic yield in most legumes. In another experiment, nitrogen content in the plant was found to increase with increasing levels of nitrogen and phosphorus together (Sampet, 1978).

2.8 ENVIRONMENTAL FACTORS AFFECTING N_2- FIXATION

The main environmental factors affecting nitrogen fixation can be grouped into (a) Physical (b) Chemical and (c) Biological factors.

2.8.1 Physical Factors.

Those physical factors that significantly influence N_2-fixation include: temperature, drought and waterlogging.

2.8.1.1 TEMPERATURE

Temperature appears to be the most important physical factors affecting N_2 fixation. Although some *Rhizobium* strains have been reported to survive at temperature around 70°C in dry soil (Marshall, 1964), excessive high temperatures in general, can kill the majority of the bacteria in the surface layers of soil. In certain parts of the tropics, the surface soil temperature can occasionally reach 65-70°C and temperatures above 50°C can be found at a depth of 5cm (Dudeja and Khrana, 1989), sufficiently high enough to

inhibit germination of seeds and kill many bacteria. High temperatures can prevent nodulation, or if nodulation does occur, can inhibit the activity of N_2-fixation in legumes (Day *et al.*, 1978) even though root nodules may be insulated from the highest temperatures by the soil. Conversely, cool temperatures lead to delayed development of plants including delays in the formation of nodules, and so decrease rates of N_2-fixation. Unlike many species of Cyanobacteria which can form spores which are highly resistant to desiccation, most heterotrophic free-living N_2-fixers and rhizobia do not possess this capability. Survival of bacteria in soils at high temperatures appears to be improved by the presence of clay particles and soil organic matter, but many of the soils where temperatures are high are sandy. Day *et al.* (1978) reported rhizobial population of only 4-40 cells/g soil at a surface layer of 5cm soil and reported a population of up to 10^4 cells/g soil at a depth of 20-25cm below the surface. The optimum temperatures for growth and N_2-fixation vary widely between legume species and reflect their environmental adaptation. Eaglesham and Ayanaba(1984),demonstrated the differences in environmental adaptation to high temperatures between rhizobia isolated from different climatic zones.

2.8.1.2 DROUGHT

Drought as a physical environmental stress has been noted to have drastic effects on nitrogen fixation in legumes. Rates of N_2-fixation are more sensitive to reductions in soil water content than other processes such as photosynthesis, transpiration, or leaf growth rates (Sinclair *et al.*, 1987). Even in species considered to be drought resistant e.g. *Acacia spp*, slight changes in the plant water potential caused a marked reduction in both the rate of N_2-fixation and in the translocation of the products of N_2-fixation to the shoot (Rao and Venkateswarlu, 1987;Venkateswarlu and Rao, 1987). In general, it has been noted that, the numbers of rhizobia in soil decline drastically as soil dries. Bushby and Marshal (1977), in their comparative study of the survival of *Rhizobium* and *Bradyrhizobium* in drying soil, indicated that, *Bradyrhizobium* strains are more tolerant of desiccation than strains of *Rhizobium* over short periods. Other workers found no simple relationship between the desiccation tolerance of fast-or slow-growing rhizobia but they did find that specific strains of each survived in much greater numbers than others (Mahler and Wollum, 1981). Rhizobia

generally survive poorly on drying in soils which contain only small amounts of clay or organic matter (Chao and Alexander, 1982). Strains which survive under greater water stress are those which retain less water within the cells (Bushby and Marshall, 1977; Al-Rashidi *et al.*, 1982).

2.8.1.3 WATER-LOGGING

Water-logging is also a major environment stress affecting N_2-fixation. Whilst rhizobia are normally aerobic organisms, some strains of *Bradyrhizobium* and *R. meliloti* possess a dissimilatory nitrate reductase which can function as an electron acceptor, and thus enable the bacteria to survive under anaerobic conditions (zablotowicz *et al.*, 1978; Daniel *et al.*, 1982). Because of the aerobic characteristic of rhizobia, the survival of rhizobia during long periods of flooding is of particular importance in cropping systems in which legumes are grown in rotation with rice. Toomson (1990), indicated that, the size of the population of rhizobia sampled from the field was generally larger (10^2-10^4 cells g/soil) when the soil was moist or fully waterlogged as compared to when the soil was dried (<10-10'3 cells g/soil). In contrast, large reductions in the numbers of

rhizobia nodulating chicken Pea which are generally fast-growing rhizobia and which may not possess a dissimilatory nitrate reductase have been reported when grown after paddy rice (Toomson *et al.*, 1982).

Lack of oxygen is also a major problem for root respiration and can rapidly result in loss of nitrogenase activity (Sprent and Gallacher, 1976; Witty *et al.*, 1986). Waterlogging can result in the rapid release into the soil of certain heavy metals like iron and manganese which are highly toxic to both rhizobia and plants in high concentration.

2.8.2 CHEMICAL CONSTRAINTS

Among the chemical factors that significantly affect nitrogen fixation include toxicity and nutrient deficiency.

2.8.2.1 TOXICITY

The bacterial symbionts may be directly affected by the acidity of their environment. Bacteria with a greater capacity to regulate their internal pH

show increased survival rates at low pH (O'Hara *et al.*, 1989). Aluminium toxicity, probably the most severe component of stress in acid soils, also has a major impact on rhizobial survival, and strains of *rhizobia* which were able tolerate a pH of 4.5 were not necessary tolerant of aluminium toxicity (Keyser and Munns, 1979b). The toxicity of aluminium to rhizobia may be due to aluminium binding to DNA and thus inhibiting DNA replication (Johnson and Wood, 1990). Strains of *Rhizobium* tolerant to aluminium had different DNA repair mechanisms to those found in strains susceptible to aluminium toxicity.

Other problems of acid soils such as manganese toxicity and calcium or phosphorus deficiency appear to have a lesser effect on *Rhizobium* survival (Keyser and Munns, 1979b). Low pH per se is not the principal cause of toxicity to plants. Rather, the problems of plant growth in acid soil result from aluminium and manganese toxicity. Franco and Munns, (1982), using solution cultures indicated that acidity and not aluminium was the principal constraint to nodulation.

Large differences in sensitivity to the toxic effects of acid soils have been found between different species of tropical pasture legumes (Andrew *et al.*, 1975; Andrew, 1976; de Carvalho *et al.*, 1981). Several species of *Stylosanthes* are tolerant of concentrations of aluminium in solution which severely depress nodulation and plant growth of other species de Carvalho *et al.*, (1981). de Carvalho *et al.*, (1981), even reported differences in aluminium tolerance among different species of *Stylosanthes*. *Stylosanthes* is nodulated by direct infection mechanism and the lowered toxic effects of aluminium on nodulation may be related to a reduction in lateral root formation reducing the number of possible infection sites (de Carvalho *et al.*, 1982). Clarkson, (1965) indicated that, the reduction in nodule initiation and development commonly seen both in *Stylosanthes* and in other legumes may be due to the reduction in meristematic activity of roots in solution high in aluminium.

2.8.2.2 NUTRIENT DEFICIENCY

Several of the nutrients essential for growth of plants or bacteria play specific roles in nodulation and /or N_2 – fixation. Thus, deficiencies in

these nutrients, or other nutrients essential for the growth of bacteria or plants can cause reduction in the numbers and size of nodules as well as the amount of N_2 fixed (Giller and Wilson, 1991). For instance, Beck and Munns (1984), indicated that there is marked variation in the ability of rhizobia to grow in low concentrations of phosphorus which appears to be due to variation in the efficiency of phosphorus uptake systems (Smart *et al.*, 1984). Strains of *Rhizobium* also differ in their ability to store phosphorus, but even in the most efficient case the amount of phosphorus stored can only support growth for 3-4 generations after removal of all phosphorus from the medium (Cassman *et al.*, 1981; Beck and Munns, 1984). In low concentrations of phosphorus, Beck and Munns (1985) indicated that, high concentrations of calcium are required for growth of rhizobia. It has been noted that, acute deficiency of phosphorus can prevent nodulation by legumes. Phosphorus and sulphur are required for nodule metabolism and tend to be concentrated in the nodules when the plant is deficient in these nutrients (O'Hara *et al.*, 1988a). It has been suggested that, as nodulated plants often have less well-developed root system than unnodulated plants, the ability of nodulated plants to capture nutrients, particularly phosphorus, is decreased (Cassman *et al.*, 1980).

Most legumes therefore depend heavily on mycorrhizas for efficient uptake of phosphorus (Hayman, 1986).

Mycorrhizas assist in uptake of phosphorus and other nutrients which are not mobile in the soil by increasing the volume of soil effectively explored by the plant. The degree of dependence on mycorrhiza for capture and uptake of phosphorus is partly determined by the root geometry of the legume; legumes with poorly-branched root system with few root hairs e.g. *Leucaena leucocephala* (Munns and Mosse, 1980), and *Centrosema pubescens* (Crush, 1974) tend to be more dependent on mycorrhizas than those plants with many long root hairs e.g. *Lotus pedunculatus* (Crush, 1974). Many researchers have indicated that, nodulation and growth of legumes growing on phosphorus –deficient soil are often stimulated by mycorrhizas inoculation (Howeler *et al.*, 1987; Arias *et al.*, 1991).

Apart from the chemical nutrient deficiencies that affect nodulation and subsequent nitrogen fixation, there is increased evidence that pesticides used in agriculture can have adverse effects on the survival of rhizobia or on nodulation of legumes (Edwards, 1989; Roberts, 1991). For instance,

pollution of soils by heavy metals has been shown to completely suppress N_2-fixation in white clover (*Trifolium repens*) due to the toxicity of the heavy metals to *Rhizobium* (McGrath *et al.*, 1988; Giller *et al.*, 1989).

2.8.3 BIOLOGICAL CONSTRAINT

The growth and survival of *rhizobia* in soil have been shown to be influenced by biological factors. Competition and antagonism by other micro-organisms are two biological factors influencing rhizobia survival and growth. Danso *et al.*, (1975) indicated that, grazing of rhizobia in soil by protozoa is responsible for the reduction in the populations of rhizobia in soil and susceptibility of strains to bacteriophages may result in their poor survival (Barnet, 1980). Also, Danso *et al.*(1974) indicated that sterilised soils support far larger population of inoculated rhizobia compared to unsterilised soils, implicating biological agents.

2.9. SOME METHODS FOR ESTIMATING NITROGEN FIXATION

An accurate method of measuring N_2-fixation is essential for any evaluation of the usefulness of different technologies, yet such a method has remained remarkably elusive. Despite active research on this subject since the discovery of N_2-fixation, many measurements of the amount of N_2 fixed in the field remain little better than informed guesses. Difficulties encounted in measuring N_2-fixation in grain and pasture legumes are magnified enormously when we consider trees. Of all the N_2-fixing symbiosis trees are the most problematic for measurement of the amount of N_2-fixed, hence most research work have been concentrated on annual herbaceous plants than trees. Danso *et al.*, (1992) have identified tow major reasons for this situation;

(a) The perennial nature of trees/shrubs

(b) The relatively large size of trees.

