D E D I C A T I O N

For a love pure and true:

To my dear wife, Joyce, and the children who had

to bear my long absence from home.

TO GOD BE THE GLORY.

STUDIES ON THE DEVELOPMENT OF *RHIZOBIUM* INOCULUM USING MOSS AS CARRIER FOR BAMBARA GROUNDNUT, *VIGNA SUBTERRANEA* (L) VERDC.

A Thesis Presented by

JOSEPH ADDO AMPOFO

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From: The Department of Botany
University of Ghana
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I hereby declare that the work presented in this thesis:

"STUDIES ON THE DEVELOPMENT OF RHIZOBIUM INOCULUM USING MOSS AS CARRIER FOR BAMBARA GROUNDNUT, VIGNA SUBTERRANEA (L) VERDC."

was done entirely by me in the Department of Botany, University of Ghana Legon from August 1990 to August 1991.

This work has never been presented either in part or completely, for any degree of this University or elsewhere.

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J.A. AMPOFO, B.Sc.(Hons)Dip.Ed.
University of Ghana
Legon.

Date: 27-11-91

.....

PROFESSOR G.C. CLERK
SUPERVISOR

Date: NOV. 27, 1991

A B S T R A C T

Research was carried out to develop an effective inoculum carrier of the nodule bacterium, **Rhizobium** sp., for the inoculation of seeds of bambara groundnut [**Vigna subterranea** (L.) Verdc. comb. nov. = **Voandzeia subterranea** (L.) Thouars] from a local material. Five out of 16 strains of **Rhizobium** sp. isolated from soils of legume farms at eight localities within a radius of 180 km from Legon were used in experiments to test the efficiency of the carrier that was developed. Plants infected by these five strains grew well, had high dry weights and high nitrogen content of the shoots and formed many large nodules.

A good **Rhizobium** carrier was developed from the moss, **Brachymenium** sp. Harvested thalli were sun-dried for 10 days and decomposed for 15 days. When stored at room temperature, the **Rhizobium** population of inoculated moss compost of 50 per cent moisture content rose to a maximum of 4.00×10^{11} cells per gram of compost after 10 weeks. The population remained stationary thereafter till the end of the 14th week without showing any signs of autolysis. Sterile compost inoculated and stored at room temperature for two weeks when the **Rhizobium** population rose to approximately 1×10^{11} cells per gram of compost was used to inoculate the seeds in the various experiments.

Ex-Ada and Ex-Tamale varieties of bambara groundnut inoculated with the five strains of **Rhizobium** sp. all nodulated. Plants of Ex-Ada variety developed best at low light intensity (1100 - 2200 lux at 9.00 am, 4030 - 6200 lux at noon and 1600 - 1900 lux at 3.00 pm). Medium light intensity (1400 - 2800 lux at 9.00 am, 6400 - 9100 lux at noon and 2900 - 3500 lux at 3.00) was less favourable and high light intensity (5300 - 6200 lux at 9.00 am, 8800- 10000 lux at noon and 4600 - 6400 lux at 3.00 pm) was least favourable. After 30 days, the respective

mean dry weights of the plants at the low, medium and high light intensities were 1.62 - 2.91g, 1.33 - 1.82g and 0.91 - 1.41g; and the respective mean number of nodules per plant was 54 - 78, 42 - 51 and 16 - 21.

Plants of Ex-Tamale variety watered once in two days showed the best growth. Those watered once in four days showed moderate growth, and growth of plants watered once in six days was greatly reduced. After 30 days, the respective mean dry weights of plants watered once in two, four and six days were 1.00 - 1.68g, 0.64 - 0.94g and 0.48 - 0.76g, and the respective mean number of nodules per plant was 43 - 56, 16 - 25 and 8 - 11. In both tests, conditions of light and moisture which encouraged higher nodulation also induced the formation of larger nodules.

It was concluded that compost of **Brachymenium** sp. is a good **Rhizobium** carrier and can be used for routine inoculation of bambara groundnut seeds. To derive the maximum benefit from the inoculation, it is desirable to identify the best strain of **Rhizobium** sp. for each variety of bambara groundnut.

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I. INTRODUCTION AND LITERATURE REVIEW

The increasing demand for food and animal feed has in the past been met largely through expanding the area of land under cultivation. But as the availability of uncultivated arable lands becomes limited, emphasis must now be shifted to raising the productivity of land already under cultivation. Improved cultivation methods, maintenance of soil fertility, use of high yielding strains and introduction of new crops are some of the recommended ways of improving productivity.

These recommendations are particularly directed at developing countries where food production lags far behind food demand. In these areas, protein deficiency is still a serious problem. Animal protein is seldom affordable by the poorer section of the societies, so legumes usually provide the chief and sometimes the only source of protein. All legumes are rich in protein (20-40 per cent), iron and the B-vitamins, which make them excellent food even when eaten in small amounts (Aykroyd and Doughty, 1964). Some have been found to contain carotene (Vitamin A) and some, for example, groundnut(**Arachis hypogea** L.), have the extra value of being rich in oil.

Tannons and Ullah (1969) indicated that the nutritive value derived from a dietary protein depends not only on its quantity in the diet but also on its quality, determined by its amino acid composition. According to Tannons and Ullah (**op.cit.**) other factors which affect the nutritive value of edible leguminous seeds include the concentration of constituent haemagglutinis and trypsin inhibitors.

Be as it may, developing countries are not just producing enough of the legumes, despite the enormous varieties, for example, bambara groundnut (**Vigna subterranea** L. Verdc = **Voandzeia subterranea** (L) Thouars), cowpea (**Vigna**

unguiculata L.), groundnut, pigeon pea (**Cajanus cajan** Millsp.) locust bean **(Ceratonia siliqua L.)**, winged bean (**Psophocarpus tetragonolobus** (L.) DC), etc., available to them. Results of seven community surveys that examined 25,000 children in Africa revealed a range of 1.7 - 7.8 per cent of severe Protein-energy malnutrition (PEM) and 5.4 - 44.9 per cent of moderate forms. Rough estimates by the World Health Organization (WHO) based on medium values of the total number of children affected by the disease in Africa as reported by Food and Agriculture Organization (F.A.O.) (1988), are 2.7 million of severe, and 16.3 million of moderate forms of PEM. PEM covers a wide spectrum of pathological conditions, ranging between the extreme forms, 'Kwashiorkor' (a malnutrition disease, primarily of children, caused by severe protein deficiency, usually occurring when the child is weaned from the breast; the symptoms are retarded growth, changes in skin and hair pigmentation, distension of the abdomen (pot belly), edema, diarrhoea, anaemia and dermatoses) on the one hand, and 'nutritional marasmus' (a condition of extreme malnutrition and emaciation, occurring chiefly in young children, characterized by progressive wasting of subcutaneous tissue and muscle) on the other. A large proportion of PEM causes an intermediate cond ition between the two, which is referred to as 'marasmic kwashiorkor'. Evidence from developing countries indicates that most children with PEM are consuming diets deficient in both energy and proteins (F.A.O., 1988).

Legumes when used as a cover crop protect the soil, increase soil fertility by mineralization of their abscissed leaves and add combined nitrogen through symbiotic nitrogen-fixation. All these result in an improvement of crops. Skerman (1977), for example, reported that at the Sisal Research Station at Tanga in Tanzania, a cover crop of tropical kudzu or puero (**Pueraria phaseoloides** (Roxb.) Benth.), growing as an inter-row crop with sisal increased the yield of sisal by 48 per cent.

It has earlier been demonstrated that legumes, as cover crop, also encouraged earlier unfurling of sisal leaves and added 635 kg of nitrogen per hectare to the soil, equivalent to the application of 1.4 tons of urea per hectare (Rijkebusch, 1967).

The practice of alley-cropping has become widespread in the Tropics in recent years. Alternating rows of crops and legume trees are raised as a means of increasing crop yield. The legume trees enrich the soil and at the same time protect the soil. In certain cases the species of legumes planted are also fodder for goats and sheep.

The Leguminosae have long been known as plants which in nature are associated with symbiotic bacteria. Although this association is now, by the new ecological terminology, more correctly a mutualism, the traditional name, symbiosis, has persisted till today, and is widely used. The bacteria occur in nodules on the roots in this mutual relationship. The Leguminosae contains three large subdivisions or families. These are the Mimosaceae and the Caesalpiniaceae, which are mostly tropical in distribution, and the Papilionaceae which contains both tropical and temperate species. Not all legumes nodulate. The ability to do so being least pronounced in the Caesalpiniaceae in which about 75 percent of the species lack nodules (Allen and Baldwin, 1954). In contrast, nodulation is almost universal among the Papilionaceae species, while the Mimosaceae occupy an intermediate position. Nodule shape and size vary considerably from species to species. Annuals usually have large nodules grouped about the taproot or the first order lateral roots. The nodules of perennials tend to be smaller and more widely distributed over the root system. New nodules are formed throughout the growing season and old nodules are sloughed off. The bacteria of legume nodules belong to the genus **Rhizobium.**

Two broad groups of angiosperms possess root nodules, one comprising the legumes and the other, certain non-legumes. The bacterial components are different in the two associations.

Root nodules are found in seven non-legume angiosperm families, namely, Betulaceae, Casuarinaceae, Coriariaceae, Eleagnaceae, Myricaceae, Rhamnaceae and Rosaceae. Their associate organism is an actinomycete. Norris (1956) advanced the hypothesis that the promiscuous "cowpea type" **Rhizobium** usually associated with the nodulation of tropical legumes is the ancestral and typical organism, and suggested that it is here that the norm of the symbiosis is to be found.

The cultivation of leguminous crops is a basic feature of most of the permanent agricultural systems of the world. From the point of view of the agronomist, the legume - **Rhizobium** symbiosis is undoubtedly the most important biological mechanism for adding nitrogen to the soil-plant system (Skerman, 1977). It is not surprising that **Rhizobium** technology has, therefore, become a specialized field of study in relation to the Leguminosae.

The strains of **Rhizobium** species have been found to differ in several properties by which they are commonly characterized. They differ mainly in their cultural and serological properties, bacteriophage susceptibility, ability to form nodules on particular host species, symbiotic nitrogen-fixing effectiveness, and in other characteristics such as the number of nodules formed on the host plant (Vincent, 1974). Sowli and Mytton (1986) have also shown that the **Rhizobium** species are host specific and each species consists of several strains. Some of these strains of the same species also vary in their ability to induce nodule formation and to fix nitrogen. They demonstrated this in experiments using strains of **Rhizobium leguminosarum** from different agricultural locations and **Vicia faba** variety minor, as host. They, thus, confirmed earlier findings of Mytton and Livesey (1983) who used clover varieties and different isolates of **Rhizobium** species. Burton, Allen and Berger (1952) provided an interesting aspect of this phenomenon many years earlier. They found that isolates of **Rhizobium phaseoli** from different nodules on the same plant varied widely.

Out of 85 strains isolated, only 12 (14.1 percent) were effective.

The development of a legume - **Rhizobium** symbiosis thus involves many specific interactions between the **Rhizobium** species and the legume host. Effective (nitrogen-fixing) nodules are formed when the host variety and **Rhizobium** strain are genetically compatible, and the appropriate interactions occur during each stage of nodule development. Conversely, ineffective (non-fixing) nodules are the result of interactions between genetically incompatible partners. Not surprisingly, the structure of ineffective nodules is frequently abnormal. The nature of the structural abnormalities and the stages at which they appear, vary from one ineffective legume - **Rhizobium** combination to another (MacKenzie and Jordan, 1974; Mosse, 1964; Pankhurst, 1974).

It follows that the association can only develop if the right strain of the appropriate **Rhizobium** species is present. But the presence of the correct strain in every locality cannot be guaranteed. The answer to the problem is to introduce the bacterium.

