AN ABSTRACT OF THE THESIS OF

Faroug Mohamed Elhadi for the degree of Doctor of Philosophy in Forest Science presented on October 7, 1987.

Title: Studies on *Acacia senegal* (L.) Wild. in Western Sudan with Special Reference to Variation Among Populations, Host x Soil Inoculum Interactions, and Host X Rhizobium Strains Interactions

Abstract approved: Signature redacted for privacy.
Dr. David A. Perry

Seeds of *A. senegal* (L.) Wild from four populations across a rainfall gradient in western Sudan were grown in two sites. One site is on an upper dune crest - (sandy soil) and the other site is on the lower slope of the same dune - (sandy loam soil). Soils significantly influenced all growth parameters except foliar N concentration. Seedlings of all populations survived and grew better on the upper dune crest (sandy soil). Populations differed only in foliar N. concentration. Although the number of first order branches did not differ among populations, there were large differences among individual seedlings in two of the populations.

In a second study four populations of *A. senegal* from western Sudan were cross inoculated with soil from the same populations. Population x soil inoculum interaction was significant for all measured growth parameters. Specific nitrogenase activity, averaging 22.2 uml/g fw/h, did not

differ significantly between populations, soil inoculum sources, or with interaction between the two. The greatest specificity between populations and source of inoculum was shown by shoot and nodule weights in populations from the extremes of the rainfall gradient. Seedlings from all populations exhibited a relatively high relationship between home soil and root length.

In a third experiement, two Acacia senegal seed lots, one collected near Elobied, a relatively low rainfall zone, and the other near Kas, a high rainfall zone (both in western Sudan), were inoculated with each of the following Rhizobium strains: strain AR 14, isolated from A. radiana, strain AN 12, isolated from A. nilotica adjestringens, strain PJ 12, isolated from Prosopis juliflora, strain TAL 1595 isolated from A. pennetula, and strain ASK isolated from A. senegal. Population x Rhizobium strain interaction was significant for shoot height. Height of Kas seedlings from the high rainfall zone was more sensitive to strain treatment than that of Elobied seedlings from the low rainfall zone. Strain main effect was highly significant for shoot weight. Seedlings inoculated with ASK strain, isolated from A. senegal, had a greater weight, especially in comparison to PJ 12 strains, which were isolated from Prosopis juliflora. Seedlings inoculated with the ASK strain nodulated earlier than seedlings inoculated with other strains.

Studies on *Acacia senegal* (L.) Wild. in Western Sudan with Special Reference to Variation Among Populations, Host x Soil Inoculum Interaction Among Populations, and Host x *Rhizobium* Strains Interactions

by

Faroug Mohamed Elhadi

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed October 7, 1987

Commencement June 1988

APPROVED:

Signature redacted for privacy.

Associate Professor of Forest Science in charge of major

Signature redacted for privacy.

Chairman of department of Forest Science

Signature redacted for privacy.

Dean of Graduate School

Date thesis is presented October 7, 1987

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. Dave A. Perry for his many hours of generous assistance and invaluble advice during the course of this study.

Gratitude is also expressed to Dr. James M. Trappe, Dr. Kermit Cromack, Jr., Dr. Roger G. Petersen, and Dr. Perry J. Brown for their suggestions and review.

Thanks are also due to Dr. X. R. Dommergues, Dr. Blanca Hernandes, Mr. Mohamed Mukhtar and Mr. Abdel Aziz Ahmed for providing the material for the study.

Acknowledgment is further extended to the author's wife Zainab Ahmed, and daughter Amy for their help and moral support.

Gratitude is also expressed to Seham Elhadi and Sadie Airth's Word Processing for typing.

Thanks is also due to the Department of Forest Science for the use of facilities.

This work was financed by the Agency for International Development (AID) and the United States Department of Agriculture (USDA) as a part of the Western Sudan Agricultural Research Project of the Agricultural Research Corporation of Sudan. The author is deeply indebted for their assistance.

The author also wishes to express his sincere and deepest gratitude and appreciation to the people of Sudan.

TABLE OF CONTENTS

LIST OF TABLES

STUDIES ON ACACIA SENEGAL (L.) WILD. IN WESTERN SUDAN WITH SPECIAL REFERENCE TO VARIATION AMONG POPULATIONS, HOST X SOIL INOCULUM INTERACTION AMONG POPULATIONS, AND HOST X RHIZOBIUM STRAINS INTERACTIONS

CHAPTER 1. INTRODUCTION

Acacia senegal (L.) Wild. produces gum arabic, Sudan's third most important commercial commodity next to cotton and oil seeds (National Report of Sudan, 1971). Sudan produces, on the average, 45,000 tons of gum per year, about 85 percent of the world total. Gum arabic is obtained from both *A. senegal* and *A. seyal*, but *A senegal* accounts for 90 percent of the total (National Report of Sudan, 1971). *A. senegal* gum has higher demand and commands a higher price on the world market than that produced from *A. seyal*. The important uses of gum arabic include: 1) additives in confectionary and other food products; 2) as a binding material in pharmaceuticals; and 3) components of metal polishes, inks, dyes, and paints.

A. senegal is important for other reasons as well. As a legume, it forms symbioses with nitrogen-fixing bacteria in the Genus *Rhizobium* (Dreyfus and Dommergues, 1981; Habish and Khairi, 1968), hence enriches soils. Drought tolerant *A. senegal* can be used in forestation programs in arid and semi-arid zones to stabilize sand dunes and stop desertification. In rural areas *A. senegal* is an important source of wood for construction and fuel. In addition, its leaves and pods provide fodder for animals. (Ahmed, 1986).

A. senegal is distributed in an east- west band between latitudes 14° N and 10° N in Sudan, forming what is called the gumbelt (Fig. 1). In Africa A. senegal is distributed throughout an area from the Red Sea to Senegal. Southwards it extends to northern Nigeria, Uganda, and Kenya. A. senegal is also found in India and Pakistan (Cheema and Qadir, 1973). The species grows best where average rainfall is 200-400 mm on sandy soils and 400-750 mm on clay soils, but it occurs in regions with less than 200 mm annual rainfall.

Phenotype, either that of an entire organism or of individual characters, can be simply expressed by the following formula:

$$P = G + E + GE$$

where P, the phenotype, is the sum of total of the effects of three components: the genetic information coded in the chromosomes (G), the environment (E), and GE, the interaction of genotype and environment (Stern and Roche, 1974). According to Perry (1978) and Stern and Roche (1974), genetically controlled geographic variation (GE) is usually large within tree species. Traits -- especially those related to adaptability, such as phenology, temperature optima for photosynthesis, seed weight and drought resistance -- vary widely with climatic clines in photoperiod, temperature and rainfall. Because of this, tree improvement programs may fail unless they are grounded in a basic knowledge of genetic structure--particularly genotype-environment interactions-- within a species.

The diversity of environmental conditions (especially moisture) under which A. senegal occurs naturally suggests that there is a great genetic variability among populations of the species. Individual trees of A. senegal exhibit a large variation in height (2-12 m) (Elamin, 1973). The crown appears in two forms, one flat and spreading, the other round and thick. Sahri (1968) reported that the species produces leaves just before rains (late June) and sheds irregularly, whereas most trees shed their leaves in November or December. Working for four years with A. senegal I have observed that many trees of A. senegal in Sudan retain their leaves after December. I also noticed considerable variation in morphologic and phenologic characteristics among populations of the species.

The likelihood of substantial genetic variation within A. senegal suggests that application of genetic technology might improve reforestation success and provide gains in traits such as gum production and nitrogen fixation. However the possibility of strong genotype-environment interactions within the species argues that genetic technology should not be applied indiscriminantly, but only with a good understanding of the degree to which populations are adapted to specific environments. In other words, genetically-improved stock will be of little use if it doesn't fit site environmental conditions. In arid and semiarid zones of Africa, tree improvement programs are very new and the majority of experiments are still too recent to have yielded precise results (Martin, 1977). To my knowledge no studies have been done to determine genotype-environment interaction of A. senegal.