Despite the problems associated with nitrogen fixation measurements, several methods for estimating N_2-fixation in plants are available although, all have some degrees of error. Most of these methods have been reviewed

by various researchers (e.g. Danso, 1985; Knowles, 1980; Rennie and Rennie,1983). The methods that have been commonly used for estimating nitrogen fixation in trees are:

(1) The total N difference method (TND)

(2) Acetylene reduction assay (ARA)

(3) ^{15}N isotope dilution method (ID).

2.9.1 TOTAL NITROGEN DIFFERENCE METHOD (TND).

This method is the most primitive and simplest of all the methods (Danso *et al.*, 1992). This method involves quantification of total nitrogen in both the fixing plant and the reference crop plant (Danso and Hardarson, 1993). The technique operates upon the assumption that two genotypically similar plants of the same isolines that differ only in the ability to nodulate and fix nitrogen should absorb the same amount of soil N whether or not the nitrogen fixing species is actively fixing under uniform environmental conditions. Thus by analysing for the total N in the two plants, the amount

of N_2 fixed could simply be estimated as the difference between the two (Danso and Herridge,1987)

i.e.: TN(fi) - TN(nfi) =Ndfa

where:

TN(fi) = Total Nitrogen in the N_2-fixing isoline.

TN(nfi) = Total nitrogen in the non-fixing isoline.

Ndfa = Amount of N_2-derived from atmosphere (fixed N_2)

One major problem with this technique is the difficulty in getting a non-fixing isoline for the reference. Indications are that non-fixing isolines of N_2-fixing trees seem not to exist (Danso and Herridge, 1987). Hence, N_2-fixing trees in non-fixing mode (e.g. uninoculated; inoculated, with killed or ineffective rhizobia; in soils devoid of homologous *Rhizobium* strains) have frequently been used as NFT references (Gautherir *et al.*, 1985).

In some cases however, unrelated species have been used as NFT controls (Pareek *et al.*, 1990) though it has been suggested that this could introduce

undesirable error in the N determination and consequently in the accuracy of the BNF estimates (Danso, 1985 Rennie and Rennie, 1983).

Furthermore, as a limitation to the assumption upon which the technique operates, it is probable that the fixing and non-fixing reference plants may be taking up different amount of soil N, particularly in N rich soils, which could be a major source of error (Danso, 1985; Rennie and Rennie, 1983). To minimize this error source therefore, it is recommended that soils with marginal N content should be used when applying the TND method (Danso, 1986). Thus serious problems or errors occur when this method is employed under conditions thought to be free of mineral N but which are in fact contaminated with N. For example, vermiculite was used as an N-free growth medium for the study of associative N_2-fixation (Rennie and Larson, 1979), but later research showed that significant quantities of mineral N can be released from vermiculite when it is incubated under warm moist conditions (Giller *et al.*, 1986).

Another limitation to this method is the fact that, the method becomes progressively inefficient and impractical as trees mature. Apart from the

difficulty in the recovery of all parts of the mature plant for analysis, including roots and litter, it is also difficult to account for the re-cycled N (Danso *et al.*, 1992). According to Danso *et al.* (1992), this might be one reason for the observed gross differences between the TND approach and other techniques in BNF estimation.

Despite the above limitations to the TND approach, the method has the advantage of giving a measure of the total amount of N fixed over the length of the experiment and is indispensable for many laboratory based studies (Giller and Wilson, 1991).

2.9.2 ACETYLENE REDUCTION ASSAY (ARA).

This method has been used widely for estimating BNF in leguminous trees (Danso *et al.*, 1992). In this method, the amount of N_2 fixed is measure indirectly by analysing for the amount of ethylene produced (nitrogenase activity/acetylene reduction activity) by detached nodules or whole plants in a gas-tight container filled with acetylene gas over a given period of time.

Based on the theoretical electron flow relationship, it was established that 3 moles of acetylene reduced are equivalent to 1 mole of N_2 reduced or fixed, and therefore Hardy *et al.*, (1973) proposed that the amount of acetylene reduced to ethylene be multiplied by a conversion factor of 3 to get the amount of reduced N.

There are, however, some serious limitations associated with this technique;

(a) The conversion factor of 3 does not conveniently apply in all cases (Hansen *et al.*, 1987). For example, Turner and Gibson (1980) cautioned that environment and other plant effects acting independently on the nitrogenase reduction of acetylene and nitrogen can sufficiently alter the 3:1 ratio.

(b) Also the technique is said to be instantaneous and may not truly reflect BNF over a long duration (Fried *et al.*, 1983).

Application of acetylene reduction assays for measurement of N_2-fixation in soils is complicated by the effect of acetylene on other microbial processes. For example, acetylene has been found to block the last step of denitrification (Balderston *et al.*, 1976) and autotrotrophic nitrificataion (Hynes and Knowles, 1978). In addition, acetylene blocks bacterial

oxidation of ethylene in soil so that "endogenous" ethylene accumulates (de Bonte, 1976; Nohrstedt, 1976). Thus, control treatments used to estimate background concentrations of ethylene in soil in which ethylene accumulation is measured in the absence of acetylene, greatly underestimate the accumulation of endogenous ethylene that occurs in the presence of acetylene. Witty (1979) demonstrated this clearly by using ^{14}C labelled acetylene for ARA measurements of N_2-fixation in soil cores. Only half of the ethylene that accumulated was from the labelled ^{14}C and the remaining unlabelled ethylene must have come from the soil.

Other problems in the application of acetylene reduction assays for measurement of low rates of nitrogenase activity in soil result from the differences in solubility and rates of diffusion of acetylene and ethylene in water (Van Berkum and Bohlool, 1980).

2.9.3 THE ^{15}N-ISOTOPE TECHNIQUE.

This method holds promise as an effective and straight forward approach for measuring nitrogen fixation in trees. It is based on the differential

dilution of the ^{15}N /^{14}N isotope ratios in NFTs with varying N_2-fixing potentials (Fried and Middelboe, 1977). Nitrogen in the atmosphere is virtually all $^{14}N_2$ (99.633%) plus a small amount (0.367%) of the natural ^{15}N (Marrioti, 1983). Any substance which has an atom % ^{15}N greater than that of the atmosphere (0.367%) is said to be enriched with ^{15}N and the ^{15}N enrichment is expressed as atom %^{15}N excess. Similarly, a material depleted in ^{15}N has an atom %^{15}N below that of the atmosphere and it is said to be depleted ^{15}N material. If a plant is grown in conditions where its sole source of N is fertiliser, which is entirely composed of ^{15}N (100 atom %^{15}N), then all the N in the plant (apart from the amount that is originally present in the bacterial inoculum) will be ^{15}N.

If the plant is able to fix dinitrogen ($^{15}N_2$) from the atmosphere then the plant will have an atom %^{15}N which is less than that of the fertiliser (i.e., less than 100 atom %^{15}N). This difference can be used to calculate the proportion of N derived from N_2-fixation and is the underlying principle behind the isotope dilution method for measurement of N_2-fixation (Giller and Wilson, 1991).

Fried and Middelboe (1977) established the following working formula for the calculation of %Ndfa in fixing plants;

$$\%Ndfa = (1 - \frac{\%Ndff(fixer)}{\%Ndff(Non\ fixer)} \times 100$$

where;

%Ndfa = percent N derived from the atmosphere by the fixing plant.

%Ndff(fixer) = percent N derived from the ^{15}N labelled fertilizer by the fixing plant.

%Ndff(Non-fixer) = percent N derived from the ^{15}N labelled fertilizer by the non-fixing plant.

Some basic assumptions in using this technique include:

i) That the ^{15}N fertilizer enrichment is even with depth in the soils under text.

ii) That the N-fixing and the non-fixing reference plants have similar N-uptake patterns (Witty, 1984).

iii) That the ^{15}N soil enrichments are the same under both the N_2-fixing and non-fixing plant.

2.10 METHODS OF ESTIMATING SOIL MICROBIAL BIOMASS

Increasing awareness that the microbial biomass of soil constitutes a major nutrient sink has underlined the need to quantify soil biomass and to understand the dynamics of soil population. Several methods have evolved over the years for estimating soil biomass. Unfortunately technical limitations associated with each method obstruct the realization of these objectives. The various methods which have been used can be grouped under the following headings (i) Conventional methods (ii) Current methods.

2.10.1 CONVENTIONAL METHODS

The conventional methods include the following :

1. Direct counting
2. Plate count

3. Most probable Number method (MPN)

The direct counting method involves the direct observation of cells on agar plates. The cells are first stained and then subjected to microscopic examination. The main disadvantage of this technique is that it overestimates the microbial biomass due to : (1) the inability to distinguish cells from stained non-microbial organic particles and (2) the failure to distinguish living from dead cells (Skinner *et. al.*, 1952).

The plate count method assumes that cells are fully dispersed into units after agitation such that every colony that developed arises from a single cell. The plate count method therefore underestimates the microbial biomass because after agitation some cells clump together with soil particles and are counted as one. Secondly, some cells are killed in the dilution medium and thirdly, there is the failure of certain spores to germinate. Further, the walls of the pipette often absorb some of the microbial cells (Gray and Williams, 1971).

The most-probable-number (MPN) method permits estimation of population density without an actual count of single cells of colonies. It is sometimes called the method of ultimate or extinction dilution or, less descriptively, simply the dilution method. The method is based on a determination of the presence or absence of micro-organisms in several individual aliquots of each of several consecutive dilutions of soil or other material. Informative discussions of the MPN method have been prepared by Halvorson and Ziegler (1933) and by Cochran (1950). The procedure involves a long period of incubation and is therefore time consuming. It is also less accurate as compared to the plate count since it is based on statistical assumption.

Generally, the conventional methods have the limitations of being inappropriate for comparing the relative contributions of different groups of soil microbes to the total soil microbial biomass and or activity. This is because the methods estimate numbers of cells and hyphae. However, fungal hyphae have higher protoplasmic weight than bacterial cell. Thus, in terms of numbers, bacteria have been estimated to exceed fungi in the ratio of 100:1 but in terms of weight, it is less than 1:1 (Gray and

Williams, 1971). Consequently, techniques have been developed to convert microbial numbers to biovolume and then to biomass giving a better index of soil microbial biomass and activity. However such conversions are time consuming and tedious.

2.10.2 CURRENT METHODS

Current methods for estimating microbial biomass are based on either direct microscopical measurement (Joones and Mollison, 1948; Paul and Johnson, 1977, Soderstrom, 1977), the fumigation-reinoculation principle (Jenkinson and Powlson, 1975) or the measurement of a cell component such as ATP (Verstraete *et. al.*, 1983).

Anderson and Domsch (1978) proposed a physiological respiratory method based on $C0_2$ production, but caliberated on the basis of the fumigation-reinoculation principle.

Sparling (1981) proposed a microcalorimetric method while Van de Werf and Verstraete proposed a method for estimating the active soil biomass, base on Oxygen consumption (physiological principle), but caliberated by

means of theoretical values that conform to microbial physiology and growth kinetics. These methods, were developed in an attempt to evolve simpler and more objective techniques for determining the soil microbial biomass (Anderson and Domsch, 1978).