The introduction of an effective strain of **Rhizobium** can mean the difference between a successful development and complete failure of the crop. Early attempts to grow legumes in new areas involved the spreading of soil from the original habitat containing the bacterium strain over the new land to be planted. Later, culture of the **Rhizobium** species in various liquid or on semisolid nutrient media was applied externally to the seeds prior to sowing. This procedure was required for not only areas being cultivated for the first time with legumes, but also for old farm lands which have not been planted with legumes for some time. For, **Rhizobium** species are facultative symbionts able to live as normal components of the soil microflora in the temporary absence of their hosts, but their continued existence as free-living heterotrophs depends on the presence of the host root (Skerman, 1977).

As knowledge of the **Rhizobium** strain requirements of each particular legume increased, it became necessary to establish type or reference cultures of each in the laboratory, and from these starter cultures are prepared for use in the field as commercial inoculants. The aim of inoculating the seed is to coat it with sufficiently high number of cells of the bacterium to provide an early and effective nodulation of the seedling. Vincent (1970) estimated that 300 cells per seed were adequate to initiate infection, but in current practice much higher numbers are used, and a range of materials has been adopted as carriers of the bacterium cells.

Finely ground peat is now being generally used in many countries as a carrier in the preparation of legume inoculants. Subba Rao (1977) described how this is done in Australia and other countries. In Australia, peat is harvested, dried in the field and ground to pass through a 200 mesh sieve. This is then neutralized with calcium carbonate ($CaCO_3$) and packed in low density polythene bags after which they are sterilized by gamma rays at a dose of 50 Kilograys - as radiation is considered to be superior to autoclaving. The **Rhizobium** strain is grown in Yeast Mannitol Broth (YEM) to attain a high population level. The recommended minimum viable count in Australia is 500×10^6 viable rhizobia per ml, although in practice the numbers usually reach the range of 1,000 - 4,000 x 10^6 viable cells per ml. The broth is added to the sterilized peat at appropriate peat volume: broth volume ratio that will raise the moisture content of the peat to 60 percent, using a syringe. The puncture made by the syringe needle is sealed with adhesive tape, and the contents of the bag are mixed by rolling the bag in the hand. The bag with its contents is incubated at 26°C for two weeks and then stored at 4°C. At the end of the incubation period, the peat would contain 10^7 - 10^8 **Rhizobium** cells per gram of peat. Non-sterile peat can also be inoculated and used. Mechanical mixers are used to mix the peat with the broth, and the final desired moisture content is 45 to 50 percent on a wet - weight basis. The peat - **Rhizobium** mixture is then sieved through a coarse sieve to remove lumps,

matured for four days at 26°C in trays covered with polythene sheets and then packaged in polythene bags.

The preparation differs in some details in other countries. In the United States of America (U.S.A.), for example, the broth is sprayed onto powdered, neutralized and flash-dried peat (partial sterilization) while it is being agitated in a ribbon or paddle-type batch-mixer. After mixing, the inoculant is spread in thin layers on the floor for 48 to 72 hours at 22-24°C. The product is then milled to break up aggregates and become finely pulverized and then packed into polythene bags. Instead of assessing the bacterium population by the Total Viable Count method, the load of bacterium cells may be determined in a very different way. This alternative method is termed the "grow-out" test. Inoculated seeds are planted in sterile sand or vermiculite supplied with nutrients other than nitrogen and the plants are harvested after 5 - 6 weeks. The roots are examined for nodules and the efficacy of the inoculant assessed. If 90 percent or more of the plants have one or more nodules on or near the primary root, the sample is considered satisfactory; with 67 to 90 per cent plants with one or more nodules, the sample is considered fair. Samples giving less than 67 per cent nodulated plants are unsatisfactory (Burton, Martinez and Curley, 1972).

In India, powdered peat or lignite (a soft material quite similar to coal) neutralized with $CaCO_3$ is passed through a 200 mesh sieve and then autoclaved at 15lb pressure (103.5 kilopascal) for four hours. Upon cooling, a shake culture is added to the peat in such a proportion so as to produce a product with 40 per cent moisture content. After curing for a few hours at room temperature the product is packed in polythene bags. The preparation usually contains a bacterium population of 10×10^7 initially, 10×10^8 after four weeks, and 10×10^{10} after 12 weeks (Subba Rao, 1977).

The minimum standards for viable rhizobia vary in other countries. Burton, Martinez and Curley (1972) reported 3 x 10^8 for Czechoslovakia, 4 - 25 x 10^9 for Holland, 1 x 10^8 for New Zealand and 5 - 10 x 10^7 for Russia.

In the inoculating process, the peat mixture is made into a slurry with water, but preferably with a dilute suspension of adhesive (1 to 2 per cent Mellofos, 15 per cent gum arabic) for greater adherence (Skerman, 1977). The seeds are mixed thoroughly in the peat - adhesive slurry. Finely ground calcium carbonate ($CaCO_3$) is then applied to the inoculated seed while it is still wet and the seed is rolled in the powder so as to obtain uniform coating of lime over it. The pelleted seed may be sown immediately but, if absolutely necessary, may be stored up to two to three weeks at temperatures not exceeding 18°C (Subba Rao, 1977).

It is important that for the inoculant to work, it must firmly adhere to the seed. As a normal procedure, therefore, adhesives are used during the application of the inoculant. The adhesives include gum arabic (40% wt/vol) (Kunelius and Umesh, 1975), carboxymethyl cellulose (4% wt/vol) (Philpotts, 1982) and wall paper glue (10% wt/vol), and others such as corn syrup, honey. powdered milk and evaporated milk (Elegba and Rennie, 1984).

Quality of legume inoculant is based on many factors: ability of the **Rhizobium** species to nodulate and fix nitrogen; presence of adequate numbers of viable effective **Rhizobium** cell in the inoculant to induce infection; longevity of the cells in the inoculant under storage conditions; the purity of the inoculum; and the ease of application.

The literature provides a long list of legumes presently inoculated before sowing as a routine practice. They include the following species: **Adesmia** DC., **Aeschynomene** L., **Alysicarpus** Neck., **Anthyllis** Riv., **Arachis** Lin., **Cajanus** DC., **Canavalia** DC., **Cassia** Tourn. ex L., **Centrosema** Benth., **Cicer** (Tourn.) L., **Desmodium**

Desv., **Glycine** L., **Hedysarum** (Tourn.) L., **Indigofera** L., **Lablab** Adans., **Lens** (Tourn.) L., **Leucaena** Benth., **Lotononis** Eckl.et Zeyh., **Lupinus**(Tourn.) L., **Medicago** Tourn. ex L., **Melilotus** Tourn ex Hall, **Onobrychis** L., **Ornithopus** L., **Phaselous** (Tourn.) L., **Pisum**(Tourn.) L., **Pueraria** DC, **Sesbania** Scop., **Stylosanthes** SW., **Tephrosia** Pers., **Teramnus** Sw., **Trifolium** (Tourn.) L, **Vicia** Tourn. ex L., **Vigna** Savi, and **Zornia** J.F. Gmel. (Skerman, 1977).

Inoculation is so inexpensive that once the correct type of carrier has been developed, it could be adopted by local farmers. Peat - based cultures are most popular because of the protection the peat offers the **Rhizobium** species, resulting in high post - inoculation survival. However, high quality peat is not easily available in some parts of the world, especially in the Tropics. This has led to the search for suitable alternative **Rhizobium**-carrier materials in places where peat is not available.

In 1968 and 1969, solid based inoculants with wet land organic soil as a peat substitute were tried in Zimbabwe (Ryder, 1984). The material was not satisfactory and was abandoned. Inoculant using finely ground rotted maize cobs (known as cob-earth), in high density polythene (HDPE) bags was also developed on an experimental basis in Zimbabwe (Corby, 1976). The cob-earth proved a suitable base. However, the natural formation of cob-earth takes as long as four years, and attempts to speed its decomposition proved futile. Work on this material was also discontinued (Ryder, 1984).

Bagasse and its fine dust component bagasillo have been tested in Zimbabwe. Bagasse inhibited multiplication of **Rhizobium** species and the cultures did not survive on it. However, bagasillo was found to be very suitable for the growth of **R. japonicum** for soybean. Inoculants for soybeans, groundnuts and other commercially important members of the cowpea group, fine-stem stylo (**Stylosanthes** erecta (guineensis), and lucerne (**Medicago satina** Linn.) are now produced on the bagasillo carrier at Grasslands Research Station, Zimbabwe.

Filter mud from sugar mills is currently being used as a carrier for inoculant production at the Nairobi **Rhizobium** MIRCEN (Microbiological Resources Centre) (Anyango, 1984). It is a by-product obtained during the filtration and clarification processes of cane juice. Filter mud consists of fine fibres, mud solids, chemical substances and has a very high water holding capacity (Philpotts, 1976). The adoption of filter mud followed earlier research studies at the University of Nairobi on locally available materials; including bagasse, bagasse and charcoal dust, coffee husks, coir dust, diatomite, filter mud and sawdust in which filter mud proved to be the most promising (Anyango, 1984). Reports on its suitability as a **Rhizobium** - carrier have also come from other sources (Talik and Subba Rao, 1974; Uriyo and Chowdhur y, 1979).

This work was done to develop a local material which will facilitate wide adoption of the inoculation method also in West Africa. Secondly, it may result in the development of a method of improving the productivity of bambara groundnut.

The most important leguminous crops in terms of production and consumption in Africa are in the order, groundnut, cowpea and bambara groundnut (Sellschop, 1962). The same order of importance of these three crops has been reported in Ghana (Guerts, 1962). According to Stanton (1968), bambara groundnuts are popular in the Northern drier areas of West Africa where the soil is too poor for the cultivation of groundnuts, and Brammer (1962) reported that bambara groundnut is among the drought resistant crops suitable for the savanna ochrosols. The importance of bambara groundnut in tropical agriculture especially those of arid savannas is thus obvious.

Bambara groundnut has various local names in different parts of Africa and Europe. The French call it "voandzou" and in Kenya and Tanzania "njuga mawe" (Swahili) (F.A.O., 1988) In Northern Ghana, it is commonly known as "semie".

It is native to West Africa and is cultivated throughout Africa; from Senegal to Kenya, from the Sahara to South Africa, and Malagasy. The greatest variation of types is thought to occur in Burkina Faso, Togo, the middle belt of Nigeria, Tanzania and Zambia. It is also found in Central and South America, in parts of Asia (Philippines and Indonesia) and Northern Austrialia (F.A.O., 1988).

The various names of bambara groundnut in different parts of Africa have been used as a criterion to trace the origin of the plant (Stanton, 1968). According to Russel (1960), **Voandzeia** which is the former generic name came from the deformation of a Malagasy term "voanjo" which means the seed that satisfies. Russel (1960) therefore, believed the crop originated from Malagasy. Cobbley and Steele (1976), however, observed that Bambara is a district in the upper Niger near Timbuktu and, therefore, the crop is probably of West African origin. They further argued that bambara groundnut is wild in isolated locations in the savanna zone in West Africa and may have been domesticated around the head water of the Niger river.

Bambara groundnut is an annual herb with either open or bunched forms, with highly branching short stem that roots at the nodes. It forms subterranean pods just beneath the surface of the ground. The leaves are compound with three leaflets which are usually held upright on a long petiole. The leaflets are elliptic to obovate, smooth, dark-green above, paler below with entire margin. The middle leaflet is usually larger than the two lateral ones. The flowers have yellow or reddish pink petals and fruits are round or ovoid pods containing one or two seeds. The near spherical seeds, about 1.5cm diameter, are smooth and very hard when dried. The seeds are of different colours, cream, brown, mottled or black-eyed, depending on the variety.

The seeds are a valuable food. Although they have less oil (6 to 12 per cent) and protein (14 to 24 per cent) than groundnuts, they have more carbohydrate, and make a well balanced food, with a calorific value equal to that of a high-quality cereal grain. The protein is relatively high in lysine and tryptophan, so makes an excellent mix with cereals. Moderate amount of B vitamins, and small amount of minerals and vitamin A are present (F.A.O., 1988). In Ghana, the seeds are soaked for 24 hours before cooking, then boiled until soft. They are then made into a type of porridge with some of the beans floating in it ("aboboe"). The porridge is often sweetened with sugar.