The performance of any legume depends not only the plant genotype but on that of its associated Rhizobium; studies that ignore the bacterial partner are, at best, incomplete, and at worst inaccurate and misleading (Gordon and Avery 1985). Successful inoculation of any legume requires concomitant concern not only with the ability of Rhizobia to nodulate a certain host but, more importantly, with how effective the symbiosis is in nitrogen fixed per unit of nodule weight (Bell and Nutman, 1971).

Interaction between A. senegal genotypes and Rhizobium strains has not been given the attention necessary to take full advantage of this biological method to fix nitrogen and stabilize soils in arid and semi-arid regions of Sudan. In particular, little is known about ecological specificity in A. senegal and associated Rhizobium strains, i.e. do symbionts (plants and bacteria) from the same site form more effective symbioses than those from differing sites? What about nodulation and nitrogen fixation when A. senegal is inoculated with Rhizobium from other Acacia species, or from woody legumes in other genera?

My interests are ultimately directed towards selecting combinations of A. senegal and Rhizobium genotypes with superior performance in particular environments. At this stage, however, my primary goal is improved understanding of genotype-environment interactions and the nature of Rhizobium strain specificity within the species. Thus, the research reported herein was designed to achieve the following objectives:

1) In a common garden study (conducted in Sudan), to measure genetic variability among seedlings from populations of A. senegal located across a rainfall gradient in western Sudan, and to determine whether populations differ in their response to two soil types (Chapter 2);

2) to investigate ecological specificity between A. senegal and associated Rhizobium strains (in soil inocula) from four locales across a rainfall gradient in Sudan, determining the effect of local vs, nonlocal inocula on nitrogen fixation and early seedling growth (Chapter 3);

3) to compare the effect of Rhizobia isolated from A. senegal, other Acacia species, and from Prosopis juliflora on nodulation rate and nodule effectiveness of A. senegal seedlings (Chapter 4).

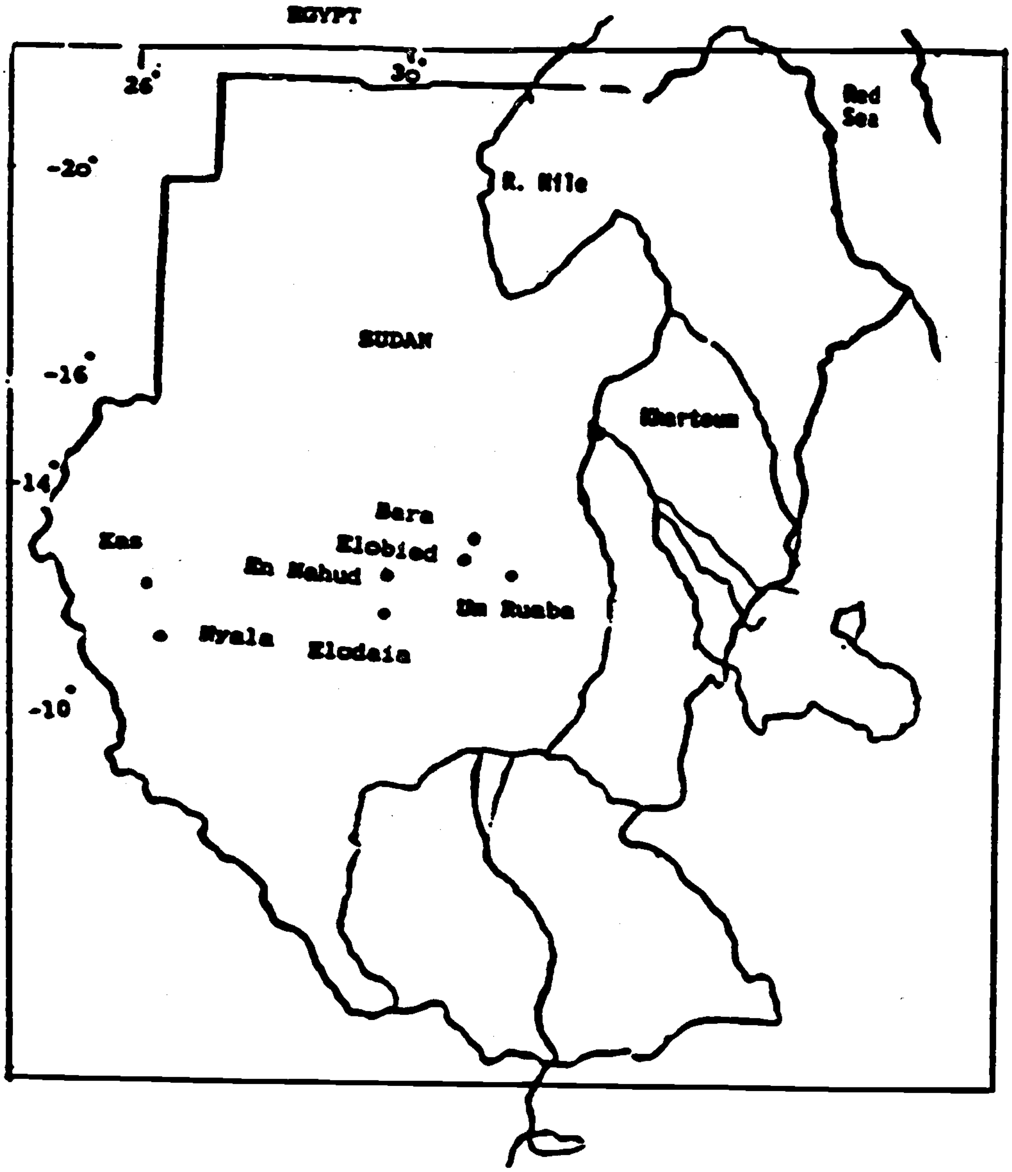

Figure 1. Map of Sudan

CHAPTER 2

GENETIC VARIABILITY AMONG SEEDLINGS OF FOUR POPULATIONS OF ACACIA SENEGAL - ACROSS A RAINFALL GRADIENT IN WESTERN SUDAN

Introduction

The great diversity in environmental conditions (especially moisture) under which *A. senegal* occurs naturally suggests that there is a great genetic variability among populations of the species. Individual trees of *A. senegal* exhibit a large variation in height (2-12 m) (Elamin 1973). The crown appears in two shapes, one flat and spreading, the other round and thick. Sahri (1968) reported that the species produces leaves just before rains (late June) and sheds irregularly, whereas most trees shed their leaves in November or December. In Sudan, many *A. senegal* trees retained their leaves after December. The study by both authors was for taxonomic objectives, and whether variation was due to the genetic make-up of the species, environment, or their interaction, was not discussed. However, working for four years with *A. senegal* I also observed variation in morphologic and phenologic characteristics between populations of the species. A study by Hagedorn and Nixon (1984) indicate that *Acacia magnium* populations vary in height, diameter and number of stems.

Geographic variability is usually large in tree species, especially for traits related to adaptability

(Perry 1978, Stern and Roche, 1974). Phenology, temperature optimum for photosynthesis, seed weight and drought resistance vary with climatic clines in photoperiod, temperature and rainfall. Genetic variation may be substantial within the extensive range of A. senegal. Hence, while the application of genetic technology could significantly effect reforestation success of the species, improved seed that do not match environmental conditions at a site could result in a disappointing outcome, which may be apparent sooner or later in the rotation.

In arid and semi-arid zones of Africa tree improvement programs are new and most experiments are still too recent to give definitive results (Martin 1977). To my knowledge genotype-environment interaction of A. senegal has not been studied.

Regeneration of A. senegal is poor throughout most areas of the gum belt due to drought, grazing and fire. Of these, drought is the major limiting factor that affects regeneration of the species and reduces the density of gum trees below the optimum level. Better understanding of genotype-environment interactions in A. senegal - particularly with respect to moisture and soils - could make management results more predictable. Choosing genotypes with increased adaptation to specific site factors may be the first step toward establishing a tree improvement program for A. senegal in arid and semi arid zones of Sudan.

Objectives of the study reported herein were: (a) to evaluate genetic variability among seedlings of four populations of Acacia senegal spanning a rainfall gradient in the Sudan; (b) to determine whether the different populations responded differently to soil types. Seedling growth parameters of major interest were survival, seedling height, root length, shoot weight, root weight, and foliage nitrogen concentration. The morphological characteristics of interest were number of first order branches per seedling, and the number of leaflets per leaf.