The method based on the extraction of cell components often fails to fully extract the desired component and the relative recovery often depends on substrate characteristics or the type of extraction procedure used. Also, the quantity of a particular cell component can vary considerably with growth conditions and within different members of the microbial population. The ratio of specific cell component to the actual biomass is therefore not necessarily constant (Anderson and Domsch, 1978).

Although the respiration method gives biomass data within six hour, it is limited by the fact that a sensitive CO_2 monitoring system is necessary to estimate hourly what is called the 'maximum initial response' of the soil. The physiological methods are also limited by the fact that a factor that links respiratory rate with biomass has not been fully derived (Anderson and Domsch, 1978).

2.10.2.1 THE CHLOROFFORM FUMIGATION TECHNIQUE

This method releases the carbon bound is microbial cells by minerlization and provides a means of calculating its weight (Jenkinson and Powlson, 1976; Anderson and Domsch, 1978).

The technique needs no special equipment and requires only the titration of CO_2 absorbed in alkali (IN NaOH). However, it requires a relatively long period of incubation (at least 10 days) before analysing for the carbon dioxide released. It offers a better means for estimating soil microbial biomass and also estimate the relative contribution of the different physiological groups to the total microbial biomass (Lynch and Panting, 1980; Anderson and Domsch, 1978).

The technique, however, breaks down in strongly acid soils (pH<4.5) and further, the choice of an appropriate 'K' factor which is used in the formula for calculating biomass is still a matter of controversy and experimental research.

2.10.2.2 SOIL ATP

Soil ATP concentration provides another satisfactory way of obtaining microbial biomass in strongly acid soils. The soil ATP concentration is determined by the luciferin-luciferase assay, using a TCA-phosphate-paraquat extractant (Jenkinson and Oades, 1979) as modified by Tate and Jenkinson (1987).

The extractant: soil ratio is 10:1 and soil is ultrasonified for 2 min with a 20 kHz, 140W, MSE sonifier with a 12.5mm dia probe operating at full power.

2.10.2.3 FUMIGATION EXTRACTION METHOD

A new, and probably the best, way of measuring microbial biomass in strongly acid soils is by fumigation extraction (Vance et al., 1987). It was proposed that the organic C rendered extractable to 0.5 M K_2S0_4 after a 24 hour chloroform-fumigation (Ec) comes from the cells of the microbial

biomass and can be used to estimate soil microbial biomass C in both neutral and acid soils.

The Ec (the difference between organic C extracted by 0.5 M K_2SO_4 from fumigated and non-fumigated soil) is closely related to the microbial biomass C measured by fumigation-incubation according to the equation:

$$\text{Biomass C} = (2.64 \pm 0.60)\ \text{Ec}.$$

However, since this method has only been tested on 10 soil samples, of which two were taken at different times from the same sites, its validity, particularly on soils of high clay content, and on a wider range of soils, is yet to be ascertained.

This new method may prove useful in acid soils, in freshly sampled soils and in soils recently amended with substrates, where the fumigation-incubation technique breaks down.

CHAPTER THREE.

MATERIALS AND METHODS.

3.1 LOCATION OF STUDY AREA.

Most of the experiments were carried out in small nursery bags set up on raised benches covered with polythene canopies situated in an open space in front of the Department of Soil Science, University of Ghana, Legon.

3.2 SOILS AND SITE CHARACTERISTICS:

Three soil types taken from the Accra plains were used for the study. The soils belong to the Toje, Hatso and Alajo series (local Names) (Brammer, 1967). The three soils occur on the same soil catena with Toje series being at the top and Hatcho and Alajo series being at the middle and bottom respectively.

Toje soils consist of more than 30 inches of red sandy loam to light clay, may overlie red iron-concretionary clay or iron pan at depth or indirectly overlie ferruginized weathered rock, mainly Togo quartzite schist (Brammer, 1967). The soils are well drained and absorb water readily except when the surface is left bare.

Hatcho soils consist of several feet of pale brown sand increasing to sandy clay with depth, humus-stained near the surface and slightly mottled orange in the subsoil, but occasionally found with seepage iron pan at a depth exceeding 2ft (Brammer, 1967). The soils 'pact' on exposure and the ground-surface becomes hard and impermeable. The soils have little retentive power and dry out rather deeply during the main dry season.

Alajo series consist of soils developed from alluvium in a marine terrace, are grey-brown, heavy plastic swelling clays containing lime concretions in the subsoil (Brammer, 1967). The soils occur on valley flats along a tributary of the Odaw stream which drains the area.

The three soil series occur under dry climatic condition with mean annual rainfall of about 1100mm which is distributed bi-modally with the long

raining peak in May/June and short peak season in September/October. The mean annual temperature is about 31^0C.

The three soils were all collected under native grassland vegetation.

3.2.1 SOIL SAMPLING AND PREPARATION:

The soil samples were taken as cores from a depth of 0-15cm after clearing the vegetation cover after which the soil samples were bulked. The soil were air-dried, pulverised and sieved through a 2mm diameter sieve.

3.2.2 SOIL ANALYSIS.

The soils were analysed for the following parameters; pH (in water), total phosphorus, available phosphorus, and total nitrogen.

3.2.2.1 pH (Water):

Ten grammes of sieved soils were weighed into a beaker and 20ml of distilled water added to give a ratio of 1 soil: 2 water. The mixture was vigorously stirred continuously for 30minutes after which it was left to stand for about 1 hour until most of the clay particles were settled and also to allow the mixture to attain room temperature. The glass electrode –pH meter was standardised using two aqueous solutions of pHs 4 and 7 and then later used to measure the pH of the prepared soil sample by carefully immersing the glass electrodes in the supernatent. The samples were replicated four times and the average pH recorded.

3.2.2.2 TOTAL PHOSPHORUS:

Five grammes of the sieved soil samples were weighed into 25ml conical flask. Ten millilitres of concentrated $HN0_3$ (aq) was added to the content of each flask, followed by 15ml of 60% $HClO_4$ The contents of the flasks were digested in a digestion cupboard until the solution became clear and the dense white fumes of $HClO_4$ had ceased. The digests; were then cooled, diluted with distilled water and filtered into 250ml flasks. The filtrates were then made up to volume and 5ml aliquot each taken for

phosphorus content determination using the molybdate ascorbic acid method of Watanabe and Olsen ((1965) involving calorimetry.

The formula below was used to calculate the phosphorus content

$$P(ppm) = \frac{(R-B)\ X\ Ve}{W\ X\ Va}$$

Where : R = Spectrometer reading of sample

B = Spectrometer reading for blast

Ve = Volume of extractant

W = Weight of soil used

Va = Volume of aliguote.

3.2.2.3 AVAILABLE PHOSPHORUS (BRAY 1 METHOD)

Five grammes of the sieved soil samples were weighed into extraction bottles followed by 30ml of the extractant ($0.3HN_4F$ + 0.1HCl). The bottles were then shaken thoroughly for 2minutes on a mechanical shaker.

The soils suspension were then filtered and 5ml aliquot of the filtrates taken for phosphorus analysis. The Phosphorus determination was done colorimetrically using the Watanabe and Olsen (1965) modified technique.

3.2.2.4 TOTAL NITROGEN:

Ten grammes of the sieved soil samples were weighed into 300ml kjedhal flasks and moistened with distilled water. Tablets of selenium were added into the content of each flask, followed by 20mls of concentrated H_2SO_4(aq).

The mixtures were then cooled and transferred with distilled water into 250ml volumetric flasks and made up to volume. Five millilitres aliquot was taken into a Markham distillation apparatus followed by 5ml of 40% NaOH (aq). The mixture was distilled and the distillate collected into 50ml flask containing 5ml of 2% H_3BO_4 (aq) indicator to which three drops of methyl red-methyl blue indicator had been added. The green solution formed was titrated against 0.01NHCl to a purplish-red end point. The percent total nitrogen was calculated using the following formula:

$$\%N = \frac{0.01 \times 50 \times 0.014 \text{ (titre value)}}{\text{Weight of soil X aliquot of digest used.}}$$

3.3 PLANT GROWTH MEDIA:

Each of the polythene bag used had a length of 15cm with an interior opening of diameter 7cm, and posterior sealed end but with three perforated holes.

Each nursery bag was filled with one kilogramme of a type of soil and each nursery bag with its content were placed in a plastic saucer after which the soil were kept semi-moist by distilled water prior to planting.

3.4 PLANTING MATERIALS:

Seeds of fourteen perennial tree and shrub legume species were collected from the Botanical Gardens, University of Ghana, Legon and the Kwadaso Agricultural Research Station. The tree species used for the initial screening exercise included: *Albizia lebbek, Sesbania specioca. Sesbania*

aculiata, Sesbania rostrata, Sammonia sammia (Pithecelobium),Leucaena leucocephela, Senna occidentalis, Tephrosia spp, Cassia occidentalis, Acacia mangium, Acacia farresiama and Tamarindus indica.

3.5 SEED PRETRATMENT:

The seeds were initially acid-scarified using concentrated H_2S0_4 (aq). The length of time allowed for the scarification varied from 5 mins to 45 mins depending on the degree of hardness of their seed coats. The seeds were then thoroughly rinsed and later soaked over night in distilled water prior to planting.

3.6 INITIAL SCREENING:

The 14 leguminous trees and shrubs were initially grown with or without P (60mg P/Kg soil) in the three soils for 10 weeks and assessed for the presence or absence of nodules. The treatments were arranged in a randomised complete block design with four replicates. The screening study was repeated with nine of the fourteen legume species that nodulated

very well in the three soils with or without P and isolates obtained from their nodules. The legume species used for the 2nd screening study include: *Albizia lebbek, Sesbania specioca, Sesbania aculiata, Sesbania rostrata, Sammania sammia (pithecelobium), Leucaena spp, Tephrosia spp, Acacia mangium and Acacia farresiana.*

3.7 INOCULATION AND N-15 STUDY:

Four of the legume species namely *Albizia lebbek, Tephrosia spp, Leucaena spp* and *Sammania sammia* (*Pithecelobium*), which were found to nodulate and showed good growth in the three soils were used for this studies.

The seeds of the legume species were scarified using procedure described in section 3.5. Four seeds were sown in each nursery bag and thinned to two after emergence. Three days after emergence, pots requiring inoculation treatment were inoculated with inoculants prepared from isolates obtained in the initial screening. The set-up was replicated four times. A week after planting, ^{15}N labelled fertilizer obtained from IITA

was applied at a rate equivalent to 5kgN/ha. The ^{15}N labelled fertilizer application was applied in three split doses, at weekly intervals.