In Cameroon, they are ground, raw or boiled, and added to soups and stews. In Tanzania the boiled seeds are crushed and mixed with groundnut paste, while in Malagasy, they are often added to meat stew with rice, or eaten with green leafy vegetable. The bambara groundnut flour has a chestnut flavour when boiled and in Southern Africa, the flour is mixed with oil or butter and eaten with meat, or it is made into balls or cakes. It is sometimes boiled with maize or millet flour to form a stiff dough which, when salted and made into balls, will keep for several days (F.A.O.,1988). The crop serves Africa very well.

African farmers usually intercrop bambara groundnut with other crops. The crop is raised from seeds. They are grown during the rainy season, with sowing carried out with the early rains. In the drier regions of the Tropics, rainfall is often the limiting factor in crop production, not only because of its scarcity but also because of the very high evaporative demand (MacCartney, Northwood, Dagg and Dawson, 1971). The growth and yield of bambara groundnuts have been found to be very sensitive to moisture stress (Billaz and Ochs, 1961; Holford, 1971).

Many factors affect nodule formation, such as soil moisture, pH and the availability of toxicity of minerals (Jardin, 1982), but the availability of phosphorus, carbohydrate serving as a source of electrons for nitrogenase activity, and an adequate plant water status can be singled out as the most limiting to nodule function in legumes (Phillips, 1980; Sprent, 1972). While drought has been shown to affect nodulation directly by desiccating delicate nodule tissues (Sprent, 1986), low soil water potential interacts with phosphorus availability (Bonetti, Montanheiro and Saito, 1984) to produce indirect effect on nodule activity mediated by the host plant.

Subba Rao (1977) reported that root temperature affected nodulation and infection processes in clovers (**Trifolium** sp) grown on agar slopes in environment controlled growth cabinets. Temperatures below 10°C and above 34°C retarded root hair infection by **Rhizobium.** Optimum temperature for bacterial tissue formation in the nodules was 20 - 30°C, but nitrogen fixation could take place from 12 to 32°C. Among the tropical legumes, effects of day temperature on root nodulation have been studied in soybean (**Glycine max** L.) and Bengal gram **(Cicer arietinum** (Tourn.) L.) in pot trails using selected **Rhizobium** strains. One of the bacterial strains was most effective at 33°C on soybean, while others showed no difference in effectiveness at 21 to 33°C. In Bengal gram, none of the bacterial strains produced nodules at temperatures above 32°C. Nitrogenase activity was best in the temperature range of 24 to 33°C.

In the investigation described in this thesis, an inoculant carrier was prepared with the moss, **Brachymenium** sp., and its efficacy was tested in a number of experiments using a number of strains of **Rhizobium** sp. from different legume - growing localities and two bambara groundnut varieties, Ex-Ada and Ex-Tamale.

II. MATERIALS AND GENERAL METHODS

i. MATERIALS

(a) Soils:

Soils were collected for two different types of experiments;

(1) **Rhizobium** species used subsequently for this investigation were isolated from soils collected in September and October, 1990, from eight different farms which were under cultivation with cowpea and soybeans:

1. a cowpea farm at Ashiaman, 25.4km east of Legon;
2. a cowpea farm at the University of Ghana Agricultural Research Station, at Kpong, 80km north-east of Legon;
3. a cowpea experimental plot in the Teaching Garden of the Botany Department, University of Ghana, Legon;
4. a cowpea plot at the University of Ghana Agricultural Research Station at Nungua, 12km north-east of Legon;
5. a soyabean plot at the University of Ghana Agricultural Research Station at Nungua, 12km north-east Legon;
6. a cowpea plot of the Plant Protection Unit, Ministry of Agriculture, at Pokuase, 19km north-west of Legon;
7. a cowpea farm at Shiashie, 4km south of Legon; and
8. a cowpea plot (plot 46) at the Weija Irrigation Project Site, 24km south-west of Legon.

A sufficiently large quantity of the soil collected at each site to a depth of 10cm, was conveyed to the laboratory in a large polythene bag (100 x 75cm) and sieved with a 2mm - mesh just before use.

(2) Loam soil used for other experiments was supplied by the Grounds and Garden Division of the Department of Botany, University of Ghana, Legon.

(b) **Bambara groundnut seeds**

Seeds of two commonly cultivated varieties in the country, Ex-Ada and Ex-Tamale, were purchased from the Makola Market in Accra and stored in small polythene bags (26 x 39cm) in the refrigerator (4°C) until needed. The Ex-Ada variety is mostly cultivated in the southern part of Ghana, in the Volta Region. The seeds are cream in colour and 0.8 - 1.2cm in diameter. The Ex-Tamale variety is cultivated in the northern part of Ghana and the seeds are also cream coloured but are black-eyed. They are slightly larger, 1.0 - 1.6cm in diameter.

Preliminary germination tests showed that the samples purchased were highly viable. A percentage germination of 90 - 96 per cent was obtained in the tests.

(c) **Rhizobium strains**

Strains of **Rhizobium** species used were isolated from the soil samples collected from the eight different legume plots. These strains have been accordingly named in this thesis after their sources of origin, viz., Ashiaman, Kpong, Legon, Nungua 1, Nungua 2, Pokuase, Shiashie and Weija strains.

The two bambara groundnut varieties were planted in the soil samples from the eight sites, and the strains of **Rhizobium** sp. isolated from effective nodules which developed on the roots as described later under General Methods. The contents of the nodules were streaked on Petri plates of Congo Red Yeast Mannitol Agar medium and incubated at 30°C for 5 days. The colonies of **Rhizobium** which developed were sub-cultured to obtain pure cultures, again, on Yeast Mannitol Agar. The cultures were then transferred to Yeast Mannitol Agar slants in McCartney tubes and incubated at 30°C for 5 days after which sterile liquid paraffin was poured onto the slants. The pure cultures were kept at room temperature.

(d) **Decomposed Moss material**

Both decomposed and fresh moss material of **Brachymenium sp.** was collected from Effiduase and New Zongo, all in Koforidua, 85.0km north-west from Legon. **Brachymenium sp.** was found to be particularly abundant on old, weathered cement blocks.

The material was thoroughly sun-dried at the Department of Botany and kept in sealed black polythene bags (90 x 60cm) and kept in the laboratory until needed.

ii. GENERAL METHODS

(a) Raising of Bambara groundnut plants

The two varieties of bambara groundnuts, Ex-Ada and Ex-Tamale, were grown in the various experiments in black polythene bags, 20cm high and 12.5cm in diameter. The polythene bags were filled with identical weights of the appropriate planting material up to a depth of 18cm. Drainage holes, 0.5cm in diameter were made at the bottom of the polythene bags.

(b) Selection of viable seeds for planting

Undamaged bambara groundnut seeds of closely similar sizes were surface-sterilized by immersing for five minutes in 0.1 per cent mercuric chloride ($HgCl$) solution and then washed in six changes of sterile distilled water. The seeds were next washed in 70 per cent Ethanol for three minutes, and rinsed twice with sterile distilled water.

The sterile seeds were then placed on water agar (1.0% agar) in large Petri plates (14cm diameter) and incubated at room temperature for three days. The vigorously germinating seeds were selected for planting.

(c) Conditions for growing the plants

The bambara groundnut plants were grown under various conditions and these would be described at appropriate places in Chapter III - Experimental Details.

(d) Culture media

Different media were used for different purposes at various stages of the work. The composition of media used were as follows:

1. **Yeast extract - mannitol agar (YMA)**

Yeast extract-mannitol agar (YMA) as described by Fred, Baldwin, and McCoy, (1932) was used for the routine cultivation of the **Rhizobium** sp.

Mannitol	10g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.2g
NaCl	0.1g
Yeast extract (eg. Difco, Oxoid)	0.4g
Agar	15g
Distilled water	1L

The medium was autoclaved at 1.1kg cm^{-2} at 121°C for 15 minutes.

2. **Congo Red Yeast extract-mannitol agar**

This contained the same ingredients as Yeast extract-mannitol agar and supplemented with 10ml of 1/400 aqueous solution of Congo red sterilized separately and added aseptically to give a final Congo red concentration of 25gml^{-1} just before use (Hahn, 1966).

3. **Yeast extract-mannitol broth (YEM)**

This contained the same ingredients as Yeast extract-mannitol agar but Agar was excluded and 4.0g Calcium carbonate ($CaCO_3$) added.

4. **Seedling Agar**

A nitrogen-free plant nutrient solution was used to make deep-layered seedling agar in large-sized test tube (19.0 x 3.5cm), according to the method of Fahraeus (1957). This medium contained:

$CaCl_2.H_2O$	0.1g
$MgSO_4.7H_2O$	0.12g
KH_2PO_4	0.1g
$Na_2HPO_4.12H_2O$	0.15g
Ferric citrate	0.005g
Agar	15g
Distilled water	1L

The medium was sterilized at 1.1kg cm-2 steam pressure at 121°C for 15 minutes.

5. **Sachs' solution**

Normal complete Sachs' solution (Sachs, 1860) used in watering the bambara groundnut plants contained:

Calcium sulphate	0.25g
Calcium phosphate	0.25g
Magnesium sulphate	0.25g
Sodium chloride	0.08g
Potassium nitrate	0.70g
Ferric chloride	Trace
Distilled water	1L

(e) **Methods of sterilization**

The seeds of the two varieties of bambara groundnut and their nodules were surface-sterilized as described earlier at (b).

Nutrient media, distilled water, McCartney tubes, measuring cylinder, Erlenmeyer flasks, glass rods and pipettes were sterilized by autoclaving at 1.1kg cm^{-2} steam pressure at 121°C for 15 minutes. Cotton wool plugs of Erlenmeyer flasks containing media or distilled water were temporarily covered with grease paper to prevent the penetration of any condensed water during autoclaving. Pipettes, measuring cylinders and glass rods were also wrapped in grease paper before autoclaving.

Petri dishes (7, 9 and 14cm diameter) were sterilized by heating at 160°C for 6 hours in an electric oven.

Tips of forceps, inoculating needles and loops were flamed to red-heat and air-cooled before use.

The inoculating room was sterilized by spraying heavily with 5 per cent aqueous dettol solution and allowed to stand for 10 minutes before use.

The planting soil was autoclaved in strong polythene sacks at 1.1kg cm^{-2} steam pressure at 121°C for 30 minutes. Autoclaving was repeated three times before the soil was used.

The decomposed moss material used as **Rhizobium** carrier was autoclaved at 1.1kg cm^{-2} at 121°C for 4 hours.

(f) **Chemicals**

All chemicals used in the preparation of media were either of the 'Analar' or the 'BDH' (British Drug House) grade.

(g) Characterization of soil samples

1. **Determination of mineral fraction:Hydrometer method**

The method described by Day (1965) was used. A hydrometer was calibrated by placing 100ml Calgon solution (Calcium hexametaphosphate, 41g; Sodium carbonate, 9g; distilled water, 1L) into a 1L measuring cylinder and the volume made up to one litre with distilled water of a temperature of 20°C. The solution was thoroughly mixed and the hydrometer was then put into it. The scale reading (RL) was recorded.

Two samples of 40g weight of air-dried sieved (with a 2.0mm mesh) soil, were carefully measured out. One lot was dried overnight at 105°C in an oven and the oven-dry weight recorded. The second lot was mixed with 100ml of Calgon solution and stirred in an electric motor mixer for 5 - 10 minutes. A few drops of amyl alcohol were added to prevent frothing. The mixture was then transferred into a 1L measuring cylinder and the volume made up to one litre with distilled water at a temperature of 20°C. The mixture was stirred with the plugger and the hydrometer put gently into the mixture.