Material and Methods

Seeds of A. senegal were collected in December 1984 from four populations across a rainfall gradient in the gum belt of Sudan. Populations were located near Bara, Elobied and En Nahud in North Kordofan province, and Nyala, in South Darfur province. Geographic locations and annual rainfall of the four populations are shown in Table 2.1.

Seeds were surface sterilized in alcohol for ten seconds and in sodium hypochlorite for three minutes, thoroughly rinsed in distilled water and sown in plastic bags in April, 1985. The planting medium was a 1:1 mixture of sand and clay soils collected near Elobied. Seedlings were grown in a nursery at Elobied until July, 1985, at which time they were transplanted to two field sites near Elobied. One site was an upper dune crest, characterized by sandy soil with clay content increasing with depth in

Table 2.1. Populations' Geographic Locations and Annual Rainfall the locales of seed collection.

No.	Location	Longitude			Latitude			Annual Rainfall
1	Bara	30°	22'	E	13°	24'	N	200 mm
2	Elobied	30°	13'	E	13°	11'	N	350 mm
3	En Nahud	28°	49'	E	12°	42'	N	400 mm
4	Nyala	24°	16'	E	11°	16'	N	500 mm

the profile and the subsoil permeable to water. The other site, on the lower slope of the dune 20 m from the first site, is characterized by sandy loams with clay content increasing with depth in the profile and a subsoil which is less permeable to water than that of the first site. Generally both soils have low organic carbon content and are deficient in nitrogen, phosphorous, calcium and magnesium (Booth, 1966).

Seedlings were outplanted at 4x4 m spacing in a randomized block design with two replicate blocks in each soil type. Each block contained ten seedlings from each population. Survival was recorded and seedlings harvested in November, 1985. Root length and shoot height were measured, number of first order branches per seedling and leaflets per leaf were counted. Shoots and roots were oven dried for 12 hours at 75°C and weighed. Foliar biomass was analyzed for nitrogen concentration by standard Kjeldahl technique according to Easting (1978).

Analysis of variance (ANOVA) was used to determine population differences, soil differences, and population x soil interaction for the following variables: (1) shoot height, (2) shoot weight, (3) root weight, (4) root length, (5) foliage nitrogen concentration, (6) first order branch number, and (7) average number of leaflets per leaf. HSD (Tukey's honesty significant difference) was used to compare means (Petersen, 1985).

Results

Results of ANOVA are shown in Table 2.2. Soils significantly influenced shoot weight ($P < .047$) height ($P < .080$), root weight ($P < .024$) and root length ($P < .0001$). However, populations differed, and responded differently to the two soils, only with respect to foliar N. Survival, which was uniform among populations, averaged 76 % on the upper dune crest and 54 % on the lower dune slope. Number of first order branches and number of leaflets per leaf did not vary significantly with soils, populations, or population x soil interactions.

Seedling sizes in the two soil types are shown in Table 2.3. Seedlings grown on the dune crest (sands) were taller (21.4 cm vs 19.4 cm), produced longer roots (46.4 cm vs 32.6 cm), and had heavier tops (1.88 g vs .51 g) and roots (1.47 g vs 1.10 g) than those grown on the lower dune slopes (sandy loams). Differences in seedling performance between the two locales were highly consistent among the four populations (Tables 2.4a-2.4d).

In contrast to their growth patterns in the two soils, populations responded differently to the two soils with respect to foliar nitrogen ($p < .10$). Seedlings from Nyala and Elobied had higher concentrations on the dune crest than on the lower dune slope, while seedlings from Bara had lower and En Nahud seedlings did not differ between the two sites (Table 2.5). En Nahud seedlings had the highest foliar nitrogen in both soil types, however, while seedlings from Bara had by far the lowest foliar nitrogen of the four populations when growing on the dune crest, they ranked second when growing on the lower dune slope. Population main effect was also significant for foliar nitrogen ($p < .02$), which tended to increase with the source's annual rainfall. However only En Nahud, averaging 3.55 %, and Bara, averaging 3.08 %, differed at the .05 level of significance (Table 2.5).

Number of leaflets averaged seven per tree. Although neither soil nor population main effects, nor their interaction, were significant for number of first order branches (averaging two per tree), seedlings from Nyala and Bara --at the two extremes of the rainfall gradient-- exhibited wide variation in this trait. Nyala seedlings had from 1 to 10 branches and Bara seedlings from 1 to 8. Such a wide range in number of first order branches was not observed for Elobied and El Nahud, which had a range of 1 to 4 and 1 to 6 branches, respectively.

Table 2.2 ANOVA: For Growth Variables Measured

Source of Variation	DF	Shoot Height (cm)	Shoot Weight (gr)	Root Weight (gr)	Root Length (cm)	Foliar N%
		Mean Squares				
Soil	1	143.99*	8.14**	5.17**	7185.66**	0.068
Population	3	52.10	1.56	1.04	309.3	1.74**
Soil x Population	3	53.64	1.69	0.454	265.9	1.25*
Error	8	49.25	2.04	1.004	308.19	0.56

* Significant at 0.10
** Significant at .05

Table 2.3. *A. senegal* Shoot Height, Root Length, Shoot Weight and Root Weight by Soil Type.

Soil Type	Shoot Ht. cm	SE	Root Length cm	SE	Shoot Wt. gr.	SE	Root Wt. gr.	SE
Sand	21.4a	(0.5)	46.4a	(6.9)	1.88a	(0.51)	1.47a	(0.35)
Clay	19.4b	(0.2)	32.6b	(6.9)	0.51b	(0.51)	1.10b	(0.35)

Means with different letters are significantly different at 0.05.

Table 2.4a. *A. senegal* Shoot Height (cm) by Soil Types within Populations, and by Populations

Population	Soil Type		Population Mean
	Sandy Soil	Sandy Loam Soil	
Nyala	20.0	20.9	20.5
En Nahud	20.9	18.5	19.7
Elobied	24.4	19.6	22.0
Bara	20.1	18.6	19.4

Pooled SE = 4.95 for soils and 2.47 for populations.

Table 2.4b. *A. senegal* Root Length (cm) by Soil Types within Populations and by Populations

Population	Soil Type		Population Mean
	Sandy Soil	Sandy Loam Soil	
Nyala	52.1	31.5	41.8
En Nahud	42.3	34.4	38.4
Elobied	48.5	35.1	41.8
Bara	42.3	29.8	36.1

Pooled SE = 12.41 for soils, and 6.2 for populations.

Table 2.4c. Shoot Weight (gr) by Soil Types within Populations and by Populations

Population	Soil Type		Population Mean
	Sandy Soil	Sandy Loam Soil	
Nyala	1.61	1.51	1.56
En Nahud	1.95	1.35	1.65
Elobied	2.44	1.46	1.95
Bara	1.56	1.40	1.48

Pooled SE = 1.07 for soils and 0.712 for populations.

Table 2.4d. Root Weight (gr) by Soil Types within Populations and by Populations

Population	Soil Type		Population Mean
	Sandy Soil	Sandy Loam Soil	
Nyala	1.53	1.04	1.29
En Nahud	1.54	1.02	1.28
Elobied	1.68	1.29	1.49
Bara	1.11	1.06	1.09

Pooled SE = 0.70 for soils and 0.50 for populations.

Table 2.5. Foliage Nitrogen Concentration (%) by Populations within Soils, by Soils and by Populations.

	Populations				
Soil Type	Nyala	En Nahud	Elobied	Bara	Soil Mean
Sandy Soil	3.45	3.53	3.35	2.86	3.35a
Sandy Loan Soil	3.28	3.57	3.10	3.29	3.31a
Population Mean	3.46ab	3.55a	3.23ab	3.08b	

Means with different letters are significantly different at 0.05. Pooled SE = 0.52 for populations, 0.26 for soils and 0.37 for soils within populations.

Discussion

All A. senegal populations survived and grew better on the upper dune crest (sandy soil) than on the lower dune slope (sandy loams). This is probably related to different moisture conditions at each site. Even though sandy soil has low water-holding capacity, it has good drainage and aeration, and water percolating to depth can be reached by the long A. senegal roots. In addition, rapid percolation reduces evaporation from sands, while the relatively dark surface resulting from greater organic matter in the sandy loams absorbs heat, raising soil temperature and increasing evaporative loss. These factors combine to make sands the more efficient moisture traps, particularly for relatively small rainfall events.