3.8 WATERING:

Watering was done thrice daily using distilled water and depending on the wetness of the soils in the polythene bags. A stock solution of the under-listed nutrient elements was prepared and applied at weekly intervals at a rate of 5ml aliquot/ litre of distilled water:

H_3HO_4 - 0.5g

$MnS0_4.4H_2O$ - 0.31g

$CoCl_2.6H_2O$ - 8×10^{-4}g

3.9 RHIZOBIA ENUMERATION:

The population of rhizobia in the three soils capable of nodulating the four tree legumes (*Albizia, Pithecelobium, Leucaena and Tephrosia)* were enumerated using the most probable number (MPN) method (Vincent,

1970) using plastic growth pouches (Weaver and Frederick, 1972). Clean seeds of the tree legumes were surfaced sterilised in 70% alcohol for 3 minutes and rinsed thoroughly in several changes of distilled water (Somasegaran and Hobeb, 1994). The seeds were pre-germinated on 1% water agar until the radicles were about 2cm long. Seedlings were planted two per porch. Ten fold dilutions of each soil sample with four replicates per dilution were used to inoculate the pouches containing N- free nutrient solution (Somasegaran and Hoben, 1994). 1 ml of the soil inoculants was used to inoculate each pouch. The pouches were randomly arranged in wooden racks and kept on a raised bench with plain polythene roofing. The plants were supplied with enough N-free nutrient solution when necessary.

The plants were assessed for nodules after 10 weeks and the most probable number of rhizobia calculated (Vincent, 1970).

3.10 CROSS INOCULATION STUDIES:

Seeds of the following four tree legumes, namely, *Albizia lebbek, Pithecelobium spp, Leucaena spp* and *Tephrosia spp* were surface sterilised in 70% alcohol for 3 mins and rinsed thoroughly in several changes of distilled water (Somasegaran and Hoben, 1994). The seeds were pre-germinated on 1% water agar until the radicles were about 2cm long. The seedlings were planted two per pouch.

Ten nodules from each of the tree legumes in the initial screening exercise were taken at random and rhizobia isolates obtained from these using standard method (Vincent, 1970).Three isolates of each tree legume were further taken at random from the initial isolates of the trees. The isolates were cultured aseptically in yeast extract manitol (YEM) broth (Vincent, 1970) at 28°C for 5 days. A suspension of each of the cultures was prepared to contain about 10^8 cells/ml. One ml each of the inoculant (suspension) was then used to inoculate the growth pouch containing N-free nutrient solution (Somasegaran and Hoben, 1994). Inoculation was done close to the base of the plant using a calibrated pipette. The pouches

were randomly arranged in wooden racks and kept on raised bench with plain polythene roofing. The plants were periodically supplied with N-free solution whenever necessary. The plants were then assessed for nodules after 10 weeks of inoculation.

3.11 HARVESTING THE TEST PLANTS

The plants were harvested after 10 weeks of growth. The shoots were separated from their roots at the collar and then put into labelled envelopes and oven-dried at 70°C for 72hours. The shoot dry weights of each plant were then taken.

The root system of each tree was recovered by washing the soil around the enclosed area. The cleaned roots were sent to the laboratory where nodules were plucked from the roots and counted. The roots were then put into labelled envelopes and oven-dried at 70°C for 72hours . The roots dry weights were then recorded.

The dried shoot of each plant was chopped into 1-5cm pieces and milled in a milling machine to be able to pass through a 1mm sieve.

3.12 LABORATORY ANALYSIS

3.12.1 TOTAL AND ^{15}N MEASUREMENTS:

Total nitrogen in the shoot of each tree was analysed by Kjeldhal method (Bremmer, 1965). The $^{15}N/^{14}N$ ratios in the shoot were analysed (Fiedler and Proksch, 1975) on an Automatic Nitrogen Analyser (1500 Carlo-Erba) coupled to a VG – Isogas mass spectrometer.

3.13 MEASUREMENT OF N_2 FIXATION:

The isotope dilution equation (Fried and middelboe, 1977) was used to estimate N_2 fixation in the above ground biomass of the various trees, using the $^{15}N/^{14}N$ ratio of the shoot of flamboyant tree (Delonix spp) as the reference plant. Percent nitrogen fixed (%Ndfa) was calculated using the following equation:

$$\%NdFa = \frac{(1 - \%\ N\ atom\ excess\ in\ fixer)}{(\%\ N\ atom\ excess\ in\ reference)} \times 100$$

3.14 STATISTICS:

All data were subjected to Analysis of variance (ANOVA) by the General Linear model of SAS (SAS, 1985) and significant differences between means were compared by the least significant Difference method (LSD).

CHAPTER FOUR

RESULTS

4.1 Soil characteristics.

The characteristics of the soils used in this study are presented in Table 1. Alajo series with a clay loam texture had the highest content of all parameters measured, while Toje soil a sandy clay loam, had the lowest pH and CEC values with Hatso, of a sandy texture, being lowest in total N, organic matter and available P content.

Table 1: Some chemical properties of the soils used.

PARAMETERS	SOIL TYPES		
	TOJE	HATSO	ALAJO
pH	5.3	6.0	6.8
Total N (%)	0.059	0.034	0.134
Organic matter (%N)	0.65	0.37	1.39
CEC (cmol/kg)	5.84	7.40	25.20
Texture	Sandy clay loam	Sandy	Clay loam
Available P (ppm)	7.44	3.68	12.3

4.2 NODULATION CAPABILITY OF 14 TREE AND SHRUB SPECIES TO NODULATE IN THREE SOIL TYPES.

The result of the initial study to assess the nodulation capability of 14 indigenous trees and shrub species is presented in Table 2. About 60% of the tree legume species nodulated with indigenous soil *Rhizobium* (ie. Eight tree legumes nodulated in one or more soil types whilst six species did not nodulate in any of the three soils). Five species, namely, *Albizia lebbek, Sesbania aculiata, Pithecelobium spp, Tephrosia spp, and Acacia farresiana* nodulated in all the three soils. In Alajo soil about 50% of the tree legume nodulated whilst in Toje and Hatso only about 43%.

Table 2. Nodulation response of fourteen tree legumes by indigenous rhizobia in three soil types.

LEGUME SPECIES	SOIL TYPES		
	TOJE	HATS O	ALAJO
Albizia adianthifola	000	000	000
Albizia zygia	000	000	000
Albizia lebbek	+++	+++	+++
Sesbania specioca	000	+++	+++
Sesbania aculiata	+++	+++	+++
Sesbania rostrata	000	000	+++
Pithecelobium spp	+++	+++	+++
Leucaena spp	+++	000	000
Senna occidentalis	000	000	000
Tephrosia spp	+++	+++	+++
Acacia mangium	000	000	000
Acacia farresiana	+++	+++	+++
Cassia occidentalis	000	000	000
Tamarindus indica	000	000	000

000 means no nodulation

+++ means nodulation

4.3 EFFECT OF PHOSPHORUS APPLICATION ON NODULATION OF NINE LEGUMINOUS TREES GROWN IN THREE SOIL TYPES.

Data on the nodulation abilities of nine leguminous trees with or without phosphorus application to soil are presented in Fig. 1 A, B and C.

Nodulation of the tree legumes with and without phosphorus application was highly variable in terms of nodule numbers and sizes. Phosphorus supplementation to the soil generally resulted in enhanced nodule numbers and sizes; average for the three soils, trees that received P fertilizer formed about 63% more nodules than the no phosphorus treatment.

Pithecelobium spp gave the highest average nodule number per plant (an average of 41/plant for the three soils), with the lowest being *Acacia farresiana*, which formed a mean of three nodule/plant. Out of the tree legumes used in the phosphorus study, 55% formed nodules in Toje soil without P application whilst 66% and 77% formed nodules in Hatso and Alajo soils respectively without phosphorus treatment (Fig. 1). S. rostrata did not form nodules in Toje soil, with or without P. Similarly, Leucaena spp and A. farresiana formed no nodules in Hatso soil with or without P. Also in Alajo soil, A. farresiana did not form any nodules with or without P treatment. For the three soils, nodulation response to phosphorus by the tree legumes was better in Toje soil, with a calculated nodulation response of about 71%, compared to the 58% for Alajo and Hatso soils (fig 2) .

Fig.1(a) : NODULATION OF NINE TREE LEGUME SPECIES WITH OR WITHOUT PHOSPHORUS APPLICATION TO TOJE SOIL.

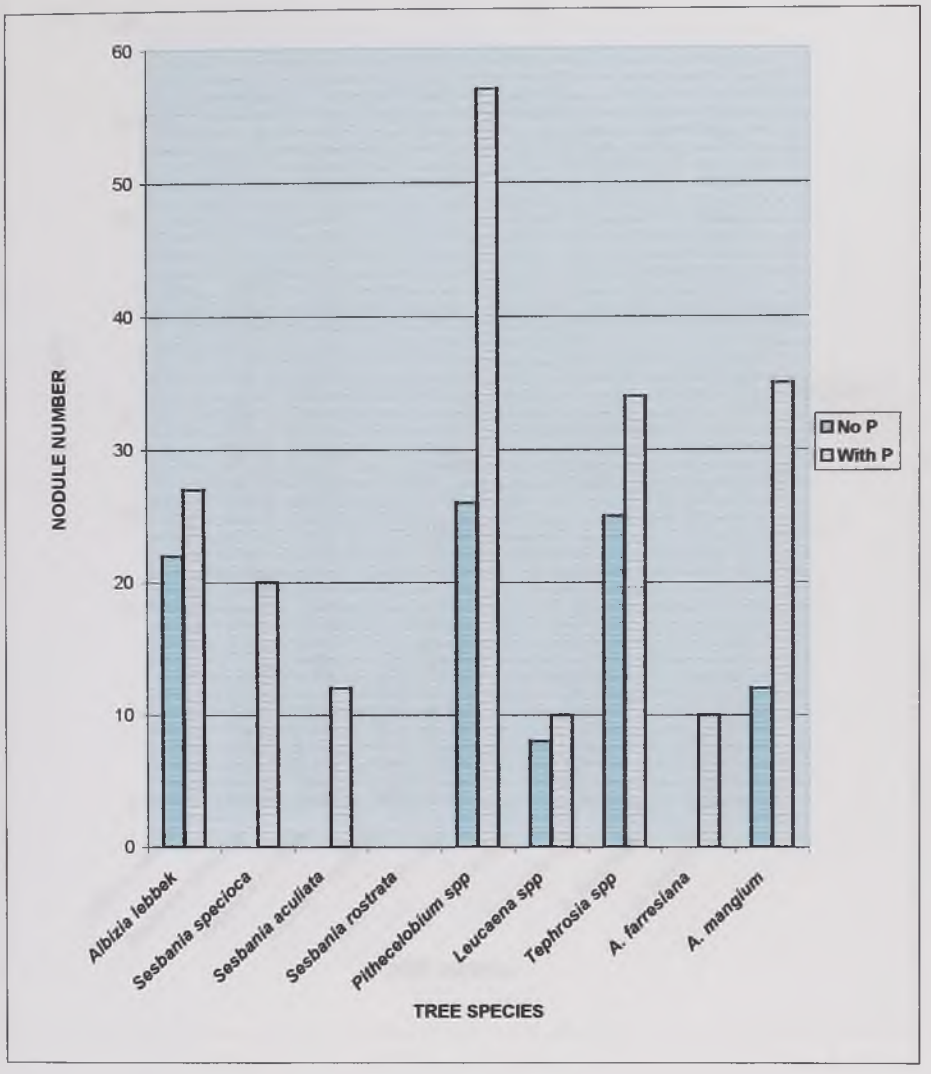


Fig.1(b) : NODULATION OF NINE TREE LEGUMES WITH OR WITHOUT PHOSPHORUS APPLICATION TO HATSO SOIL.