The hydrometer reading (R) was recorded after 30 seconds and again after one minute. Without remixing the suspension, further hydrometer readings were made after 3, 10, 30, 90, 270 and 720 minutes. After the last reading the suspension was stirred and poured into a 450 um-mesh sieve and washed under the running tap. The residual soil particles were scooped out, dried in an oven overnight at 105°C and weighed to obtain weight of total sand. The experiment was carried out at 20°C.

The concentration of suspension in g/litre (C) was calculated using the equation:

$$C = R - RL$$

And the summation percentage (P) from the equation:

$$P = 100\ (C/C_o)$$

where Co is the oven-dry weight of soil in g/litre of suspension.

The corresponding particle sizes were calculated from the equation:

$$X \text{ (microns)} = {}^{O}/_{(t)^{\frac{1}{2}}}$$

where O is the sedimentation parameter from Table of values of O (Day, 1956) using corresponding values of R. The letter 't' is the sedimentation time in minutes.

A graph of P against X was plotted on a semi-logarithmic axis. The summation percentage (P) at different values of X were read. Values for P_2, P_6, P_{20} and P_{60} were provided by X values of 2, 6, 20 and 60 respectively.

Percentage clay	= P_2
Percentage fine silt	= $P_6 - P_2$
Percentage medium silt	= $P_{20} - P_6$
Percentage coarse silt	= $P_{60} - P_{20}$
Percentage total sand	= 100-(% clay + % total silt)

2. **Estimation of oxidizable organic matter in soil: Walkley and Black's Rapid Titration method**

The method described by Hesse (1972) was employed. Half a gram of sieved air-dry soil (using 0.5mm mesh) was put in a 500ml Erlenmeyer flask and 10ml of 1N Potassium dichromate solution (49.04g $K_2Cr_2O_7$ in 100ml of distilled water) added, followed by 20ml of concentrated sulphuric acid (98 per cent). The content of the flask was agitated for one minute and allowed to stand for 30 minutes on asbestos sheet. Two hundred millilitres of distilled water, 10ml of phosphoric acid, and 1.0ml of diphenylamine indicator solution (0.5g diphenylamine; 100ml concentrated Sulphuric acid and 20ml distilled water) were then added in that order. The contents of the flasks were titrated against 1N Ferrous ammonium sulphate (87.44g Ferrous ammonium sulphate; 500ml, distilled water; 20ml concentrated sulphuric acid made up to one litre of solution) until the colour changed from orange to green and finally to reddish-brown. A blank determination made without soil, served to standardise the Ferrous ammonium

sulphate solution. Percentage oxidizable organic carbon was calculated from the formula:

$$\% \text{ Organic carbon} = \frac{(\text{blank titre} - \text{actual titre})}{\text{weight of soil}} \times 0.3 \times N$$

where N is the normality of Ferrous ammonium sulphate solution.

Percentage oxidizable organic matter was obtianed by multiplying the value of percentage oxidizable carbon by the factor 1.724.

3. **Estimation of Total Nitrogen in soil**

The method described by Hesse (1972) was employed. One gram of air-day soil was placed in 300ml Kjeldahl flask and moistened with a few drops of distilled water. A spatulaful of catalyst (K_2SO_4, 10g; $CuSO_45H_2O$, 1.0g; Selenium, 1.0g) was added, followed by 10ml of concentrated sulphuric acid and digested over an electric heater for 2½ hours or until the digest clarified. The digest was allowed to cool and the volume made up to 100ml with distilled water in a volumetric flask. Amounts of 5ml each of the diluted digest were pipetted into Markham distillation apparatus and 2ml of 50% Sodium hydroxide solution added. The mixture was distilled and the distillate collected in 4ml of 2% Boric acid - indicator (20g Boric acid crystals dissolved in 900ml hot water), cooled and a mixed indicator solution (prepared by dissolving 0.1g bromocresol green and 0.07g methyl red in 100ml 95 per cent Ethanol) added until the colour of the Boric acid-indicator changed from green to pink. The distillate was then titrated against 0.01N Sulphuric acid. Values presented in this thesis are means of three replicate titres.

The whole process was repeated using 0.2g cane sugar in place of soil to correct for any nitrogen compound present in the reagents. Total nitrogen in soil sample was determined from the equation:

$$\% \text{ Nitrogen} = \frac{\text{Meq. of acid} \times \text{Meq. of N} \times \text{Vol. of extract}}{\text{weight of sample} \times \text{volume of diluted digest}} \times 100$$

where;

Meq. of acid	=	Normality of acid x Titre volume
Normality of acid	=	0.01
Meq. of N	=	0.014
Vol. of extract	=	50ml

(h) **Determination of Nitrogen content of plants**

The same procedure described above for the estimation of total nitrogen in soil was used, to determine the nitrogen content of dried and powdered plant material.

(i) **Isolation of Rhizobium strains**

The root systems of the two bambara groundnut varieties were washed carefully under gentle running water. Well formed, and firm nodules on the tap root of each plant were carefully detached for the isolation of the bacterium. The nodules were put in sterile distilled water contianing a little clean acid-washed sand in McCartney tubes and the tubes vigorously shaken to remove gross surface contamination.

The nodules were next surface-sterilized for five minutes in 0.1% HgCl solution and repeatedly rinsed in six changes of sterile distilled water. They were further put in 70 per cent Ethanol for three minutes and finally rinsed with sterile distilled water.

The nodules were then crushed with a sterile glass rod in a few drops of sterile distilled water in sterile McCartney tubes. Five serial dilutions of 1/10, 1/100, 1/1000, 1/10000, and 1/100000 of the suspension were prepared and the 1/100000 dilution streaked on Petri plates of Congo red YMA medium and incubated at 30°C, for five days.

At the end of the incubation period, isolated colonies of the **Rhizobium** sp. were sub-cultured on YMA slants in McCartney tubes. The McCartney tubes with the pure cultures were filled with sterile liquid paraffin to completely submerge the slant and stored in the refrigerator (4°C).

(j) **Preparation of decomposed moss material**

The fresh **Brachymenium** sp. material collected from Koforidua was sun-dried for 30days. It was then washed in a bowl of tap water to remove the adhering soil particles and fragments of foreign plant matter. The bulk was next cured by storing in sealed black polythene bag at room temperature for 15 days.

After curing, the moss material was sun-dried for 10 days and ground to pass through a 200 um mesh.

The ground moss material was neutralized with Calcium carbonate ($CaCO_3$) powder and aliquotes of 10g were put into polypropylene bags, 12.5cm high and 15cm diameter, and the mouth of the bags tied with twine.

These were autoclaved at 1.1kg cm^{-2} at 121°C for four hours, the period also used for peat and lignite (Subba Rao, 1977). The bags with their contents were stored in the refrigerator (4°C) until needed.

(k) **Preparation of the Rhizobium inoculum.**

Shake cultures of **Rhizobium** sp. were grown in Yeast extract-mannitol broth in 250ml Erlenmeyer flasks for 10 days. A half, ¼, and $^1/_8$ dilutions of the suspension of each strain were prepared, and were used, together with the undiluted suspension as separate treatments, to inoculate the cured moss material.

(l) **Viable Total Cell Count of Inoculated Moss.**

Appropriate dilutions of the suspension of the inoculated moss material were prepared and 1ml aliquotes were of each dilution used to inoculate individual Petri plates of Congo red YMA medium. The plates were incubated at 30^0C for 48 hours and the number of colonies that developed on a plate was counted. The number of cells in the original suspension was obtained by multiplying the mean number of colonies per plate by the dilution factor.

(m) **Planting sheds of different light intensities.**

Three sheds were constructed with plywood battens in such a way as to give shades of different light intensities. Each was 8m long, 1½m wide and 1m high with battens on the four sides and the top. The sheds stood parallel to each other on a concrete platform with the two ends facing east and west. The battens of shed No.1 were 2.5cm apart. The battens of shed No.2 were 5cm apart. Shed No. 3 had no battens and plants placed in this were fully exposed to sunlight. All the sheds were covered on the top and the east and west sides with polythene sheets to keep off rain. The two remaining sides were left uncovered to allow free circulation of air. The northern sides of the two sides of the two sheds with battens were loosely nailed so that they could be easily removed when watering or measuring the light intensity.

The light intensity under the three sheds was measured every other day with an "Eel" Portable Photoelectric photometer (Evans Electroselenium Ltd. Halstead, England). Three measurements were made in each shed, 2m apart along the median east-west transect, and the mean calculated.

(n) **Assessment of extent of growth of experimental plants.**

The following records were made of the bambara groundnut plants for the assessment of the effects of the treatments:

1. Number of leaves.
2. Length and width of the middle leaflet.
3. Number and diameters of the nodules .
4. Dry weight of the plant.
5. Nitrogen content of plant top.

(o) **Determination of plant dry weight**

Uprooted plants were thoroughly washed under the tap to remove all soil particles. They were then put individually in metal pans and dried in an electric oven at 80°C for 48 hours and then weighed with Precisa 300C Weighing machine (PAG. DERLIKON AG., Zurich, Switzerland).

(p) **Measurement of pH.**

Hydrogen ion concentration of soil suspension, powdered moss suspension and the media were measured with a Pye Unicam Model 290 pH meter (EDT Instruments Ltd, Dover England).

(q) **Statistical analyses.**

Results were statistically analysed where appropriate.

(r) **Experimental precautions.**

1. Glassware were kept scrupulously clean. Glassware which had already been cleaned with water and detergents were rinsed several times under the tap and three times with distilled water and allowed to drain dry before use or sterilization.

2. The room used for raising the plants in the 'Seedling Agar' tubes was sprayed thoroughly with 5 per cent aqueous solution of chlorophenol and allowed to stand for a day before being used.

3. **Rhizobium** suspension for plating was always shaken with WhirliMixer TM (FISONS Scientific Equipment, England) for one minute and sample withdrawn immediately thereafter for plating.

4. Soil for growing the plants was autoclaved just before use.

5. To prevent cross contamination with different strains or different densities of suspensions of the same strain, hands were washed in warm water and then with 70 per cent Ethanol and rinsed several times with sterile distilled water when the inoculated seeds were being planted.

III. EXPERIMENTAL DETAILS

A. DETERMINATION OF PROPERTIES OF THE DIFFERENT SOIL SAMPLES

The first exercise which was carried out was to determine some of the properties of the eight samples of soil collected from legume farms at Ashiaman, Kpong, Legon, Nungua, Pokuase, Shiashie and Weija.

Nodulation is affected by a variety of edaphic factors, some of which act through their influence on the nutrition of the host. For example, calcium and phosphorus play an important part in the legume - **Rhizobium** relationship. Phosphorus deficient plants do not nodulate properly and in addition an adequate supply of soil phosphorus helps to maintain the population of nodule bacteria in the soil at a high level. Calcium is important for the nutrition of both legumes and bacteria (Anderson and Moye, 1952). Plants which are grossly nitrogen deficient nodulate sparingly or not at all while a high level of availability of soil nitrogen depresses nodulation (Vincent, 1965) and reduces fixation. Furthermore, an increase in soil pH increases rhizobial numbers and results in good nodulation (Bond, 1951). Any large differences in the properties of the eight soil samples may cause significant differences in nodulation. The nitrogen content, organic matter content, pH of the soil, soil mineral fraction, and **Rhizobium** population counts, of each sample were assessed.

Soil Nitrogen Content Test

This was carried out with each of the eight soil samples. Five grams of each soil sample were digested in each of three Kjeldahl flasks, as described under 'Material and Methods'. Each of the three replicates was distillated separately and titrated. The mean of the

three titres was then determined. The mean titre was used to calculate the Percentage Nitrogen of the soil sample.