The populations used in our study exhibited surprising uniformity in average seedling size, particularly in view of the fact that they spanned a wide precipitation gradient. In common garden studies, early growth rate of temperate tree species generally correlates closely with growing conditions at the population home locale (Perry 1979). Hagedorn and Nixon (1984) found significant differences in seedling height and diameter among A. magnium populations. My inability to demonstrate differences is probably due to a combination of low replication and high within-population variability. The latter possibility is supported by the wide range of branches occuring in individuals within two of the populations. Large differences in branch number are most likely accompanied by differences in at least top weight.

Populations of A. senegal did differ significantly in foliar nitrogen concentration. Variability among populations for foliage nitrogen concentration was also reported by Hielman (1985) in a provenance study of black cottonwood and its hybrids. In my study, differences may be due to different interactions of local Rhizobium strains with different populations (Chapter 3, Chapter 4). Optimum nitrogen concentrations generally result in seedlings that exhibit enhanced field survival and height growth (Duryea and McClain, 1983; Fisher and Mexal, 1983). What the "optimum" may be for A. senegal is not known, however.

It is not surprising that branch number and leaflet number did not vary with soils. Characters that form rapidly, such as reproductive structures and leaf shapes, are fixed at an early stage of development and are therefore less influenced by environment than characters formed by long periods of meristematic activity, such as size of vegetative parts and stem elongation (Stephen and Burton 1980).

Patterns of adaptive variation have direct implications for forest management (Rehfeld, 1985). A major goal of A. senegal improvement programs should be to select superior tree genotypes and compatible Rhizobium strains suited to particular site conditions and aimed at achieving specific ends. This goal can be achieved if variation among A. senegal genotypes is recognized and packaged into individual genotypes with the desired performance. For example, early vigorous root growth of A. senegal is important for seedling establishment and field survival in arid and semi-arid zones of western Sudan. Early nodulation is probably also important for survival (Dobereiner and Campelo 1977, Wilson and Coutts 1985, Chapter 4). If livestock browsing is an intended use, high foliar nitrogen will provide nutritious forage. Having a large number of branches per tree increases the number of sites that can be tapped without injuring the tree, hence enhances gum production (unpublished report by Elobied Gum Arabic Station).

Tree improvement programs may be an important aid in establishing A. senegal plantations in order to stabilize sand dunes, help stop desertification, increase gum arabic production, and perhaps enhance agroforestry uses for the species. The wide range in number of first order branches within some populations of this study suggests that, like many other tree species, sufficient genetic variation exists within populations of A. senegal to allow selection of genotypes that are both superior performers and site-adapted. Such selection, however, should not be at the expense of the ecologically important genetic variability within populations. Particularly in today's uncertain and rapidly changing global environment, genetic diversity, because of the buffering capacity it provides, is itself an important trait to be selected for (Perry 1978, 1985, Perry and Maghembe 1988).

CHAPTER 3

INTERACTION BETWEEN SEEDLINGS OF FOUR POPULATIONS OF _A. SENEGAL_ AND FOUR SOIL INOCULA-ACROSS A RAINFALL GRADIENT - FROM WESTERN SUDAN.

Introduction

Acacia senegal is an important tree in savanas and arid regions of Sudan (Elhadi 1983). The species produces gum arabic, Sudan's third most important commodity following cotton and oil seeds. _A. senegal_ trees protect soils against wind erosion and, in rural areas, are an important source of wood for both construction and fuel. The ability of the species to fix atmospheric nitrogen (N), coupled with a deep and extensive root system, allow it to flourish on drought, infertile sites that are unsuitable for most crops. _A. senegal_ enriches soils in both N and carbon (C) (Booth 1966, Ahmed 1986) and leaves and pods provide fodder for domestic animals.

The expense of commercial fertilizers, and advantages of multipurpose tree crops combine to make N-fixing trees an important component of the agricultural systems of developing countries (Domingo 1983, Gordon and Avery 1985, Robinson 1985). Moreover, drought tolerant, N-fixing trees and shrubs may offer the best opportunity to stop or at least slow the spread of deserts, and to reclaim lands lost to desertification.

Understanding the relationship between tree and endophyte is a necessary prerequisite for optimizing the value of N-fixing trees in either agroforestry systems or reclamation (Gordon and Avery 1985), however interaction between leguminous trees and Rhizobium strains has received scant attention (Wison and Couts 1985). Dreyfus and Dommergues (1981), who studied the ability of 13 Acacia, species to nodulate with fast and slow-growing strains of Rhizobium, did find significant host X endophyte interaction. However, virtually nothing is known about the degree of ecological specificity in the symbioses, i.e. do trees from a given population perform best when inoculated with soils from the population locale--or a locale with a similar environment--or is such specificity insignificant. The objective of this study was to investigate the degree to which such ecological specificity existed in A. senegal populations and associated Rhizobium from different rainfall zones in western Sudan. We hypothesized ecological specificity would exist; i.e. that growth, nodulation, and nitrogenase activity of seedlings from a given population would correlate positively with proximity of the inoculum source (within the rainfall gradient) to the population's home locale, reaching maximum with soil from the home locale.

Material and Methods

Seeds of Acacia senegal were collected in November 1984 from four natural stands (populations) located near

Um Ruaba, Elobied, En Nhaud, and Elodaia in North Kordofan Province, Sudan. Population locales span a rainfall gradient of 325 to 450 mm annually. Geographic locations and rainfalls are shown in Table 3.1. Soils were collected in October 1985. In each stand, mineral soil to 100 cm depth was collected from five points separated by at least 100 m. These five collections were then composited to give a single soil sample per locale.

Table 3.1. Geographic Locations And Annual Rainfall Of Sampled Stands.

No.	Name	Longtitude	Latitude	Annual Rainfall (mm)
1	Um Ruaba	31° 14' E	12° 56' N	325
2	Elobied	30° 13' E	13° 11' N	350
3	En Nahud	28° 40' E	12° 42' N	400
4	Elodaia	28° 13' E	12° 00' N	450

Seeds were surface sterilized in 95% alcohol for ten seconds and in sodium hypochlorite for three minutes, then thoroughly rinsed in distilled water. Modified Leonard jars (Vincent 1967) were used to grow the seedlings. Seeds were planted in surface sterilized plastic pots filled with perlite and inoculated with 100 g of soil from one of the population locales. After planting, a 2 cm layer of sterilized sand coated with paraffin was spread on the perlite surface to minimize aerial contamination and reduce surface evaporation. The five seedlings allowed to establish per pot were thinned to two by snipping tops following emergence. Watering was with a reservoir system. Each pot was fitted within a second, plastic-lined pot, to which water and nutrient solution were periodically added. A cloth wick facilitated movement of the nutrient solution from the reservoir to the planting pot. Irrigation water contained the following concentrations of mineral salts (per liter of distilled water): 0.20 g $CaHPO_4$, 0.04 g each of K_2HPO_4, $MgSO_4 \cdot 7H_2O$ and Nacl, and 0.02 g $Fecl_2$. The pH was adjusted to 7.0. Immediately prior to use, irrigation water was autoclaved for 45 min. at 120°C.

The experiment was carried out in a glass greenhouse. Automatic timers controlled temperature, light, and air movement. Temperature was automatically maintained at 25°C during the day and 20°C at night. A photoperiod was programmed at 12 hours. Ventilation was controlled by automatic fans to insure uniform air distribution.

Seedlings from each population received one of six soil inoculum treatments: one from each of the four population locales, one with no soil added but with 10 mg/l KNO_3 in irrigation water (high N treatment), and one with no soil and 2 mg/l KNO_3 in irrigation water (low N treatment). Added soils contained nutrients as well as various biological inocula; it is reasonable assume that a significant portion of seedling response to a given soil was due to the addition of Rhizobium. Each population X soil inoculum treatment combination was represented by five replicate pots, while the high N treatment was replicated four times and the low N treatment three times within each population. This gave a total of 108 pots in the experiment. To reduce the risk of cross contamination, pots from each inoculation treatment were separated by at least 30 cm. Periodic rotation of pot locations within the greenhouse minimized the chance of experimental error due to location within the greenhouse. Pot locations within a given inoculum treatment were completely random.