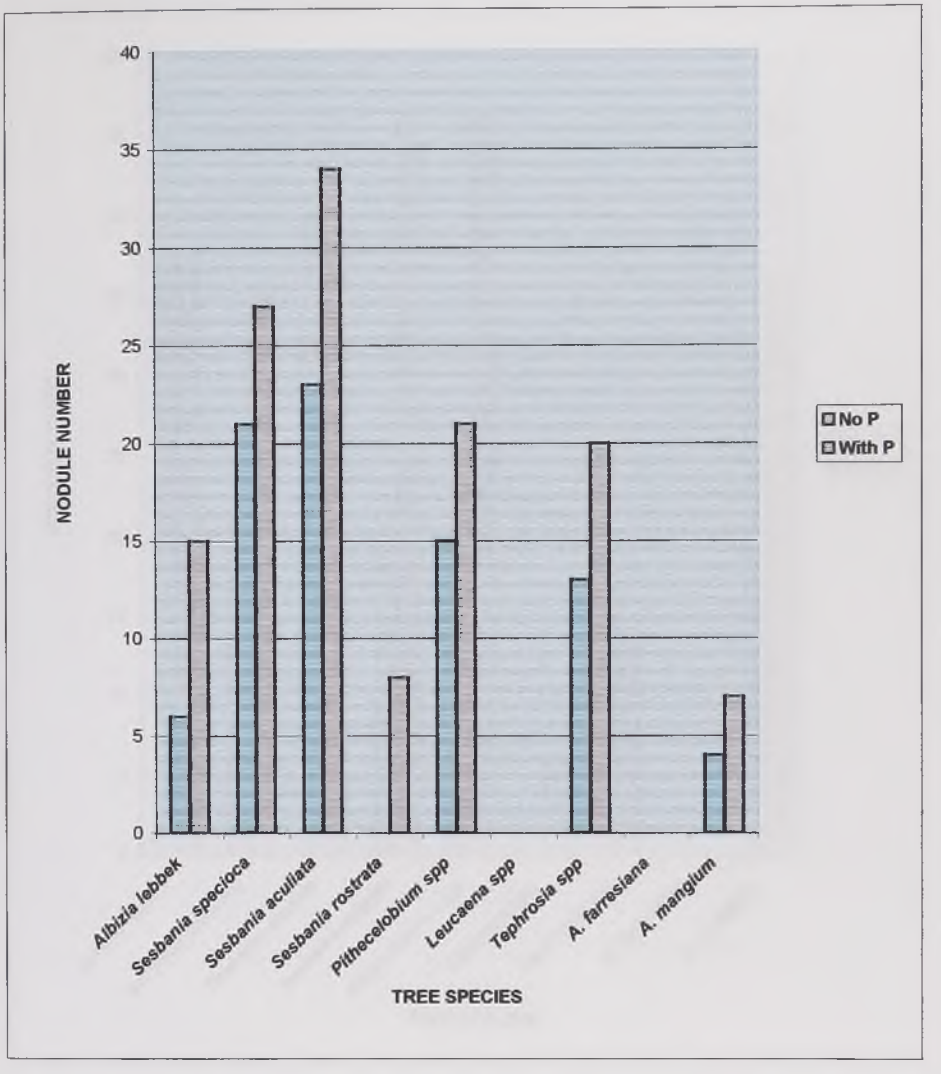


Fig. 1(c) : NODULATION OF NINE TREE LEGUMES WITH OR WITHOUT PHOSPHORUS APPLICATION TO ALAJO SOIL.

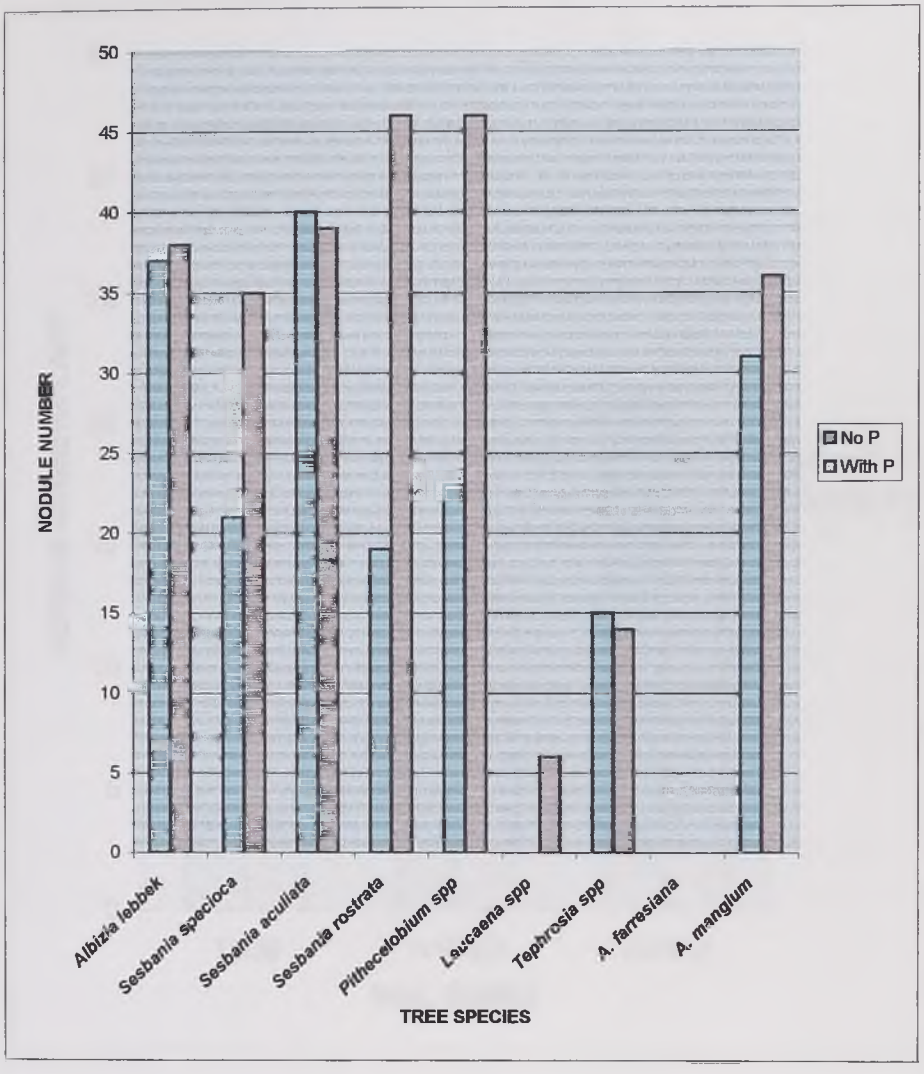
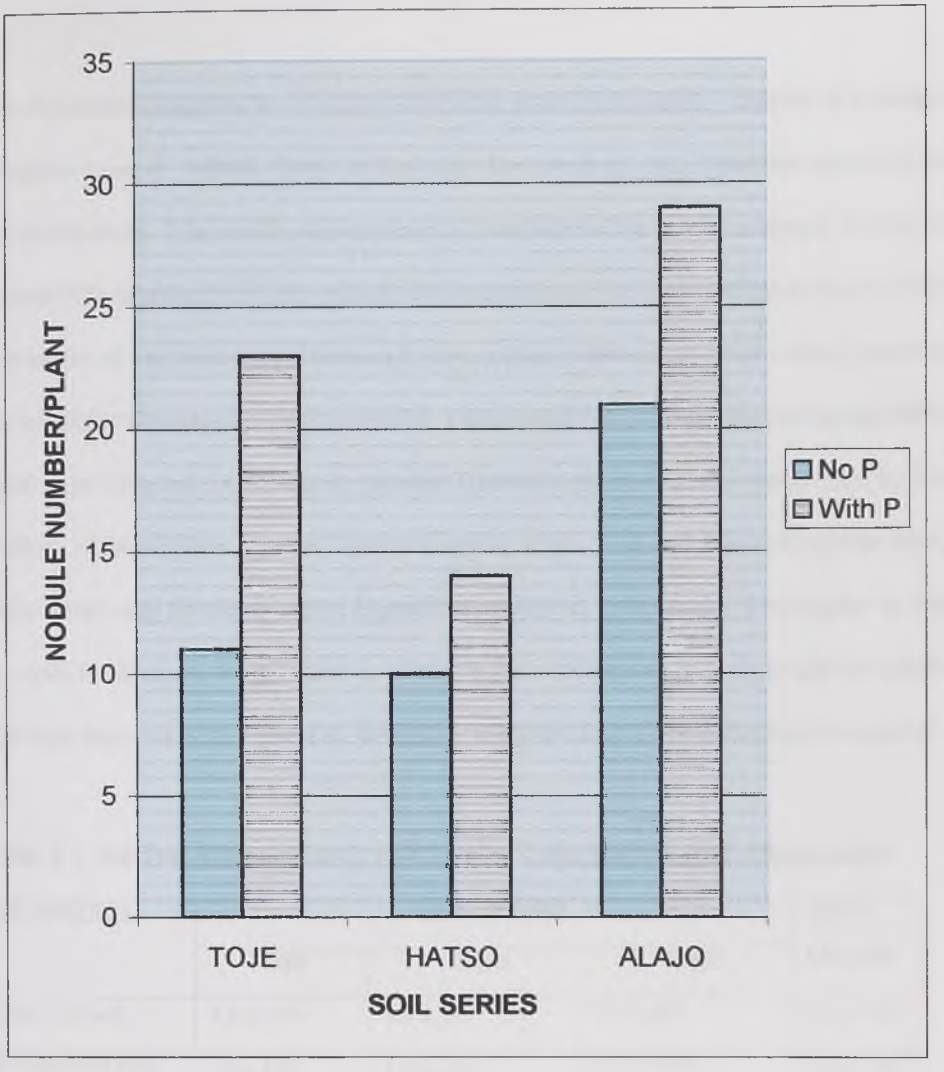


Fig. 2 : Summary of the ability of the three soils Toje, Hatso and Alajo to support nodulation of nine tree legumes, with or without P application.



4.4 MOST PROBABLE NUMBER COUNTS OF NATIVE RHIZOBIA CAPABLE OF NODULATING SELECTED LEGUME TREES.

Table 3 provides information on native *Rhizobium* population density capable of nodulating four of the legume trees, *A. lebbek, Pithecelobium spp, Leucaena spp and Tephrosia spp* in the three soils used for the study. The results indicate that in two thirds of the cases examined, the soil contained in excess of 100 rhizobia/g soil and except for *Leucaena spp* for which no homologous rhizobia were found in two of the three soils (Hatso and Alajo), native *Rhizobium* strains were present in the three soils for all the four legume trees examined. The numbers of rhizobia counted by the MPN method ranged from 31/g soil for *Leucaena spp and Tephrosia spp* in Toje and Hatso soils, to 1700/g soil for *A.lebbek , Pithecelobium spp and Tephrosia spp* in Alajo, Toje and Alajo soil series. Also, except for *Pithecelobium spp* for which native *Rhizobium* capable of nodulating it were higher in Toje soil than Alajo soil, in all cases, the populations of native *Rhizobium*/gram of soil capable of nodulating each of the four legumes were highest in Alajo soil, while the least *Rhizobium* counts occurred in Hatso soil.