Organic Matter Content

The organic matter content of 0.5g soil was determined by first establishing the percentage Carbon content which was then multiplied by 1.724 to give the Percentage Organic Matter of the sample. Three tests were made for each soil sample, according to the method described under 'Materials and Methods' to provide three titres from the titration of the digested soil. The mean titre value was then used to calculate the percentage carbon content, which was used for the determination of a single value of Percentage Organic Matter Content.

pH of Soil

The pH of soil solution prepared with samples of soil from each locality was determined. Three determinations were made for each soil sample and the mean pH calculated. The soil solution was prepared by shaking 40g soil in 100 ml distilled water with an electric motor mixer for one hour, and the supernatant used for the pH measurement after the large particles had settled.

Soil Mineral Fraction

The method used has been fully described under 'Materials and Methods'. The original solution for the determination of the mineral fraction consisted of 40g soil and 100 ml Calgon solution.

Three determinations were carried out with each of the eight soil samples, which provided three values for each mineral fraction. The Table of Results (Table 1) contains the means of each set of three determinations.

B. ISOLATION OF STRAINS OF RHIZOBIUM SP. FROM THE DIFFERENT SOIL SAMPLES

The two varieties of bambara groundnut plants, Ex-Ada and Ex-Tamale were planted in each of the eight soil samples. Black polythene bags, 20cm high and 12.5cm diameter, with drainage holes at the bottom were filled up to 18cm high with the different soil samples.

Five seeds were sowed in each bag, and the seedlings were thinned to one after they had emerged. There were five replicates for each soil sample and plant variety. The bags were labelled appropriately and arranged randomly, on the eastern verandah of post-graduate laboratory. The plants, therefore, received full sunlight up to 11.00 am each day and were protected from rains. They were watered daily with 20ml tap water per bag. Once a week, each bag received, in addition, 10ml Sachs' solution to augment the nutrient content in the soil. The plants were sown on September 17, 1990 and harvested on October 21, 1990.

After the plants had been harvested and the roots thoroughly washed, the nodules were detached to be counted and measured, and the largest ones of each lot selected for isolation of the **Rhizobium** sp. following the method described under 'Materials and Methods'.

To provide additional details on the performance of the plants in the various soil samples, the dry weights of the plants and their relative percentage nitrogen content were determined.

C. COMPARATIVE INEFFECTIVE ABILITY OF FIVE STRAINS OF RHIZOBIUM SP. ISOLATED FROM BAMBARA GROUNDNUT PLANTS

The soil properties studied in Experiment 4 differed among the eight soil samples. The extent of nodulation of the plants growing in the different soils was, therefore, determined by other factors in addition to the ability of the strains to infect bambara groundnut. The nodule - forming abilities of the strains isolated from nodules formed in the different soil samples were, therefore, compared in this experiment under the same growing condition.

Broth cultures were raised with strains of **Rhizobium** sp. isolated from the nodules and used to inoculate tubes of 'Seedling Agar'.

The Large boiling tubes were filled to a depth of 12.0cm with the 'Seedling Agar'. A piece of non-absorbent cotton wool was inserted into each tube to just above the surface of the medium and the tube was then plugged with a non-absorbent cotton wool and autoclaved.

The medium was allowed to set after autoclaving with the tube in an upright position and allowed to stand for 24 hours. Each tube was inoculated with 1.0ml of 10-day old culture broth of the appropriate strain. The tip of the pipette containing the inoculum was pushed through the non-absorbent cotton wool inserted into the boiling tube and the inoculum placed directly on the surface of the 'Seedling Agar' medium.

A 2-day old surface-sterilized and germinating bambara seed of the Ex-Ada variety was aseptically placed upright on the wad of cotton wool inside the tube, pushing the radicle through the cotton wool so that it touched the

'Seedling Agar' medium. The cotton wool plug was then put back in place, and the 12-cm column of the 'Seedling Agar' covered with black polythene sheet. For the control 1ml sterilized distilled water was used instead of broth culture. Three replicates of each strain, and the control were set up. The tubes were kept in the laboratory near the windows to receive adequate light, from November 11, 1990 to December 18, 1990. On November 20, 1990 the cotton wool plugs were removed to allow the plants to extend out of the tubes.

The plants were examined at the end of the experiment, and

(a) plant with nodules were identified,
(b) nodules on nodulating plants were counted, and
(c) tops of nodulating plants were dried at 80°C for 48 hours and weighed.

D. PREPARATION OF THE MOSS COMPOST AS CARRIER AND STUDY OF SHELF LIFE ON INOCULUM CARRIER

The identification of promising **Rhizobium** strains among those isolated was followed by the preparation of **Brachymenium** sp. compost as **Rhizobium** inoculum carrier according to the procedure outline in 'Material and Methods

The sterile compost in the polythene bags was inoculated with undiluted, ½, ¼ and $^1/_8$ dilutions of the broth culture, using a volume broth culture that adjusted the moisture content of the compost to 50 per cent. By a previous determination, it was established that 20ml of the broth inoculum brought 10g of the compost to 50 per cent moisture content. Subba Rao (1977) used a preparation with 45-60 per cent moisture content.

After the inoculum had been added and the mouth of the polythene bag tied again with twine, the bag was kneaded with fingers to mix the contents thoroughly. They were appropriately labelled and left in the inoculating room at 28°C for two weeks to mature, a viable cell count was made, and then used in the various subsequent nodulation tests. Those which were not used immediately and those used in studies on the shelf life of inoculated moss carrier were stored in the refrigerator (4°C) until needed.

Subba Rao (1977) reported that he obtained the highest **Rhizobium** cell count of 10 x 10^8 per gram after four weeks storage. It was considered necessary to find out whether the period of incubation of two weeks adopted here could be extended if desired without autolysis and decline of the cell population setting in.

Inoculated bags containing moss with inocula of the different concentrations were kept in the laboratory at room temperature (28°C). Samples were withdrawn at 7-day intervals over 14 weeks for the determination of the cell population, using Congo red YMA for the total viable cell count tests.

E. NODULATION OF PLANTS INOCULATED WITH DIFFERENT STRAINS OF RHIZOBIUM SP. IN MOSS CARRIER UNDER DIFFERENT WATER STRESS CONDITIONS

Since moisture content is known to affect the development of leguminous plants (Benthlenfalvay, Brown, Mihara and Stafford, 1987; Seliskar, 1987), it was selected as one of the factors that could be used in trials to test the efficiency of **Brachymenium** sp. compost as **Rhizobium** sp. inoculum carrier. Besides, the viability of rhizobia is affected by moisture content of the soil and rhizobia are sensitive to excessive drying when exposed to open air. However, small numbers survive for a long time

in air-dried soil, probably in the film of hygroscopic moisture which surround the soil particles. Excess water may limit aeration and hence survival of the bacteria. Skerman (1977) reported that maximum growth and nodulation of legumes generally occur in soil with a water content between 75 and 85 per cent of its water - holding capacity. This test was carried out using Ex-Tamale variety which is cultivated mostly in Northern Ghana where low soil moisture levels occur for a greater part of the year.

The bambara groundnut plants were raised in soil of three different water regimes Surface - sterilized bambara groundnut seeds pre-germinated on 1% water agar were coated with the moss inoculum carrier, by stirring 30 seeds in a mixture of 3g of moss carrier inoculum, 1.2ml of 40 per cent gum arabic and 1.0g $CaCO_3$. The inoculated seeds were spread in large Petri dishes for one hour to allow the inoculum to dry properly before they were sown. Germinated seeds for the control were not inoculated. The seeds were sown in equal volumes of the autoclaved soil at 5 seeds per pot which were thinned to one per pot after germinating.

The planted seeds were divided into three batches: Batch No.1 was watered each other day; Batch No.2 was watered once in four days; and Batch No.3 was watered once in six days. The time table for watering is shown in Appendix A. The pots were arranged randomly on a veranda of the laboratory facing east and received full sunlight each morning till 11.00 am. There were five replicates for each treatment.

The plants were harvested 30 days after germination (grown from March 13, 1991 to April 17, 1991) and (a) size of the middle leaflets, (b) number of leaves on each plant, (c) dry weight of the plants, and (d) number and diameters of the nodules, of each treatment and control were recorded.

F. NODULATION OF PLANTS INOCULATED WITH DIFFERENT STRAINS OF RHIZOBIUM SP. IN MOSS CARRIER UNDER DIFFERENT LIGHT INTENSITIES

An optimum light intensity is necessary for maximum nodulation and nitrogen fixation. This may be related to the optimum carbohydrate levels in the plants. For it has been shown that, under shaded conditions spraying the plants with sugar solution increases nitrogen fixation (Skerman, 1977). The bambara groundnut plants were, in this experiment, grown under three light intensities to find out which of the three would be considered optimum for this crop. The Ex-Ada variety was used for this experiment as it is the variety grown widely under mixed farming in southern Ghana.

Inoculated germinated seeds, prepared as in Experiment E, were planted in the black polythene bags and put in the sheds of the three light intensities. The control bags were planted with uninoculated germinated seeds. There were five replicates of each treatment. The plants were watered daily in the late afternoon with tap water.

Light intensities in the three sheds were measured every other day with the 'Eel' Portable Photoelectric Photometer, at 9.00 am, 12.00 noon and 3.00 pm.

The plants were harvested after 30 days (grown from April 10, 1991 to May 15, 1991). In this experiment also (a) size of the middle leaflets, (b) number of leaves on each plant, (c) dry weight of the plants, and (d) number and diameters of the nodules, of each treatment and control, were recorded.

VI. RESULTS.

A. DETERMINATION OF PROPERTIES OF THE DIFFERENT SOIL SAMPLES.

The results of this experiment presented in Table 1a show that the pH's of the soils were similar. The pH of the eight soil samples ranged from pH 6.48 (Legon) to pH 6.84 (Weija). Also with the exception of Kpong, Legon and Weija soils, the soil samples were sandy loam, as shown in Table 1b, and determined by the Prescott Triangle (Appendix A) from the 'Particle size distribution' data in Table 1a. In contrast, the Percentage Nitrogen and Mean Percentage Organic matter varied considerably. The Percentage Nitrogen of the Ashiaman soil was only 0.025 per cent, while it was as high as 0.132 per cent in the Pokuase soil. The Kpong and Legon soils which came next to the latter had approximately only half the level of Percentage Nitrogen in the Pokuase soil.

Likewise, while the highest Mean Percentage Organic Matter recorded was 4.513 per cent in the Pokuase soil, the least which occurred in the Ashiaman soil was as low as 0.468 per cent.

There was a clear relationship between Percentage Nitrogen and Mean Percentage Organic Matter. Percentage Nitrogen increased with increasing Mean Percentage Organic Matter. Thus, the Ashiaman soil had the lowest Percentage Nitrogen and Mean Percentage Organic Matter, and Pokuase soil had the highest Percentage Nitrogen and Mean Percentage Organic Matter.

Total viable count studies using Congo red YMA produced the **Rhizobium sp.** populations indicated in Table 2. There were high populations, between 111 x 10^4 and 108 x 10^4 cells per gram of soil in soils from Kpong, Legon, Nungua(1), Pokuase and Weija. The remaining soils had very low populations not exceeding 20 x 10^4 per gram of soil. Photographs of Congo red YMA inoculated with suspensions of soils from the eight localities and incubated at 30^0C for 5 days are shown in Plates 1a and 1b.

TABLE 1a

Some properties of soils from legume plots from different localities.

Source of soil	Particle size distribution (% by wt)			% Nitrogen	Mean Organic Matter (%)	Mean pH
	Sand	Silt	Clay			
Ashiaman	82.33	3.42	14.25	0.025	0.468	6.60
Kpong	51.10	9.65	39.25	0.067	2.009	6.80
Legon	74.83	3.42	21.75	0.069	1.733	6.48
Nungua(1)	75.24	5.51	19.25	0.049	1.472	6.67
Nungua(2)	80.68	3.82	15.50	0.039	0.977	6.82
Pokuase	82.30	0.95	16.75	0.132	4.513	6.76
Shiashie	81.30	4.45	14.25	0.028	0.578	6.66
Weija	69.73	6.02	24.25	0.055	2.064	6.84

TABLE 1b

Kinds of soils obtained from legume plots from different localities as determined with the Prescott Triangle.
(Based on data of Particle size distribution in Table 1a)

Source of Soil	Type of Soil
Ashiaman	Sandy loam
Kpong	Sandy clay
Legon	Sandy clay loam
Nungua (1)	Sandy loam
Nungua (2)	Sandy loam
Pokuase	Sandy loam
Shiashie	Sandy loam
Weija	Sandy clay loam.