Seedlings were grown for 12 weeks. Upon lifting, roots were separated from the shoots and subjected to acetylene reduction assay to estimate nitrogenase activity (Hardey et al 1968; Hardy et al 1973). Freshly harvested unwashed intact nodules were placed in jars; each jar was sealed with a lid fitted with a rubber serum stopper. Purified acetylene, generated from calcium carbide and water, was injected into each jar to 10% (V/V), then the jars were immediately swirled and left to stand at 28°C. After one hour 0.1 ml gas samples from each jar were removed and analyzed for C_2H_2 and C_2H_4 with Hewlett-Packard 5830A gas chromatograph fitted with 2 m x 2.1 mm 80-100 mesh Porapak R column. Injection and flame ionization detector temperatures were adjusted to 100°C. Nitrogen carrier gas flow rate was adjusted to 40 ml per min.

Acetylene reduction rates were measured as umoles C_2H_2/g nodule fresh weight/h. Shoot and root lengths were recorded, and nodules separated from roots and weighted. Seedling tops and roots were oven dried for 12 hrs at 70°C then weighed.

Data were analyzed as a factorial set of experiment in a completely randomized design. Within each population, means for each soil inoculation treatment were compared to that of the "home" inocula (soil from that population locale) using Fisher's Protected LSD (Petersen 1985).

Results

Only one nodule formed on one of the noninoculated seedlings, indicating minimal cross-pot contamination. Neither populations nor inoculation treatments (main effects, inoculation treatment including soil inocula and the two N treatments) differed significantly for any variable. However, interactions between populations and inoculation treatment were significant for shoot weight ($p < .005$), height ($p < .002$), root weight ($p < .002$), root length ($p < .016$), total plant weight ($p < .002$), and nodule weight ($p < .016$) (Table 3.2). Specific nitrogenase activity, averaging 22.2 umol/g fw/h, did not vary significantly between inoculum sources, populations, or with interaction between the two. Across all treatments, shoot weight correlated closely with height ($r^2 = .77$), and total plant weight (r^2 - .88); nodule weight correlated closely with plant weight (r^2 = .88). For shoot weight the greatest specificity between populations and source of inoculum was in Elodaia and Um Ruaba, the two populations from the extremes of the rainfall gradient. Within the Elodaia population, seedlings inoculated with Elodaia soil were significantly larger than those inoculated with En Nahud and Um Ruaba soil, and were also larger than uninoculated seed in both the high and low N treatments. Um Ruaba seedlings inoculated with Um Ruaba soil had significantly heavier shoots than those of all other treatments. The two populations from the middle of the precipitation gradient showed no such home soil preference. Source of soil inoculum did not affect top growth of En Nahud seedlings, while, unlike seedlings from Elodaia and Um Ruaba, their top growth was significantly improved by the high N treatment. Growth of Elobied seedlings was significantly enhanced (relative to home soil inocula) by both the high N treatment, and by inoculation with Um Ruaba soil (Table 3.3).

Root weights followed the same general pattern as shoots, but with one major exception. Whereas shoot growth of Um Ruaba seedlings was best in Um Ruaba soil, root growth was insensitive to both inoculum source and fertilization (Table 3.4). In contrast to shoot and root weights, seedlings from all populations exhibited a relatively strong relationship between home soil and root lengths

(Table 3.5). In all but the Elobied population, home soil tended to produce shorter roots than other soils or than the two N treatments. Elobied seedlings, in contrast, grew longer roots when inoculated with home soil. Patterns of nodule weights closely followed those of shoot weights (Table 3.6). Elodaia and Um Ruaba seedlings both produced the greatest nodule weights when inoculated with their own soils. The difference was striking with Um Ruaba seedlings, which formed from 2.5 to 6 times greater nodule weights with their own than with other soils. Elobeid seedlings, on the other hand, produced 60% less nodule weight with their own soils than with soils from both Elodaia (the wettest site) and Um Ruaba (the driest site). Elodaia soils produced nearly twice as many nodules on En Nahud seedlings as En Nahud soils, however Um Ruaba soils produced slightly but significantly fewer.

Table 3.2. ANOVA for Growth Variables

Source of Variation	DF	Shoot Weight	Root Weight	Root Length	Nodule Weight
		Mean Squares			
Inocula	3	1.082*	1.141**	649.287**	9.803**
Population	3	0.929**	0.968*	235.484**	4.394
Inocula x population	9	0.510**	0.519*	74.692**	8.301**
Error	54	0.206	0.366	35.515	3.190

* Significant at .10
** Significant at .05

Table 3.3. Shoot Dry Weight (g) By Populations and Soil Inoculant

Inoculant	Populations			
	Elodaia	En Nahud	Elobied	Um Ruaba
Elodaia	1.34	0.83	0.68	0.17**
En Nahud	0.44**	0.67	0.26	0.13**
Elobied	1.03	0.68	0.60	0.37*
Um Buaba	0.72**	0.55	1.24**	0.86
+N	0.58**	1.60**	1.16*	0.36*
-N	0.36**	0.30	0.15	0.29*

*, ** = mean differs significantly at 0.1 or at .05 (respectively from mean for "home" inocula (underlined). Pooled SE for soil inoculants = 0.20, for +N = 0.23, and for -N = 0.26.

Table 3.4. Root Dry Weight (g) by Population and Soil Inoculant

Inoculant	Populations			
	Elodaia	En Nahud	Elobied	Um Ruaba
Elodaia	0.99	0.33	0.45	0.18
En Nahud	0.30**	0.27	0.18	0.38
Elobied	1.22	0.76**	0.33	0.85
Um Ruaba	0.46**	0.39	1.02**	0.46
+N	0.68	1.41**	0.90	0.38
-N	0.36**	0.58	0.17	0.15

** = mean differ significantly at .05 from mean for "home" inocula (underlined). Pooled SE for soil inoculants = 0.17, for +N = 0.19, and for -N = 0.22.

Table 3.5. Root Length (cm) by Population and Soil Inoculant

Inoculant	Populations			
	Elodaia	En Nahud	Elobied	Um Ruaba
Elodaia	16.8	22.4**	12.8**	10.8**
En Nahud	18.0	17.0	17.5**	17.0
Elobied	34.0**	28.2**	38.4	25.4**
Um Ruaba	26.6**	28.4**	27.2**	19.0
+N	25.3**	26.3*	24.0**	22.0
-N	20.3**	20.3**	17.6**	11.3**

** = mean differs significantly at .05 from mean for "home" inocula (underlined). Pooled SE for soil inoculants = = 1.1, for +N = 1.2, and for -N = 1.4.

Table 3.6. Nodule Fresh Weight (mg) by Populations and Soil Inoculant

Inoculant	Populations			
	Elodaia	En Nahud	Elobied	Um Ruaba
Elodaia	130	130**	100**	70**
En Nahud	50**	70	70**	110**
Elobied	100**	70	40	40**
Um Ruaba	110**	60**	100**	270

** = mean differ significantly at .05 from mean for "home" inocula (underlined). Polled SE = 7.8.

Discussion

Despite the limited number of populations and soil inoculum investigated in this study, significant population x soil inoculum interactions were found for all measured parameters except rate of C_2H_2 reduction. Significant interaction between host genotype and Rhizobium strains has also been reported for herbaceous legumes (Caldwell and Vest 1977, Hobbs and Mahon 1982, Pulver *et al* 1985). In our study, neither population nor soil inoculum (main effects) differed significantly, suggesting that independent selection of plant or rhizobial strain genotypes is not the most efficient way to improve biological nitrogen fixation (c.f. Wilson and Couts 1985).

Shoot weight and nodule weight relationships to hosts and home inocula were much stronger in the two populations from the extremes of the rainfall gradient than in the two from the middle. The relationship of of nodule formation to population was especially strong in trees from the driest site--Um Ruaba--which formed from 2.5 to more than 6 times more nodule weight with home soil than with other inocula sources. In contrast, trees from Elobeid and En Nahud, in the middle of the gradient, did exhibit preference, but not for home inocula. In fact, Elobeid trees had poorest nodule formation when inoculated with home soils. This was not due to lack of inoculium, because Elobeid soils produced good nodule formation on Elodaia trees. Pulver et al (1985) suggest that nodule weight indicates the degree of compatibility between host and rhizobium strain, while dry weight measures the N-fixing effectiveness of the symbiosis. The patterns that we detected suggest that, at least in western Sudan, particular attention should be paid to properly matching hosts and symbionts in relatively dry or relatively wet environments.