Table 3 : Native *Rhizobium*/g soil in the Toje,Hatso and Alajo soils.

TREE SPECIES	SOIL TYPES			TREE MEANS
	Toje	Hatso	Alajo	
Albizia lebbek	5.8×10^2	3.1×10^2	17×10^2	8.6×10^2
Pithecelebium spp.	17×10^2	1.0×10^2	5.8×102	7.9×10^2
Leucaena spp.	3.1×10^1	0.00	0.00	1.0×10^1
Tephrosia spp.	3.1×10^2	3.1×10^1	17×10^2	6.8×10^2
Soil means.	6.55×10^2	1.10×10^2	9.95×10^2	

4.5 CROSS INOCULATION STUDIES

The results from this study are presented in Table 4 .

The isolates from *Tephrosia spp* were the most promiscuous, and except for *Leucaena spp* , they nodulated all the other tree species under study. Isolates from *Leucaena spp* were also able to nodulate *A. lebbek* but not *Pithecelobium spp* and *Tephrosia spp*, but the isolates from *A. lebbek* and *Pithecelobium spp* were specific only for their respective host plants.

Whereas the strain isolated from *A. lebbek* appeared highly specific, this tree was nodulated by isolates obtained from *Leucaena spp* and *Tephrosia spp*. *Pithecelobium spp* and *Tephrosia spp* on the other hand could not be nodulated by rhizobia isolated from any of the other tree species. *Leucaena spp* was nodulated in addition, by the isolates obtained from *Tephrosia spp.*

Pithecelobium spp and *Tephrosia spp* were however not nodulated by any of the isolates except isolates obtained from their respective host.

Table 4 : Cross inoculation response by some tree legumes.

INOCULANTS	TREE SPECIES			
	Albizia lebbek	*Pithecelobium spp*	*Leucaena spp*	*Tephrosia spp*
A (3)	+++	000	000	000
A (7)	+++	000	000	000
P (17)	000	+++	000	000
P (18)	000	+++	000	000
P (20)	000	+++	000	000
L (29)	+++	000	+++	000
L (31)	+++	000	+++	000
T (44)	+++	000	+++	+++
T (46)	+++	000	+++	+++
T (47)	+++	000	+++	+++

A (3) and A (7) - Isolates obtained from *Albizia lebbek.*

P (17) , P (18) and P (20) - Isolates obtained from *Pithecelobium spp.*

L (29) and L (31) - Isolates obtained from *Leucaena spp.*

T (44) , T (46) and T (47) - Isolates obtained from *Tephrosia spp.*

4.6.0 NITROGEN FIXATION RESPONSE BY FOUR TREE LEGUMES TO INOCULATION AND PHOSPHORUS IN THREE SOIL SERIES.

The total amount and proportion of N fixed by the four tree legumes (*A. lebbek, Pithecelobium spp, Leucaena spp*, and *Tephrosia spp*) varied and were influenced by inoculation (fig. 4) , phosphorus fertilizer application (fig. 5) as well as soil series (fig. 6).

4.6.1 (a) Nitrogen fixation in *A. lebbek, Pithecelobium spp, Leucaena spp* and *Tephrosia spp* as influenced by inoculation.

The N fixed by the four tree legumes to inoculation is given in Fig 4. Inoculation of the tree legumes generally resulted in an increase in both percent and total N fixed, however the increase in both %N and total N fixed was not significantly (P = 5%) for the tree species. Inoculation resulted in over 20% increase in %N fixed and 56% increase in total N fixed for the tree species under study.

For all the tree legumes the response to inoculation in terms of increase in total and percent N fixed followed similar trend. The range of increase in total N fixed was from 38.8% for *Tephrosia spp* to 123% for *Leucaena spp* ,whilst the range was from 8% for *Tephrosia spp* to 59% for *Leucaena spp* in terms of increased in percent N fixed .The increases in both %N fixed and total N fixed for the tree legumes as a result of inoculation followed the trend *Leucaena spp* > *A. lebbek* > *Pithecelebium spp* > *Tephrosia spp.*

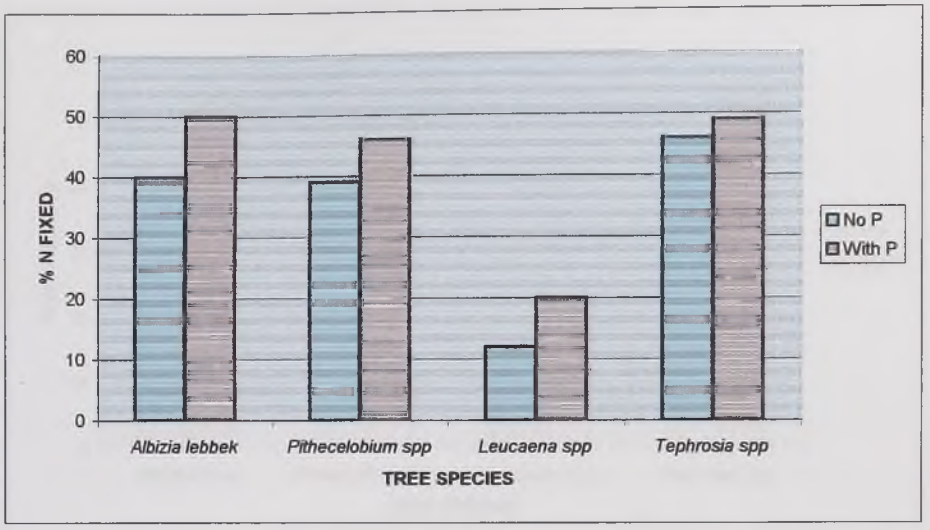
Tephrosia spp, however, gave on the average the highest biological nitrogen fixation (BNF) with the least being *Leucaena spp.*

4.6.2 (b) Nitrogen fixation in *A. lebbek, Pithecelobium spp, Leucaena spp* and *Tephrosia spp* as influenced by P application.

The N-fixed by the tree legumes in response to phosphorus is shown in Fig. 5. Application of phosphorus to the tree legumes resulted in variable response in terms of both percent and total N fixed ; however, in general phosphorus application resulted in significant increases (p=5%) in both percent and total N fixed, however, for the various tree species, there was no significant difference between with and without phosphorus application. Phosphorus application however resulted in more than 38% higher %N fixed and 44% increase in total N fixed by the tree species.

Albizia lebbek gave the highest response to phosphorus application in terms of increased total N fixed (82%) followed by *Pithecelobium spp* (64%) and *Tephrosia spp* (52%), with the least being *Leucaena spp* (27%)

Fig. 4:Nitrogen fixation response of *A. lebbek, Pithecelobium spp, Leucaena spp* and *Tephrosia spp* to inoculation.



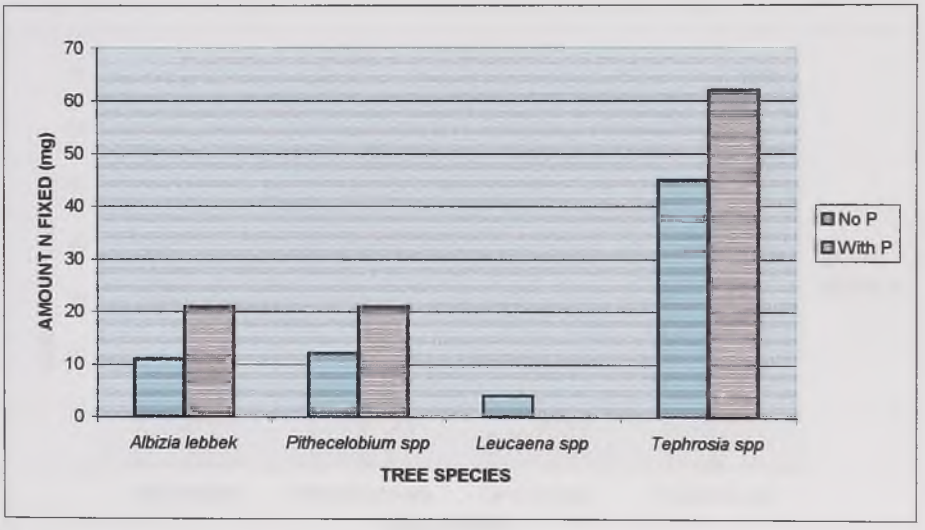
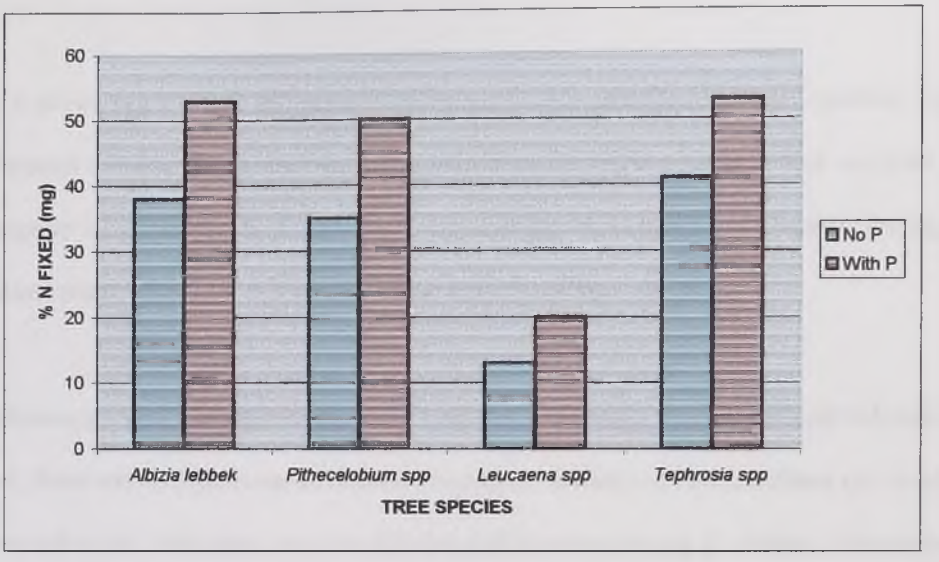
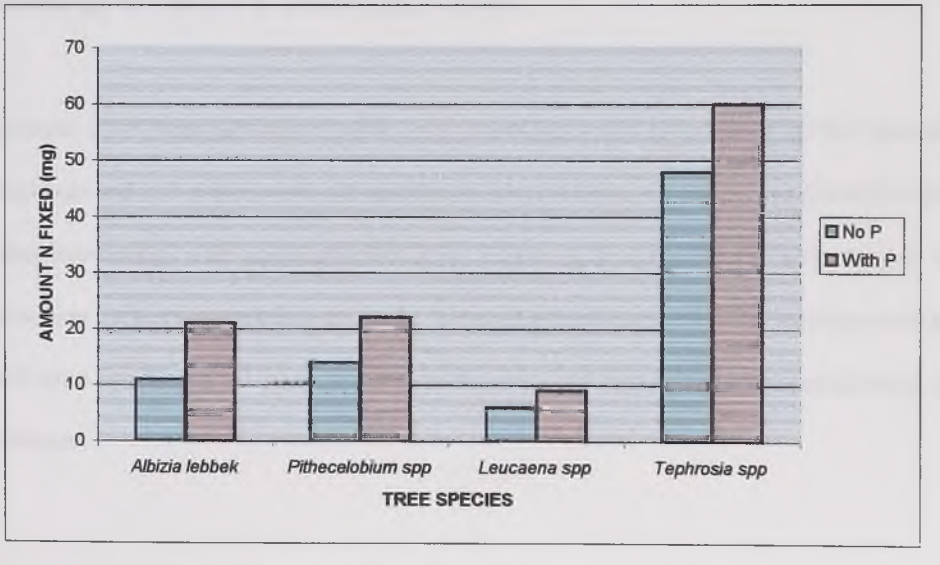