TABLE 2

Rhizobium population in soils from the different localities.

Source of Soil	Mean No. of ***Rhizobium*** population (X 10^4) g^{-1} soil
Ashiaman	15
Kpong	180
Legon	138
Nungua (1)	112
Nungua (2)	20
Pokuase	111
Shiashie	19
Weija	165

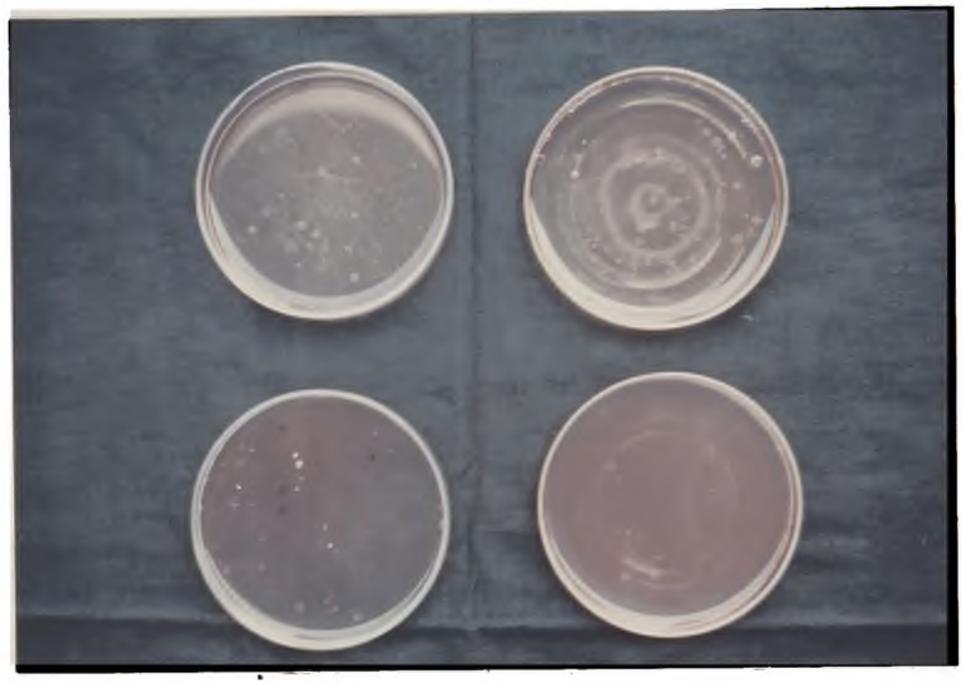
1a. TOP: Inocula of Nungua (2) soil (left) and Pokuase soil (Right).

BOTTOM: Inocula of Ashiaman soil (Left) and Shiashie soil (Right).

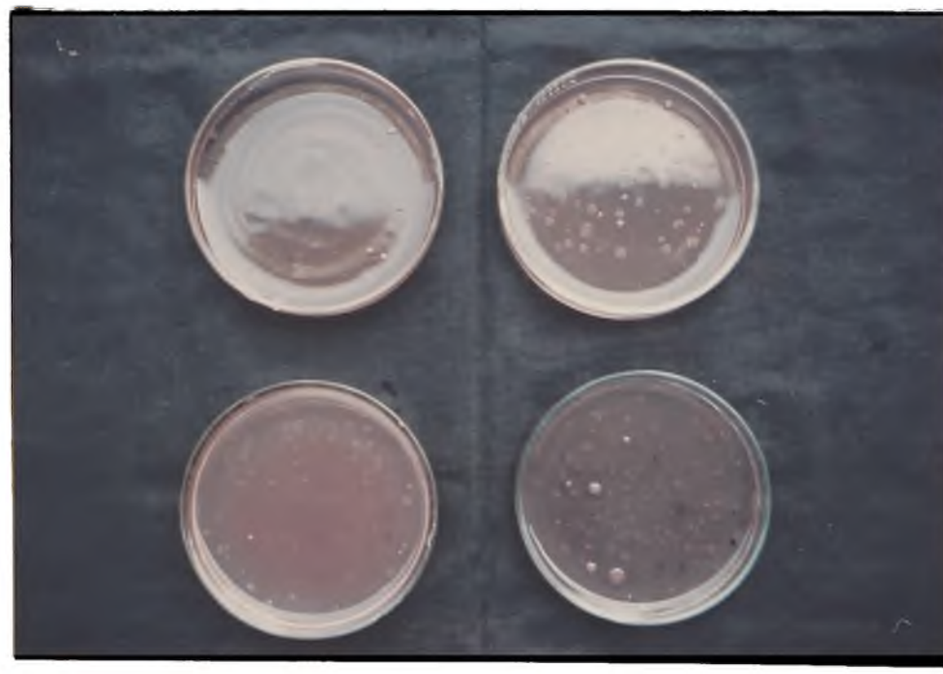
1b. TOP: Inocula of Weija soil (Left) and Legon soil (Right).

BOTTOM: Inocula of Weija soil (Left) and Legon soil (Right).

PLATE 1. Photographs of Petri plates of Congo red YMA inoculated with suspensions of soils from eight different localities showing colonies of **Rhizobium** sp. after incubation at 30^{0}C for 5 days. (X 1/3).



1a



1b

B. ISOLATION OR STRAINS OF RHIZOBIUM sp. FROM THE DIFFERENT SOIL SAMPLES

The preceeding tests showed that **Rhizobium** species were present in all the eight soils. This experiment showed that they were capable of infecting bambara groundnut plants and causing nodulation when the plants were raised in the soils. But they did this to varying degrees. Most of the observations and results of the experiment are recorded in Table 3 and Figs. 1 and 2.

On the basis of plant growth and the extent of nodulation recorded in Table 3, the plants could be separated into three groups as follows:

Group 1. Plants which grew luxuriantly with deep-green foliage, had highest Mean Shoot dry weight, (between 1.80g and 2.36g) and four of them had mean root dry weight (between 0.31g and 0.39g). They also formed the highest number of nodules. The bambara groundnut varieties and associated **Rhizobium** species were:

i Ex-Ada variety and Legon **Rhizobium** sp. strain
ii Ex-Ada variety and Weija **Rhizobium** sp. strain
iii Ex-Tamale variety and Kpong **Rhizobium** sp. strain
iv Ex-Tamale variety and Nungua(1) **Rhizobium** sp. strain
v Ex-Tamale variety and Pokuase **Rhizobium** sp. strain

Their nodules were the largest with respective mean diameters of 1.81, 1.41, 2.47, 1.50 and 1.80mm. The class-diameters of these nodules are presented in the histograms in Fig. 2.

Group 2. Plants with moderate growth and nodulation:

i Ex-Ada variety and Kpong **Rhizobium** sp. strain
ii Ex-Ada variety and Nungua(1) **Rhizobium** sp. strain
iii Ex-Ada variety and Pokuase **Rhizobium** sp. strain
iv Ex-Tamale variety and Legon **Rhizobium** sp. strain
v Ex-Tamale variety and Weija **Rhizobium** sp. strain.

Group 3. Plants with stunted growth and yellowish-green foliage and formed the smallest number of mean number of nodules per plant, 3 - 10 nodules, compared to 30 - 44 mean nodules per plant of the associations of Group 1.

i Ex-Ada variety and Ashiaman **Rhizobium** sp. strain
ii Ex-Ada variety and Nungua(2) **Rhizobium** sp. strain
iii Ex-Ada variety and Shiashie **Rhizobium** sp. strain
iv Ex-Tamale variety and Ashiaman **Rhizobium** sp. strain
v Ex-Tamale variety and Nungua(2) **Rhizobium** sp. Strain
vi Ex-Tamale variety and Shiashie **Rhizobium** sp. strain.

It was obvious that infection by strains of **Rhizobium** sp. of soils from Ashiaman, Nungua(2) and Shiashie plots was poor and were not used anymore in the subsequent investigations. It can also be seen from Table 3 and Fig. 1 that the lowest Percentage Nitrogen content of the shoots was recorded in the plants, both Ex-Ada and Ex-Tamale varieties, grown in the Ashiaman and Shiashie soils

Fig. 1 illustrates the relationship between extent of nodulation of the plants of the various variety-strain associations, their dry weights and per cent nitrogen of their shoots.

There was a highly significant positive correlation between the mean dry weight of shoot and the percentage nitrogen of the shoot ($r = 0.8616$; $t_{14} = 12.5130$; $P<0.001$). There was no correlation between the mean dry weight of root and the percentage nitrogen of the shoot ($r = 0.1947$).

Considering all the observations, it was decided that the **Rhizobium** sp. strains of the associations of Group 1 would be used in all the subsequent experiments. These are:

(a) Kpong **Rhizobium** sp. strain in nodules of Ex-Tamale variety
(b) Legon **Rhizobium** sp. strain in nodules of Ex-Ada variety
(c) Nungua(1) **Rhizobium** sp. strain in nodules of Ex-Tamale variety
(d) Pokuase **Rhizobium** sp. strain in nodules of Ex-Tamale variety
(e) Weija **Rhizobium** sp. strain in nodules of Ex-Ada variety.

TABLE 3

Growth and nodulation of two varieties of Bambara groundnut raised in soils from legume plots from different localities for 30 days under normal day/night regime.

Source of Soil	Plant variety	Mean Dry weight (g)(± S.E) of plant: Shoot	Mean Dry weight (g)(± S.E) of plant: root	% Nitrogen of shoot	Mean No. of nodules per plant (to the nearest whole no.)
Ashiaman	Ex-Ada	0.44 ± 0.01	0.06 ± 0.01	0.37	5
	Ex-Tamale	0.61 ± 0.15	0.08 ± 0.01	0.29	2
Kpong	Ex-Ada	0.39 ± 0.06	0.07 ± 0.02	0.40	11
	Ex-Tamale	2.36 ± 0.37	0.38 ± 0.03	0.46	38
Legon	Ex-Ada	2.03 ± 0.35	0.39 ± 0.05	0.49	41
	Ex-Tamale	0.51 ± 0.03	0.14 ± 0.02	0.41	14
Nungua (1)	Ex-Ada	0.72 ± 0.12	0.11 ± 0.02	0.37	11
	Ex-Tamale	1.81 ± 0.19	0.33 ± 0.05	0.48	32
Nungua (2)	Ex-Ada	0.35 ± 0.06	0.07 ± 0.01	0.39	10
	Ex-Tamale	0.35 ± 0.13	0.09 ± 0.01	0.41	8
Pokuase	Ex-Ada	0.67 ± 0.12	0.07 ± 0.01	0.37	12
	Ex-Tamale	2.08 ± 0.37	0.31 ± 0.06	0.53	30
Shiashie	Ex-Ada	0.34 ± 0.17	0.07 ± 0.01	0.33	3
	Ex-Tamale	0.61 ± 0.15	0.11 ± 0.02	0.38	3
Weija	Ex-Ada	1.80 ± 0.59	0.10 ± 0.00	0.49	44
	Ex-Tamale	0.85 ± 0.05	0.38 ± 0.04	0.40	17