Rate of H_2C_2 reduction by nodules of A. senegal (22.3 umol/g fw/h) resembles that of A. pellita (22.8 umol/g x fw/h) Langkamp (1979), and exceeds that reported by Monk et al (1981) for A. pulchella (11.5 umol/g fw/h). H_2C_2 reduction by A. senegal also exceeds that reported by Schubert and Evans (1973) for 19 nonwoody legumes, which ranged from 1.25 to 17.37 umol/g fw/h. Hobbs and Mahon (1982) also found that differences in nitrogen fixation rate of pea genotypes with different Rhizobium strains were due to nodulation rather than nodule activity.

For various reasons, growth patterns within the controlled environment of the greenhouse might differ from those in the field, especially in soil moisture. Future studies might explore effects of water deficiencies on inoculam effectiveness. Hence, field assessment is a necessary step after this initial greenhouse study. The importance of A. senegal in maintaining the integrity of arid and semi-arid regions in Sudan in the face of potential deforestation by wind erosion, fire, animals, and humans, make such field research worthwhile.

CHAPTER 4

INTERACTION BETWEEN SEEDLINGS OF TWO POPULATIONS OF A. SENEGAL - FROM HIGH AND RELATIVELY LOW RAINFALL ORIGINS IN WESTERN SUDAN - AND FIVE RHIZOBIUM STRAINS

Introduction

Acacia senegal is the most important tree in arid and semi arid regions of Sudan. Drought-tolerant and capable of fixing atmospheric dinitrogen (Dreyfus and Dommergues 1981, Habish and Khairi 1986), the species enriches arid and semi arid soils in nitrogen and organic carbon (Booth, 1966). Forestation with *A. senegal* has considerable potential for stabilizing sand dunes, hence slowing desertification. *A. senegal* is important economically as well as ecologically. It produces gum arabic, Sudan's third most important commodity following cotton and oil seeds, and is an important source of construction and fuel wood in rural areas. In addition, its leaves and pods provide fodder for animals (Ahmed, 1986).

As with any leguminous tree, the success of *A. senegal* in forestation programs may well depend on the accompanying *Rhizobium* strain (Gordon and Avery 1985, Wilson and Couts 1985). *Rhizobium* exhibits considerable specificity, both with different host species and with different genotypes within a species (Wilson and Couts 1985). Understanding and utilizing this specificity has considerable potential. For example, Dobereiner and Campelo (1977) found that good nodulation in the nusery nearly doubled survival of *Mimosa* *caesalpiniaefolia* upon outplanting, and increased growth by 60 percent. Despite potential benefits, however, little is known about specificity between leguminous trees and *Rhizobium* strains.

Elsewhere we have shown that populations of *A. senegal* from extremes of a rainfall gradient within western Sudan do exhibit specificity for local *Rhizobium* strains (soil inocula), while populations from the center of the rainfall gradient generally do not (Chapter 3). Specificity in this case was expressed as nodule formation and growth, but not specific activity of nodules. In the study reported herein we tested the specificity of seedlings from two *A. senegal* populations for *Rhizobium* isolates from several associated species. Isolates were from *A. senegal*, *A. nilotica*, *A. raddiana*, *A. pennetula* and *Prosopis juliflora*. We hypothesized that *A. senegal* would form more effective symbioses with *Rhizobium* isolated from *A. senegal* than with isolates from other host species. Variables of interest were shoot weight, shoot height, rapidity of nodulation and nitrogenase activity.

Material and Methods

Two seed lots of *Acacia senegal* were obtained from the gum arabic research station, Western Sudan Agricultural Research Project, Elobied, Sudan. One seed lot had been collected near Elobied, a relatively low rainfall zone, and the other near Kas, a relatively high rainfall zone (Table 4.1).

Table 4.1. Geographic Locations and Annual Rainfall of Sampled Stands.

No.	Name	Longitude	Latitude	Annual Rainfall (mm)
1.	Elobied	30° 13' E	13° 11' N	350
2.	Kas	24° 13' E	12° 30' N	600

Strain AR 14, isolated from A. raddiana, strain AN 12, isolated from A. nilotica adstringens, and strain PJ 12, isolated from Proscopis juliflora, were obtained from Dr. Y. Y. R. Dommergues (Laboratory of Microbiology - Dakar, Senegal). Strain TAL 1594, isolated from A. pennetula was obtained from Dr. Blanca Hernandes - University of Panama. Strain ASK was isolated in our laboratory - Forest Science, Oregon State University - from fresh nodules of pot grown A. senegal seedlings which were raised from Elodaia - Sudan (450 mm annual rainfall) - seeds and inoculated with Elodaia soil. Nodules were washed free of soil and surface sterilized by immersion in 95% ethanol for 30 sec followed by treatment with 0.2% HCl (acidified with 5 ml/l con. Hcl) for 4 min., then were rinsed in six changes of sterile distilled water. Surface-sterilized nodules were dissected under aseptic conditions and the fluid from within the nodules spread over plates of yeast-extract manitol agar (YEMA) and incubated at 26°C for four days (Vincent 1970). Cultures were maintained in the YEMA which was used as a solid nmedia for petri dishes. YEMA has the following mineral salt composition per liter of distilled water: 1.0 g K_2HPO4, 0.2 g NaCl, 1.0 g $MgSO_4 \cdot 7H_2O$, 0.5 mg $FeSO_4$, 1.0 mg $CaCl_2$ and 1.0 g yeast-extract (Vincent 1970).

A. senegal seeds were surface sterilized in 95% alcohol for ten seconds and in sodium hypochlorite for three minutes, then thoroughly rinsed in distilled water. Inoculation was performed immediately prior to sowing. Each seed lot was set out in a small sterile dish which then received a suspension of the test strain. Seeds were then grown in autoclaved plastic pouches with an absorbent paper towel inserted (Miller 1988). Seedlings were planted in a fold at the upper rim of the pouch. Three seeds were planted per pouch and thinned to one after emergence. Roots developed within the pouch, and were easily visible, and plant tops grew in the open air. Irrigation water contained the following concentrations of mineral salts (per liter of distilled water): 0.20 g $CaHPO_4$, 0.04 g each of K_2HPO_4, $MgSO_4H_2O$ and NaCl, and 0.02 g Fcl_3. The pH was adjusted to 7.0. Immediately prior to use, irrigation water was autoclaved for 45 min. at 120°C. Pouches were put in a light room with overhead illumination using regular flourescent lamps. A light timer programmed to provide 12 hours photoperiod was used, and temperature was maintained at 25°C.

Seeds from each population received seven treatments: inoculation with each of the five Rhizobium strains, no inoculation but with 10 mg/l KNO_3 in irrigation water (plus N treatments), and neither inoculation nor added N (minus N treatment). Throughout, we refer to these seven as the "strain" treatments. Each population X strain treatment combination was represented by five replicate pouches, giving a total a 70 pouches in the experiment. Pouch locations were completely randomized.

Seedlings, grown for 5 weeks, were scored for nodule formation once each day. Upon lifting, roots were separated from the shoot to estimate nitrogenase activity by acetylene reduction assay (Hardy et al, 1968; Hardy et al, 1973). Freshly harvested unwashed roots with intact nodules were placed in jars, which were then sealed with a lid fitted with a rubber serum stopper. Purified acetylene, generated from calcium carbide and water, was injected into each jar to 10% (V/V), then the jars were immediately swirled and left to stand at 28°C. After one hour 0.1 ml gas samples from each jar were removed and analyzed for C_2H_2 and C_2H_4 with a Hewlett-Packard 5830A gas chromatograph fitted with 2 m x 2.1 mm 80-100 mesh Porapak R column. Injection and flame ionization detector temperatures were adjusted to 10°C. Nitrogen carrier gas flow rate was adjusted to 40 ml per min. Acetylene reduction rates were measured as moles C_2H_4/seedlings (nodules were too small to accurately weigh). Shoot length was recorded, and tops were oven dried for 12 hours at 70°C, then weighed.