Fig. 5:Nitrogen fixation response of A. lebbek, Pithecelobium spp, Leucaena spp and Tephrosia spp to phosphorus





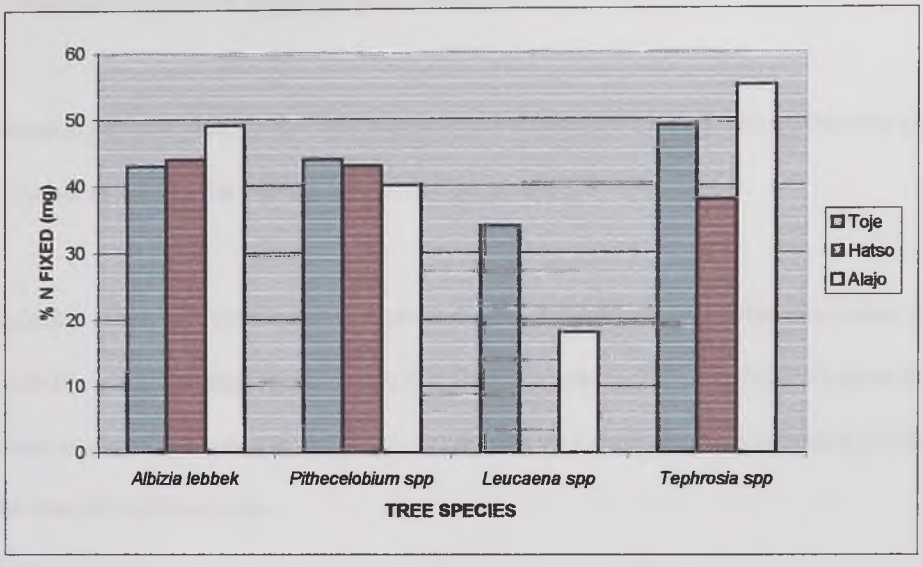
4.6.3 (c) Nitrogen fixation in *A. lebbek, Pithecelobium spp, Leucaena spp* and *Tephrosia spp* and how this is affected by soil series.

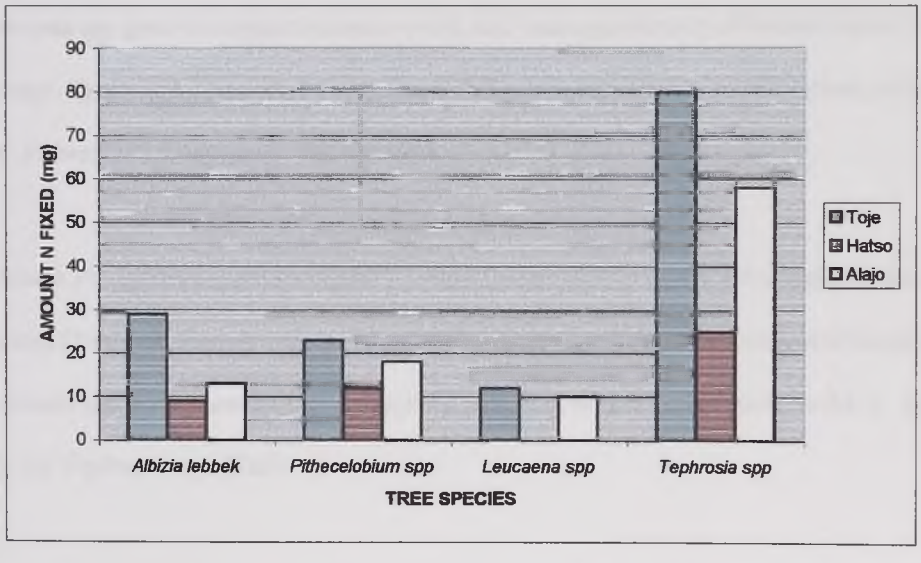
Fig. 6 shows that soil type influenced the nitrogen fixing abilities of the tree legumes. Significant differences (P=5%) were observed among the three soil types in terms of %N and total N fixed. Except for %N fixed for *A. lebbek* and *Tephrosia spp* in Alajo soil, in all cases, the highest N_2 fixation occurred in Toje soil series and the lowest in Hatso soil series.

Tephrosia spp gave the highest N_2 fixed in all the 3 soil series. In terms of both %N and total N fixed, there was no significant difference between *A. lebbek* and *Pithecelebium spp* in Alajo and Hatso soil series. Also there were no significant differences among *A. lebbek, Pithecelebium spp* and *Leucaena spp* in Toje soil in terms of total N fixed.

In general, apart from the interaction between inoculation and trees as well as the interaction between Phosphorus and soil which were not significant for %N fixed, all factors considered as well as their various interactions were significant (P=5%) . However, in terms of total N fixed, apart from the interactions between inoculation and soils, between phosphorus and soils, between soils and trees which were significant, all other interactions between and among the factors considered were not significant .

Fig. 6 : Nitrogen fixation response of four tree legumes on three soil types.





4.7 TOTAL DRYMATTER YIELD OF FOUR TREE LEGUMES TO INOCULATION AND PHOSPHORUS IN THREE SOIL SERIES.

The total dry matter yield of the tree legumes was influenced by both inoculation and phosphorus application as well as the soil types used for the study.

Inoculation of the four tree legumes in general resulted in increases in total dry matter yields (Table 5), with the increasse ranging from 41% for *Tephrosia spp* to 21% for *Pithecelobium spp.* The increase in shoot biomass was, however, not significant (p=5%) for the various tree legumes except in the case of *Tephrosia spp.*

Tephrosia spp gave the highest biomass yield, and was significantly different from all the other tree legumes. There was, however, no significant difference between the total biomass yield of *Leucaena spp, Pithecelobium spp,* and *Albizia lebbek.*

Although Phosphorus application also increased considerably the total dry matter yield of the tree legumes (Table 6), these increases were not significant (p=5%) for the various tree species, except for *Tephrosia spp.* In percentage terms, the yield increases ranged from about 2% for *A. lebbek* to about 45% for *Tephrosia spp* (Table 6)

The increases in total dry matter yield of the tree legumes due to inoculation were on the average similar to those observed for phosphorus application .There was, however, no significant difference between phosphorus and tree interaction as well as phosphorus and inoculation interaction(Table 7).

Total dry matter yield for the tree legumes was also influenced by the type of soil used (Table 8). Significant differences (P=5%) were observed among the soil types in terms of total dry matter yield, There was, however, no significant interaction between soil and tree.

With the exception of *Leucaena spp* in Toje soil, for all the tree legumes, the highest total dry matter yield for each tree species was observed in the Alajo soil series, with the lowest yields occurring in Hatso soil series (Table 8). On the average *Tephrosia spp* gave the highest total dry matter yield in the three soils with the lowest being *A. lebbek*

Total DM yield was significant for inoculation and soil interaction (Table 9) as well as phosphorus and soil interaction (Table 10). Also total DM yields were significant among inoculation treatment, phosphorus treatment and tree interaction as well as the interaction among inoculation, soil and tree. There was also significant difference among phosphorus, soil and tree interaction. Total DM yield was however not significantly affected by inoculation, phosphorus and soil interaction.

Table 5 : Total drymatter yield of four tree legumes as influenced by inoculation

INOC.(I)	TREE SPECIES (T)				Inoc means
	Albizia lebbek	*Pithecelobium spp*	*Leucaena spp*	*Tephrosia spp*	
No Inoc.	1.37	1.63	1.88	4.39	2.32
Inoc.	1.72	1.97	2.29	6.18	3.04
Tree means	1.55	1.80	2.09	5.29	

LSD (5%)

Inoc =0 .27
Tree =0.38

I X T =0.54 (NS)

Table 6 :Total drymatter yield of four tree legumes as influenced by phosphorus.

PHOS.	TREE SPECIES (T)				Phosp means
	Albizia lebbek	*Pithecelobium spp*	*Leucaena spp*	*Tephrosia spp*	
No Phos	1.52	1.65	1.88	4.06	2.47
With Phos	1.58	1.94	2.29	6.52	2.88
Tree means	1.55	1.80	2.09	5.29	

LSD (5%)

Phos = 0.27

Tree = 0.38

P X T = 0.54 (NS)

Table 7: Total drymatter yield of four tree legumes as influenced by three soil types.

SOIL TYPES	TREE SPECIES				Soil
	Albizia lebbek	*Pithecelobium spp*	*Leucaena spp*	*Tephrosia spp*	means
Toje	1.21	1.81	3.68	4.44	2.73
Hatso	1.11	1.58	1.24	4.07	2.00
Alajo	2.32	2.01	1.58	7.34	3.30
Tree means	1.55	1.80	2.09	5.29	

LSD (5%)

Tree (T) = 0.38
Soil (S)= 0.33

T X S = 0.66 (NS)

Table 8 : Total total drymatter yield of four tree legumes as influenced by inoculation in three soils.

INOC.	SOIL TYPES			Inoc.
	Toje	Hatso	Alajo	Means
No inoc.	2.41	1.91	2.63	2.32
With inoc.	3.06	2.09	3.97	3.04
Soil means	2.73	2.00	3.30	

LSD (5%)

Inoc(I). = 0.27

Soil (S) = 0.33

IXS = 0.47 (NS)

Table 9 : Total drymatter yield of four tree legumes as influenced by phosphorus in three soils.

Phosphorus	SOIL TYPES			Phosp. Means
	Toje	Hatso	Alajo	
No Phosp.	2.65	2.05	2.72	2.47
With Phosp.	3.40	1.36	3.88	2.88
Soil means	3.03	1.71	3.30	

LSD (5%)

Phosp.(P) = 0.27

Soil (S) = 0.33

P X S = 0.47

CHAPTER 5

5.0 DISCUSSION

The results of the nodulation studies in the initial screening exercise indicated that, 50% of the tree species that did not nodulate in any of the three soils belong to the sub-family Caesalpinoideae. There is evidence in the literature that majority of non-nodulating legumes occur in the Caesalpinoideae sub-family (Allen and Allen 1981). It is therefore not surprising that of the four tree species from Caesalpioideae examined in this study, only one (25%) nodulated in all the three soils. Earlier studies had shown that *C. occidentalis* and *Tamarindus indica*, as well as another popular multipurpose tree in Ghana, *Tetrapleura tetraptera*, failed to nodulate in several soils examined in the Laboratory of Danso, S.K.A. (personal communication). Based on these studies and the lack of any report on the nodulation on these trees in the literature, it is likely that these trees could be non-nodulating. The pattern of nodulation observed is suggestive of diversity in *Rhizobium* populations, in host range and in the symbiotic efficiencies of the indigenous *Rhizobium* species. The lack of nodulation observed in some soils but not in others and the significant inoculation responses observed for many soils reveal the need to seriously consider seed or soil inoculation with the appropriate effective *Rhizobium* strains for maximum nodulation and nitrogen fixation for many tree legumes (Halliday, 1980; Danso, *et al.*, 1992). The nodulation of *Leucaena spp* in Toje soil suggests that *Leucaena* rhizobia are quite specific, and that, *Leucaena spp* or some

native legumes belonging to the same cross inoculation group as *Leucaena spp* may have grown previously in the Toje soil used for the study, thus accounting for the nodulation of Leucaena in this soil but not others. This is supported by the report that Leucaena usually requires inoculation with specific rhizobia in soils with no history of Leuceaena cultivation (Date and Halliday, 1980).