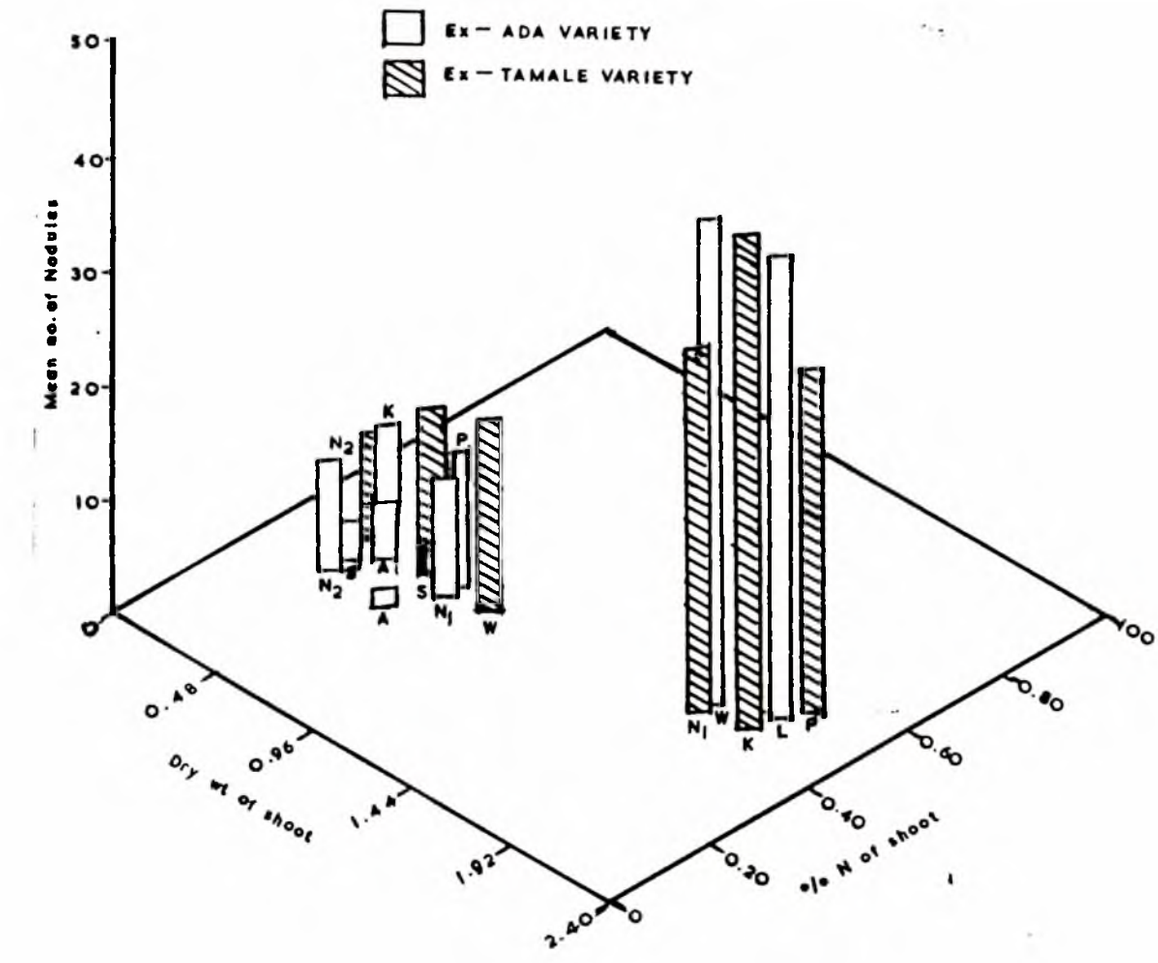


Fig. 1 The graph of nodulating ability of two varieties of Bambara groundnut plants raised in soil samples for 30 days.

A - Ashiaman soil; K - Kpong soil; L - Legon soil;

N_1 - Nungua (Plot 1) Soil N_2 - Nungua (Plot 2) soil P - Pokuase soil;

S - Shiashie soil; W - Weija soil

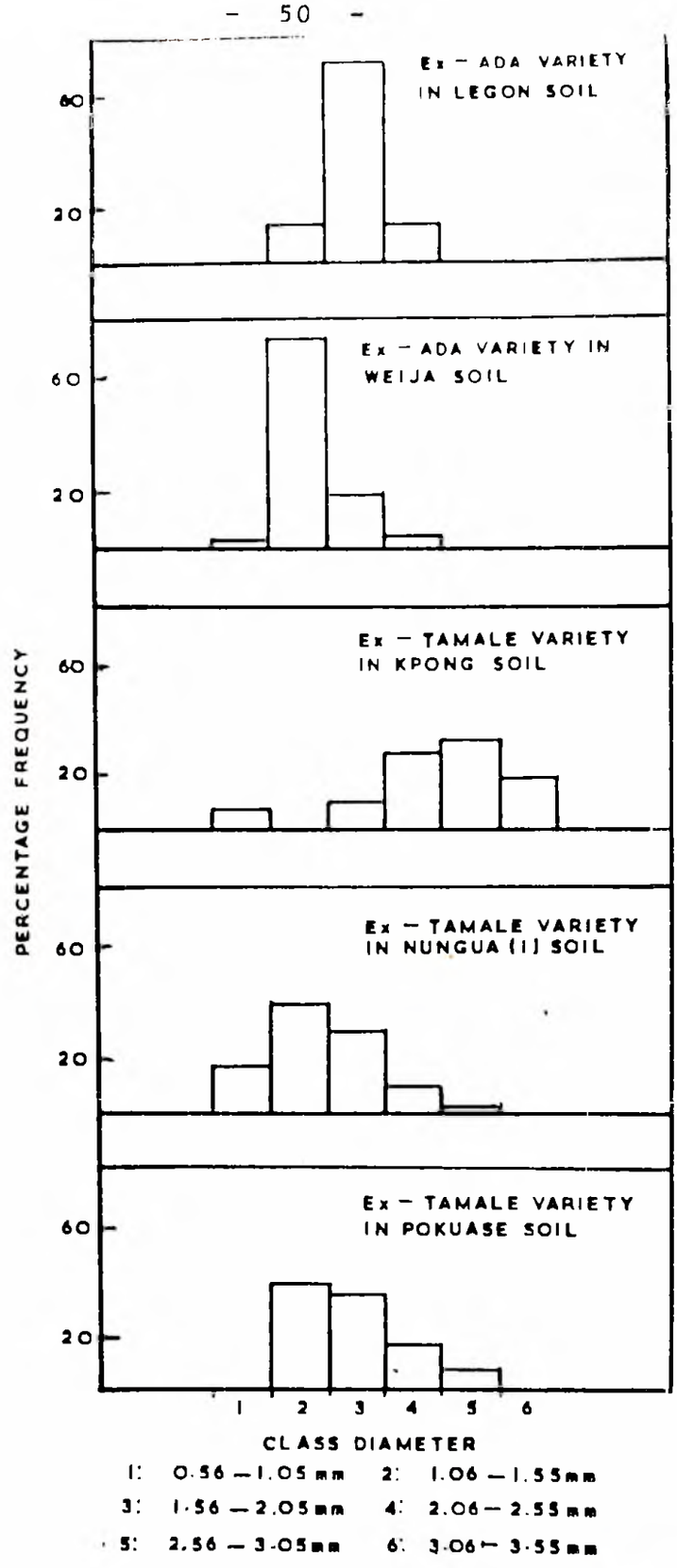


Fig. 2 Distribution of class-diameters of nodules of Bambara groundnut plants of the Ex-Ada and Ex-Tamale varieties grown in soils from Kpong, Legon, Nungua(1), Pokuase and Weija plots for 30 days under normal day/night regime.

C. COMPARATIVE INFECTIVE ABILITY OF THE FIVE STRAINS OF RHIZOBIUM sp. ISOLATED FROM BAMBARA GROUNDNUT PLANTS

'Seedling Agar' proved to be a good medium for this experiment in which the infectivity of the five strains of **Rhizobium** sp. was studied. Ex-Ada variety plants inoculated with the Group 1 strains of **Rhizobium** sp. of Experiment B all grew well as can be observed in Plate 2.

The mean dry weight of the shoots of the different treatments, mean number of nodules which developed on the plants and the mean diameter of the nodules are tabulated in Table 4. Fig. 3 shows the nodule class-diameter distribution for the various treatments.

The values for mean shoot dry weights were quite close; the least was 0.41g for plants inoculated with Pokuase-Ex-Tamale strain, and the highest was 0.49g for plants inoculated with the Legon-Ex-Ada strain. There was no significant difference between the mean dry weights of the inoculated plants. They were, however, all significantly different from the value for the control. Values for the mean number of nodules per plant for the five treatments also fell within the narrow range of 28-34.

There was, however, considerable variation in mean diameters of the nodules. The histograms in Fig. 3 also illustrate this very well. The greatest mean diameter of 1.46mm of plants inoculated with the 'Kpong-Ex-Tamale' strain is far greater (39 per cent increase) than the smallest mean diameter of 1.05mm of plants inoculated with the Legon-Ex-Ada strain.

The control uninoculated plants were smaller in size and they did not nodulate.

TABLE 4

Dry weight and extent of nodulation of inoculated Bambara groundnut plants, Ex-Ada variety, raised on 'Seedling Agar' for 20 days at 28°C.

Strain of *Rhizobium* sp from nodules of	Mean Dry weight of shoot (g)	Mean No. of Nodules per plant (to the nearest whole no.)	Mean Diameter of Nodules (mm)
Ex-Ada in Legon soil	0.49[a]	28	1.10
Ex-Ada in Weija soil	0.45[a]	34	1.23
Ex-Tamale in Kpong soil	0.44[a]	30	1.05
Ex-Tamale in Nungua(1) soil	0.47[a]	33	1.46
Ex-Tamale in Pokuase soil	0.41[a]	30	1.42
Uninoculated plants (control)	0.25[b]	0	-

By the calculated Confidence Limits at 95% (Kershaw, 1973) means bearing the same letter are not significantly different.

From Left:
1. Inoculation with Pokuase strain of **Rhizobium** sp.
2. Inoculation with Legon strain of **Rhizobium** sp.
3. Inoculation with Nungua(1) strain of **Rhizobium** sp.
4. Inoculation with Kpong strain of **Rhizobium** sp.
5. Inoculation with Weija strain of **Rhizobium** sp.
6. Uninoculated (Control).

PLATE 2. Photograph of Bambara groundnut plant, Ex-Ada variety grown in 'Seedling Agar' inoculated with different strains of **Rhizobium** sp., under normal day/night regime at room temperature for 12 days. (X 1/5).