Data were analyzed as a factorial experiment in a completly randomized design. Means were compared to that of the "ASK strain" (isolated from A. senegal) using Fisher's Protected LSD (Petersen, 1985).

Results

Noninoculated seedlings did not nodulate, indicating no bacterial contamination between pouches. Population x strain interaction was significant only for shoot height ($p < 0.05$) (Table 4.2), height of Kas seedlings being much more sensitive to strain treatments than that of Elobied seedlings (Table 4.3). Within the Kas population (high rainfall), all strain treatments except TAL 1594 (from A. pennetula) were shorter than ASK (the A. senegal isolate). In contrast, among Elobied seedlings only those in the -N treatment were shorter than ASK-inoculated seedlings.

Strain main effect was highly significant for shoot weight ($P < 0.0002$), due primarily to low weight of seedlings inoculated with PJ 12 (from P. juliflora), and of seedlings in the -N treatment (Table 4.4). Although population-strain interaction was not significant at the .05 level, the pattern of variation in shoot weights resembled that of heights: Kas seedlings exhibited greater response to the ASK isolate than seedlings from Elobeid. In fact, among the four Acacia isolates, Elobied seedlings had the lowest average weight when inoculated with ASK (Table 4.5).

Strain main effect for mean of number of days to first visible nodules followed the same pattern as shoot height (Table 4.6): seedlings inoculated with ASK nodulated earlier than seedlings inoculated with other strains. The mean number of days to first visible nodules for ASK strains (16 days) was not significantly different from that of seedlings inoculated with TAL 1594 strain (17 days), but did differ significantly from that of seedlings inoculated with AN 12, AR 14 and PJ 12 (averaging 19, 21 and 24 days to visible nodulation, reespectively). The two populations were quite similar in this case (Table 4.7). There was a strong negative correlation (r = -.97) between mean number of days to first nodules and shoot dry weight.

Only seven seedlings showed C_2H_2 reduction, with no pattern by treatment. The seedlings which did reduce C_2H_2 had a higher average shoot weight than the average of all nodulated seedlings (85.5 g vs 64.5 g).

Table 4.2. ANOVA - For Growth Parameters

Source of Variation	DF	Shoot Height	Shoot Weight	Number of Days to First Visible Nodulation
			Mean Squares	
Population	1	4.128	1320.228	0.0200
Strain	4	85.857*	3507.561*	97.65*
Population x strain	4	25.295*	1019.761	1.70
Error	30	11.121	646.521	7.200

*Significant at .05.

Table 4.3. Shoot Height of A. senegal Seedlings by Populations and Strain or N Treatment

Strain or N Treatment	Rhizobium Source	Shoot Height (cm) Population Kas	Elobeid
ASK	A. senegal	15.6	13.4
TAL 1594	A. pennetula	15.8	12.2
AN 12	A. nilotica	9.0*	13.0
AR 14	A. raddiana	10.4*	15.0
PJ 12	P. juliflora	9.4*	10.8
+N		10.2	11.4
-N		6.8*	4.8*

*Significant at 0.05 from means for ASK strain "isolated from A. senegal" (underlined). Pooled SE = 1.5

Table 4.4. Shoot Weight of A. senegal Seedlings by Strains or N Treatment

Strain or N Treatment	Rhizobium Source	Shoot Weight (mg)
ASK	A. senegal	79.3
TAL 1594	A. pennetula	76.7
AN 12	A. nilotica	68.9
AR 14	A. raddiana	67.9
PJ 12	P. juliflora	49.2*
+N	-	59.0
-N	-	26.4*

*Significant at 0.05 from means for ASK strain "isolated from A. senegal" (underlined). Pooled SE = 8.0.

Table 4.5. Shoot Height of A. senegal Seedlings by Populations and Strain or N Treatment

Strain or N Treatment	Rhizobium Source	Shoot Weight (mg) Population	
		Kas	Elobeid
ASK	A. senegal	90.4	68.2
TAL 1594	A. pennetula	76.6	76.6
AN 12	A. nilotica	47.6*	90.2
AR 14	A. raddiana	59.0*	76.8
PJ 12	P. juliflora	40.8*	57.6
+N	-	59.0*	67.0
-N	-	26.4*	24.2*

*Significant at 0.05 from means for ASK strain "isolated from A. senegal" (underlined). Pooled SE = 11.41.5

Table 4.6. Number of Days to First Visible Nodules of A. senegal Seedlings by Strains

Strain or N Treatment	Rhizobium Source	Days to first Visible Nodulation
ASK	A. senegal	16
TAL 1594	A. pennetula	17
AN 12	A. nilotica	19*
AR 14	A. raddiana	21*
PJ 12	P. juliflora	24*

*Significant at 0.05 from means for ASK strain "isolated from A. senegal" (underlined). Pooled SE = 0.87

Table 4.7. Number of Days to First Visible Nodulation of A. senegal Seedlings by Populations and Strains or the N Treatment

Strain or N Treatment	Rhizobium Source	Days to first Visible Nodulation Population	
		Kas	Elobeid
ASK	A. senegal	16	16
TAL 1594	A. pennetula	17	18
AN 12	A. nilotica	19*	19*
AR 14	A. raddiana	21*	20*
PJ 12	P. juliflora	24*	24*

*Significant at 0.05 from means for ASK strain "isolated from A. senegal" (underlined). Pooled SE = 1.2

Discussion

All Rhizobium strains used in this study were capable of nodulating seedlings from both Acacia senegal populations, suggesting that, when nodulation alone is used as a criterion, the species possesses low specifity for Rhizobium--at least for those strains that are able to infect other woody legumes. This conclusion is supported by the study of Habish and Khairi (1968), who cross inoculated 21 species of legumes commonly found in Sudan, including A. senegal A. seyal, A. mellifera and A. albida. They found that A. mellifera, A. seyal and A. senegal cross inoculated among themselves and produced effective nodules, while strains from A. albida cross inoculated and produced effective nodules only with A. albida and A. senegal. A. senegal failed to form nodules, however, when inoculated with strains isolated from nonwoody legumes in the cowpea and soybean groups.

Though all strains in our study were capable of infecting and nodulating A. senegal, however, not all were equally effective in producing rapid nodule formation and seedling growth. Herrera (1980) found that Prosopis chilenses formed more effective symbioses when inoculated with its own Rhizobium than when inoculated with Rhizobium isolated from Acacia cyanophylla or A. melanoxyloan. Robinson (1969) crossed-incoulated red clover and subterranean clover and found that plants of either host species nodulated faster and more effectively when incoulated with cultures isolated from homologous host than did plants inoculated with hetrologous hostg. Our results, and the findings by Herrera (1980) and Rabinson (1969) support the theory which suggests that genetically interrelated plants generally form more productive symbioses with Rhizobium strains from each other than they did with Rhizobium strains from unrelated plants (Mytton 1975).

Although the greatest degree of incompatibility between A. senegal and the various Rhizobium strains was with the Prosopsis isolate, Acacia isolates clearly differed in their effect on height growth of seedlings from the Kas population. Only the A. pennetula isolate produced height growth equal to that of the A. senegal isolate. No such specificity existed in the Elobeid seedlings, however, supporting our previous findings that _A. senegal_ populations from extremes of the rainfall gradient in western Sudan exhibit greater specificity for _Rhizobium_ than those from the middle of the rainfall gradient (Chapter 3). Interestingly, of the four _Acacia_ species tested, _pennetula_ is the only one that is not native to Sudan, suggesting that compatability may be more related to ecological similarity than to geographic proximity. Compared to _A. senegal_, _A. auddiana_ occurs in drier habitats, along rivers and seasonal valleys and on loamy or gravelly soil within savanna grassland, while _A. nilotica_ occurs in relatively wet, clayey soils, usually on the inside bend of rivers, or on mudflats formed by periodic flooding (Sahni, 1968; Elamin, 1973).