In general, the interior of nodules were observed to be pink, indicating the presence of leghaemoglobin in the nodules and signifying that they were effective in N_2 fixation. Also, nodules occurred mainly on the lateral roots especially for those legumes grown in Hatso soil, indicating late nodulation probably due to low level of bacterial population in these soils (Table 3).

The response to phosphorus by the tree legumes were variable and depended on the tree species involved as well as the parameter examined.The general inadequacy of P in the three soils is revealed by the fact that the application of 60kg P/ha on the average resulted in significantly improved nodulation (about 63%) as compared to the no phosphorus application. Interestingly, *Sesbania specioca, S. aculiata, and A. farresiana* did not nodulate with the indigenous rhizobia when no phosphorus is applied in Toje soil but did nodulate after phosphorus application. Similarly, *S. rostrata* and *Leucaena spp* nodulated in Hatso and Alajo soils, respectively only after phosphorus application. These results are interesting, as the application of this limiting nutrient alone, without any rhizobium inoculation was capable of changing the nodulation status of a tree.

Phosphorus application probably improved the efficiency of the symbiosis, although the possibility that increases in the populations of soil rhizobia to levels high enough to effect nodulation at the latter stages of the growth of the trees cannot be excluded and should be examined. Despite the general response by the tree to phosphorus,some species, like *A. lebbek, S. aculiata* and *Tephrosia spp* did not respond to phosphorus application on Alajo soil. Similarly, *Leucaena spp* did not respond to application in Toje soil. This finding might give an indication that there were enough compatible rhizobia in those soils and thus inoculation was unnecessary for optimum nodulation (Danso and Owiredu, 1988). The general low response of nodulation to phosphorus applicaton by the tree legumes in Alajo soil compared to Toje soil might be due to the existence of already high number of compatible rhizobia in the soil (Table 2) coupled with the relatively high available phosphorus content of Alajo soil (Table 1).

Nitrogen fixed in terms of both %N and total N fixed for the tree species increased with phosphorus application, except for *Leucaena spp* which did not show significant response to phosphorus application in term of total N fixed. The increase in % N fixed due to phosphorus application in general was not significant, and was in accordance with the findings by Sanginga *et al.* (1990) who observed that % N fixed was little affected by increased levels of P. The very high increase in total N fixed for *A. lebbek* and *Pithecelobium spp* with P application is also in line with the data of Sanginga *et al.* (1990). This was attributed mainly to the increased total dry matter yield when P was supplied.

The results obtained suggest that P is an essential nutrient which needs to be added to many soils in Ghana, for maximum benefits to be derived from N_2 fixation. The results gave an indication for P requirement for growing *A. lebbek* and *Pithecelobium spp.* especially on P- limiting soils since this could also affect nodulation and nitrogen fixation. This agrees with previous finding that a low level of P is among the main chemical constraints for establishing tree legumes on tropical soils (Sanginga, 1985) and that the availability of P is closely associated with nitrogen fixation. Nitrogen fixation in terms of both percent N and total N fixed was highest in Alajo soil and least in Hatso soil. The high fixation in Alajo soil might be due to a relatively high available P in Alajo soil as compared to Hatso soil as well as the high indigenous rhizobia population in Alajo soil.

Inoculation of the tree legumes generally, resulted in an increased number of nodules formed. This is probably due to the higher efficiency and competitive ability of the introduced strains an against the indigenous strains. The various tree species response to Inoculation was generally not statistically significant in terms of both %N and total N fixed, this could be attributed to the high and probably effective indigenous rhizobia population in the soils used for the study. This findings is in accordance with the studies by Singleton and Tavares (1986), which showed that statistically significant inoculation response could be eliminated in the presence of as few as 20 indigenous rhizobia /g soil, and as long as this population contained some effective strains. Thies *et al.* (1991a, 1991b) also showed that yield enhancement with inoculation decreased dramatically with

increasing numbers of indigenous rhizobia. Without inoculation, none of the four tree species examined derived up to 50% of its N from fixation. The implications is that, without inoculation, the soil rather than atmospheric N_2 was the major source of N for the growth of all these trees. Even with inoculation only, *A. lebbek* derived 50% of its N from fixation. These results are in contrast to some of the very high proportions of N in trees reported to be fixed in many trees (Sanginga *et al.*, 1989; Awonaike *et al.*, 1990). It is apparent that further studies are needed to find out factors that accounted for such low percentages of fixation, and how to rectify them.

The tree species gave a similar trend in response to inoculation in terms of increase in percent N fixed and total N fixed. *Leucaena spp* responded highest to inoculation compared to the other tree species with the least being *Tephrosia spp*. Inoculation of *Leucaena spp* and *Tephrosia spp* on average resulted in increases of more than 123% in total N fixed, respectively. This finding suggests that inoculation of *Leucaena spp* with effective *Rhizobium* strain may be more important for maximum nitrogen fixation than in *Tehrosia spp*. Thus the result further suggests that in situations like this it is unnecessary to inoculate the *Tephrosia spp* for maximum nitrogen fixation.

Inoculation also resulted in a general increase in both shoot and root dry production. *Tephrosia spp* responded highest to inoculation in terms of total dry matter yield with the least being *Pithecelobium spp.*

Total biomass production for the tree species was highest for *Tephrosia spp* with the least being *Albizia lebbek* . With the exception of *Tephrosia spp* there were no significant differences among *Albizia lebbek, Leucaena spp* and *Pithecelebium spp* in terms of total dry matter yield. The high biomass production by *Tephrosia spp* indicates that this legume species has a high genetic potential for high yield. It is also likely that both Tephrosia and especially *Leucaena spp* have high soil N uptake capacities than *A. lebbek* and *Pithecelebium spp.*

In all cases total biomass production by the tree species was highest in Alajo soil except in the case of *Leucaena spp* in which highest yield occurred in Toje soil (Fig.9). The high total biomass production by *Leucaena spp* in Toje soil might partly be due to the high nodulation response by *Leucaena spp* on this soil and that the genetic potential for yield of Leucaena in this study was greatly affected by nitrogen fixation.

By comparing and combining nodulation, growth and nitrogen fixation of the four tree legumes in the three soils, *Tephrosia spp* was found to have better nitrogen fixing potential than any of the other tree legumes with the least being *Leucaena spp*, this is because of the ability of Tephrosia to combine high biomass production with high nitrogen fixation.

CHAPTER 6

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Although nodules are important for nitrogen fixation in legumes, most indigenous tree legumes have not been assessed for their nodulation ability with the indigenous soil rhizobia. To contribute to this gap in knowledge, some species of indigenous trees and shrubs were assessed for their nodulation, growth and nitrogen fixing potential in three soil types. The influence of phosphorus and inoculation on these parameters were also assessed.

One kilogramme of each soil series was placed into nursery bags and used as the growth medium. Fourteen species of indigenous trees and shrubs were initially screened for nodulation by growing each of the tree and shrub species for 10 weeks in nursery bags filled with each soil series. The experiment was repeated with nine species of the legumes but in addition two levels of phosphorus (0 and 60mgP/kg) were applied to each soil series in the nursery bags.

A further study was carried out using four of the tree legumes, namely, *Albizia lebbek, Pithecelobium spp, Leucaena spp* and *Tephrosia spp*. These legumes were assessed for capability to fix N_2 as response to inoculation and phosphorus application. Rhizobial isolates were obtained from their respective host plants in the initial screening exercise. A basal amendment was prepared according to Dr.

Grahams new pot experiment formula and applied to the tree legumes. ^{15}N – labeled ammonium sulphate was added to each nursery bag 3 days after emergence. The experiment was allowed to run for 10 weeks after which both the above ground parts (shoot and leaves) and the roots were harvested and the oven dried weights recorded. The dried shoot was milled and passed through 0.5mm sieve-mesh for nitrogen analysis. Nitrogen fixed was determined by the ^{15}N methodology using flamboyant (Delonix) as the reference plant.

Phosphorus application on average resulted in an increase in nodule numbers of the tree legumes by about 63%. It however did not result in significant increase in both %N (about 38%) and total N (about 44%) fixed. Also, with the exception of *Tephrosia spp,* P application did not result in significant increase in total dry matter yield of the tree legumes.

Nodulation generally was highest in Alajo soil with the least in Hatso soil.However, nodulation response to phosphorus was higher in Toje than Alajo owing to the already high P content of Alajo soil series as compared to Toje soil series. Alajo soil on the average contained the highest Rhizobial population of about 1000/g of soil with the least Rhizobial population of about 110/g of soil recorded in Hatso soil.

Inoculation resulted in more than doubled nodule numbers of the tree species, it however did not result in significant increase in both total N

fixed and %N fixed. It also did not significantly increase total dry matter yield except in the case of Tephrosia, a tree species that also responded least to inoculation in terms of %N fixed, with *Leucaena spp* showing the highest responds. Inoculation resulted in an increase of 123% total N fixed for *Leucaena spp.*

In general however, *Tephrosia spp* gave the highest BNF followed by *Pithecelobium spp* and *Albizia lebbek* with the lowest being *Leucaena spp* in terms of both percent and total N fixed.

From the experiments conducted and the results obtained, it can be concluded that many potential nitrogen fixing trees were not fixing nitrogen because they were not nodulated in the soils used for the study. However, inoculation and phosphorus application (60mgP/kg) generally resulted in nodulation of many trees, with consequential increase in nodulation, total biomass and N fixed by these trees. Also, it can be concluded that, *Leucaena spp* although widely published to be a high nitrogen fixer hence suitable for integration in most agroforestry systems, its use must be critically examined especially in those soils used for the study since it gave the least nitrogen fixation.

On the basis of results reported in this research, it is recommended that a lot more soils be screened with multipurpose trees like *A. lebbek, Pithecelebium spp, Leucaena spp and Tephrosia spp* for their nodulation and nitrogen fixing potential

with the view to recommending their use in agroforestry systems in Ghana. It is also recommended that future studies would involve both the pot and field studies to check whether results obtained in pots are comparable to those in the field. Finally, it is also recommended that future studies involving trees and phosphorus would allow enough time for crop growth, a minimum of 3 months is suggested.

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