Pulver _et al_ (1985) suggested that shoot dry weight is a measure of the effectiveness of a given host-_Rhizobium_ association. In our study, shoot weight did not differ significantly among seedlings inoculated with the various _Acacia_ isolates. However, we believe that it would be premature to conclude that strains do not differ in their effectiveness. Mean differences in shoot weight followed the same pattern as height differences, and more replication may well have established statistical significance. The strong negative correlation between shoot weight and days to nodulation supports the idea that there are subtle, but real, differences in the effectiveness of the various _Acacia_ isolates. Hobbs and Mahon (1981), who inoculated ten genotypes of Pisum sativums with two strains of Rhizobium leguminosarum under environmentally controlled conditions, demonstrated that highly effective plants nodulate slightly earlier than less effective plants. Our study was also conducted under controlled conditions with ample resources. In field environments, rapidity of nodulation and vigor of top growth could be a critical to survival (Dobereiner and Campelo 1977), and subtle incompatabilities more obvious.

This and our other studies (Chapter 3) indicate that identifying proper host-symbiont combinations can be an important aid to successful forestation with A. senegal. Future research should be performed in field situations, and must be aimed not only at identification of suitable symbionts, but toward further clarification of the degree to which populations of A. senegal differ in their specificity for Rhizobium strains.

LITERATURE CITED

Ahmed, E. A. 1986. Some aspects of dry land afforestation in the Sudan with special reference to Acacia tortilis (Forsk.) Hayne, A. senegal Willd. and Prosopis Chilensis (Molina) stuns. Forest Ecology and Management. 61:209-221.

Bell, F., and P.S. Nutman. 1971. Experiments on nitrogen fixation by nodulated lucerne. Plant and Soil, special volume. 231-264.

Booth, G. A. 1966. Survey of forests in Central Kordofan. Report issued for F.A.O. and the Min. of Agr., Khartoum Sudan.

Caldwell, B. E., and Grant Vest. 1968. Nodulation interaction between soybean genotypes and serogroups of Rhizobium japonicum. Crop Science 8:680-682.

Cheema, M.S.Z.A., and S. A. Qadir. 1973. Autecology of Acacia senegal (L.) Willd. Vegetatio. 27:131-162.

Dobereiner, J. and A. B. Campelo. 1977. Importance of legumes and their contribution to tropical agriculture. In: A Treatise on Dinitrogen Fixation. Section 4: Agronomy and Ecology, R. W. F. Hardy and A. H. Gibson (ed.) 191-220. New York: John Wiley.

Dreyfus, B. L., and Y. R. Dommergues. 1981. Nodulation of Acacia species by fast- and slow-growing tropical strains of Rhizobium. Appl. Environ. Microbiol. 41:97-99.

Duryea, M. L., and K. M. McClain. 1983. Altering seedling physiology to improve reforestation success. In: Seed Physiology and Reforestation Success. M. L. Duryea, and G. N. Brown (eds.) Martinus Nijhoff/Dr. W. Junk publishers. Boston, pp. 77-114.

Easting, E. F. 1978. Total nitrogen determination for plant materials. Anal. Biochem 85:591-594.

El-Amin, M. H. 1973. Sudan Acacias. Forest Research Institute. Bulletin No. 1. Information Department, Khartoum.

Elhadi, F. M. 1984. The effect of presowing treatments of Acacia senegal seeds on germination and early seedling establishment. MS Thesis. Washington State University, Pullman.

Fisher, J. T., and J. G. Mexal. 1983. Nutrition management: A physiological basis for yield improvement. In: Seed Physiology and Reforestation Success. M. L. Duryea, and G. N. Brown (eds.). Martinus/Dr. W. Junk publishers. Boston, pp. 269-298.

Gordon, J. C. and M. E. Avery. 1985. Improving tree crops using microorganisms in designed systems. In: Attributes of Trees as Crop Plants. M.G.R. Cannel (ed.), Institute of Terrestrial Ecology, Huntigdon England.

Habish, H. A., and S. M. Khairi. 1968. Nodulation of legumes in The Sudan: cross-inoculation groups and the associated Rhizobium strains. Exp. Agric. 4:227-234.

Hagedorn, S. F., and K. M. Nixon. 1984. A provenance trail of Acacia mangium in Zululand. Report, Wattle Research Institute, University of Natal.

Hardy, R. W. F., R. C. Burns, and R. D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry 5:47-81.

Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. 43:1185-1207.

Heilman, P. E. 1985. Sampling and genetic variation of foliar nitrogen in black cottonwood and its hybrids in short rotation. Can. J. For. Res. 15:1137-1141.

Herrera, M. A., E. J. Bedar, and J. Olivares. 1985. Host specificity of Rhizobium strains isolated from nitrogen-fixing trees and nitrogenase activities of strain GRH2 in symbiosis with Prosopis chilenssis. Plant Science. 42:177-181.

Hobbs, S. L., and J. D. Mahon. 1982. Effect of pea (Pisium sativum) genotypes and Rhizobium leguminosarum strains on N_2 (C_2H_2) fixation and growth. Can. J. Bot. 60:2594-2600.

Langkamp, P. J., B. L. Swinden, and M. J. Dalling. 1979. Nitrogen fixation (acetylene reduction) by Acacia pellita on areas restored after mining at Groote Eylandt, Northern territory. Aust. J. Bot. 27:353-361.

Martin, B. 1977. Eucalypts as exotics: recent progress in problems of genetic improvement of tropical forest trees. D. G. Nickles (ed.), Oxford University.

Miller, S.L., C. Lanphear-Cook, and O.K. Miller. 1988. Ectomycorrheal synthesis in the genus Rhizopogon mycotaxon (in press).

Monk, D., J. S. Pate, and W. A Loneragan. 1981. Biology of *Acacia pulchella* R. Br. with special reference to symbiotic nitrogen fixation. Aust. J. Bot. 29:579-592.

Mytton, L. R. 1975. Plant genotype X *Rhizobium* strain interactions in white clover. Ann. Appl. Biol. 80:103-107.

National Report of Sudan. 1971. United Nations Conference on "The Human Environment". The National Councel for Research, Khartoum.

Perry, D. A. 1974. Ecotypic variation in *Pinus contorta* Dougl. var. *Latifolia* Engelm. (Lodgepole pine). Ph.D. thesis, Montana State University.

Perry, D. A. 1978. Variation between and within species. In: The Ecology of Even-aged Plantations. Proceedings of the Meeting of Division 1 International Union of Forestry Research Organization, D.C. Ford.

Perry, D. A. 1985. The Competition Process in Forest Stands. In: Attributes of Trees as Crop Plants. M.G.R. Cannell & J.E. Jackson (eds.). Institute of Terrestrial Ecology, Abbots Ripton, Huntingdon, England.

Perry, D. A. and J. Maghembe. 1988. The ideotypic community paradigm. Submitted to forest Ecology & Management, Oregon State University, Corvallis.

Petersen, R. G. 1985. Separation of Means. In: Design and Analysis of Experiments, D. B. Owen (Ed.), Marcel Dekker, Inc., New York. pp. 72-111.

Pulver, E. L., E. A. Kueneman, and V. Ranga-Rao. 1985. Identification of promiscuous nodulating soybean efficient in N_2 fixation. Crop Science. 25:660-663.

Rehfeldt, G. E. 1985. Ecological genetics of *Pinus contorta* in the Wasatch and Uinta Mountains of Utah. Can. J. For. Res. 15:524-530.

Robinson, A. C. 1969. Host selection for effective *Rhizobium trifollii* by red clover and subterranean clover in the field. Australian Journal of Agricultural Research. 20:1053-1060.

Sahni, K. C. 1968. Species distribution and illustrations. In: Important Trees of the Northern Sudan. Khartoum University Press, Khartoum.

Stephen, H.S., and V. B. Burton. 1980. Forest Tree Variability and Diversity. In Forest Ecology. pp. 11-46. John Wiley and Sons, New York.

Stern, K. and Roche, L. 1974. Adaptive strategies. In: Genetic of Forest Ecosystem. J. Jacobs (ed.). Springer Verlag, New York.

Vincent, J. M. 1970. A manual for the practical study of root-nodule bacteria. IBP Handbook No. 15. Blackwell Scientific Publications, Oxford.

Wilson, J. and M. P. Coutts. 1985. Exploiting tree crop-symbiont specificity. In: Attributes of trees as crop plants. M.G.R. Cannel and J.E. Jackson (ed.). Institute of Terrestrial Ecology, Abbots Ripton, Huntigdon, England.