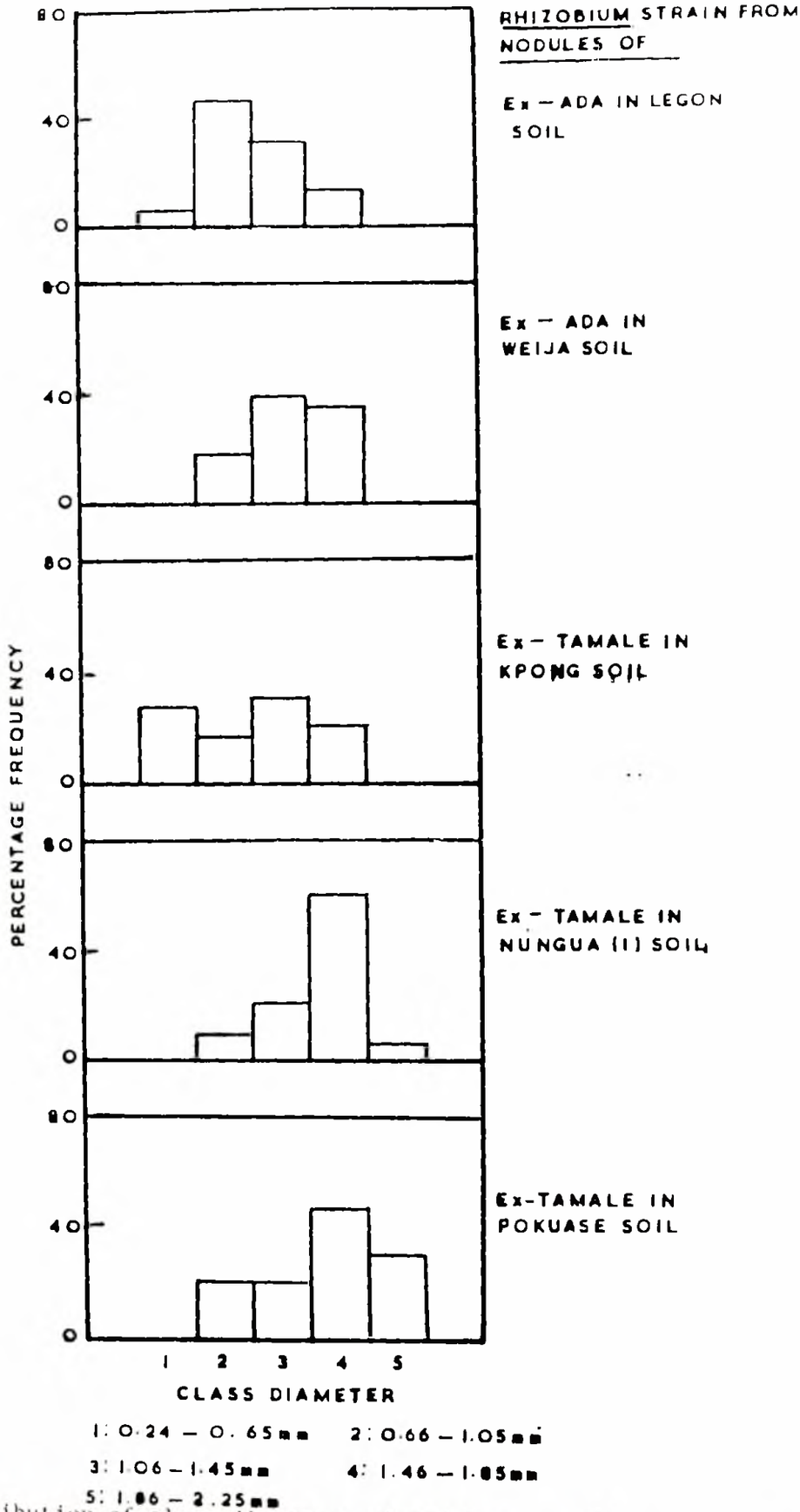


Fig.3 Distribution of class-diameters of nodules formed by 20 day-old inoculated seedlings of Bambara groundnut plants, Ex-Ada variety, raised on Seedling Agar' at 28 C. Plants inoculated with Rhizobium sp. strains from indicated source.

D. PREPARATION OF THE MOSS COMPOST AS CARRIER AND STUDY OF SHELF LIFE OF INOCULUM CARRIER

Cell populations of the five strains of **Rhizobium** species selected for the rest of this research evidently multiplied at different rates during 10 days of incubation in Yeast-extract mannitol (YEM) Broth. The populations ranged from 619 x 10^5 cells ml^{-1} (Weija strain) to 973 x 10^5 cells ml^{-1} (Nungua (1) strain), as shown in Table 5.

The five strains fell into two groups. Legon and Nungua(1) strains showed considerably greater populations than Kpong, Pokuase and Weija strains.

Undiluted, and ½, ¼, and 1/8 dilutions of the various broth cultures were used in inoculating, individually, samples of the ground moss compost. The inoculated compost was incubated for two weeks at room temperature. Viable cell counts of the **Rhizobium** sp. of the various strains cultured in the moss carrier for two weeks are presented in Table 6.

Each contained 10g of moss compost. To this was added 20ml of broth culture which brought the moisture content to 50 per cent. By mixing the contents of the bags very thoroughly, 1.0g of compost could be assumed to contain 2.0ml of the culture suspension added. It was possible, therefore, to determine the approximate initial cell population at the beginning of incubation. These calculated values have been recorded in Table 6 to show the extent of growth of the rhizobia in each treatment. There were three remarkable findings. First, growth of the population of all strains was tremendous. Secondly, inocula of the four different concentrations produced almost similar population levels at the end of 14 days, and thirdly, population counts, of strains with initial

smaller cell numbers (Ex-Ada in Weija soil, Ex-Tamale in Kpong soil and Ex-Tamale in Pokuase soil) and those of strains with initial greater cell numbers (Ex-Ada in Legon soil and Ex-Tamale in Nungua(1) soil) were quite close.

Shelf life studies made with moss compost inoculated with undiluted broth culture and half-diluted broth culture only, showed gradual increase in population counts with all and half-diluted broth culture the strains of **Rhizobium** sp. in the moss carrier within 14 weeks. There was no sign of autolysis in the 14 weeks, but by the 10th week all the strains had reached the phase of Stationary Growth.

Plate 3 shows abundant growth of the cells of the various strains when suspensions of the various moss carriers were prepared after six weeks' storage and plated on Congo red YMA. The weekly population counts recorded are presented graphically in Fig. 4, while the values appear in Appendices.

Contamination of the moss carrier during storage was negligible. The very few contaminants could be identified because they become strongly coloured with the dye. No attempt was made to identify them, nor to quantify their numbers.

TABLE 5

Population of strains of **Rhizobium** sp. grown in broth of YEM medium at 30°C for 10 days.

Strains of **Rhizobium** sp. from nodules of	Mean No. of **Rhizobium** population. ($X10^5$) ml^{-1}
Ex-Ada in Legon soil	852
Ex-Ada in Weija soil	619
Ex-Tamale in Kpong soil	675
Ex-Tamale in Nungua(1) soil	973
Ex-Tamale in Pokuase soil	676

TABLE 6

Multiplication of strains **Rhizobium** sp. in moss carrier incubated at 30°C for 2 weeks.

Strains of **Rhizobium** sp from nodules of	Inoculum concentration	Approximate initial Population ($X10^9$) g^{-1} carrier	Mean No. of **Rhizobium** population present ($X10^9$) g^{-1} carrier
Ex-Ada in Legon soil	Undiluted	0.0852	242
	½ Dilution	0.0426	192
	¼ Dilution	0.0213	207
	1/8 Dilution	0.0107	215
Ex-Ada in Weija soil	Undiluted	0.0619	240
	½ Dilution	0.0310	189
	¼ Dilution	0.0155	199
	1/8 Dilution	0.0077	210
Ex-Tamale in Kpong soil	Undiluted	0.0675	246
	½ Dilution	0.0338	194
	¼ Dilution	0.0169	213
	1/8 Dilution	0.0084	243
Ex Tamale in Nungua(1) soil	Undiluted	0.0973	221
	½ Dilution	0.0487	196
	¼ Dilution	0.0243	228
	1/8 Dilution	0.0122	206
Ex-Tamale in Pokuase soil	Undiluted	0.0676	216
	½ Dilution	0.0338	190
	¼ Dilution	0.0169	224
	1/8 Dilution	0.0085	195

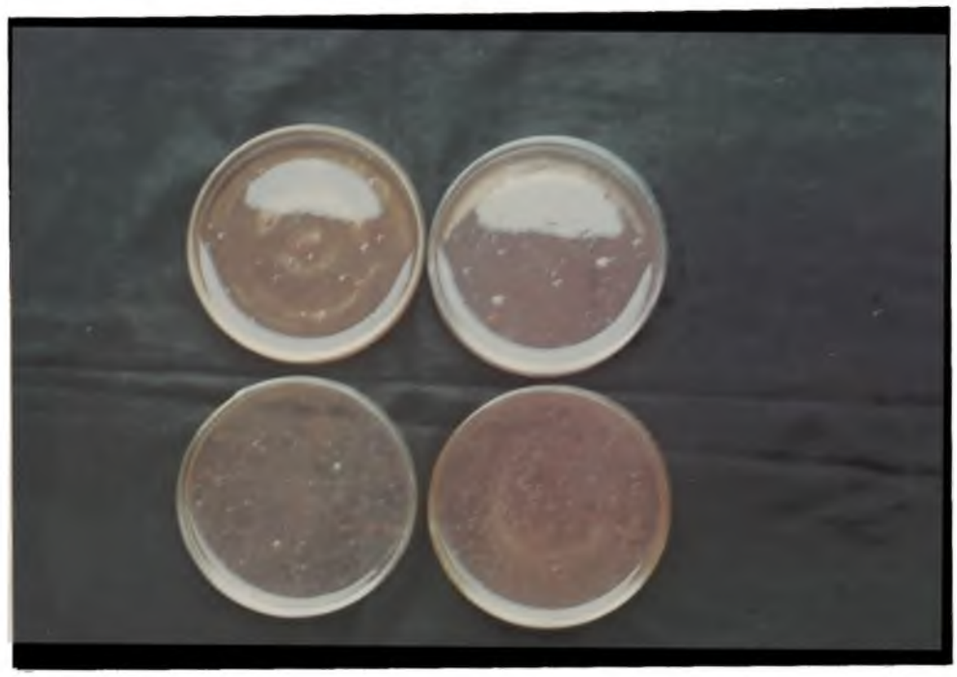
3a. TOP: Inocula of Kpong-Ex-Tamale strain (Left) and Pokuase-Ex-Tamale strain (Right).

BOTTOM: Inocula of Kpong-Ex-Tamale strain (Left) and Pokuase-Ex-Tamale strain (Right) using the ½ dilutions as inocula. ($X^1/_3$).

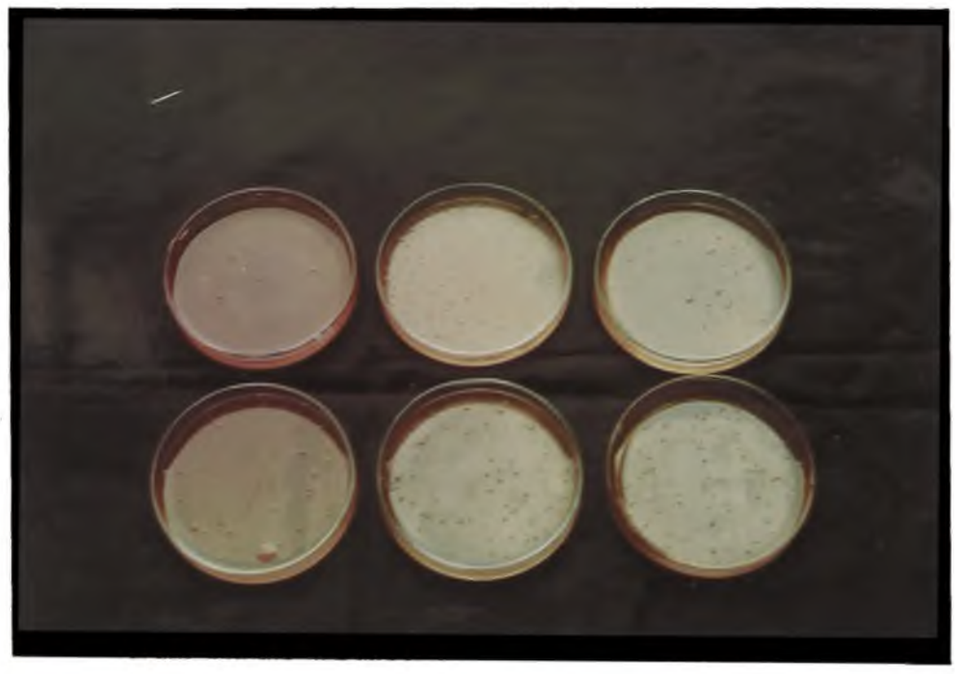
3b. TOP: Inocula of Weija-Ex-Ada strain (Left), Legon-Ex-Ada strain (Middle) and Nungua(1)-Ex-Tamale strain (Right).

BOTTOM: Inocula of Weija-Ex-Ada strain (Left), Legon-Ex-Ada strain (Middle) and Nungua(1)-Ex-Tamale strain (Right), using the ½ dilutions as inocula. ($X^1/_4$).

PLATE 3. Photographs of Petri plates of Congo red YMA inoculated with suspensions from the moss carrier after 6 weeks on the shelf. Incubation at 30°C for 5 days.



3a



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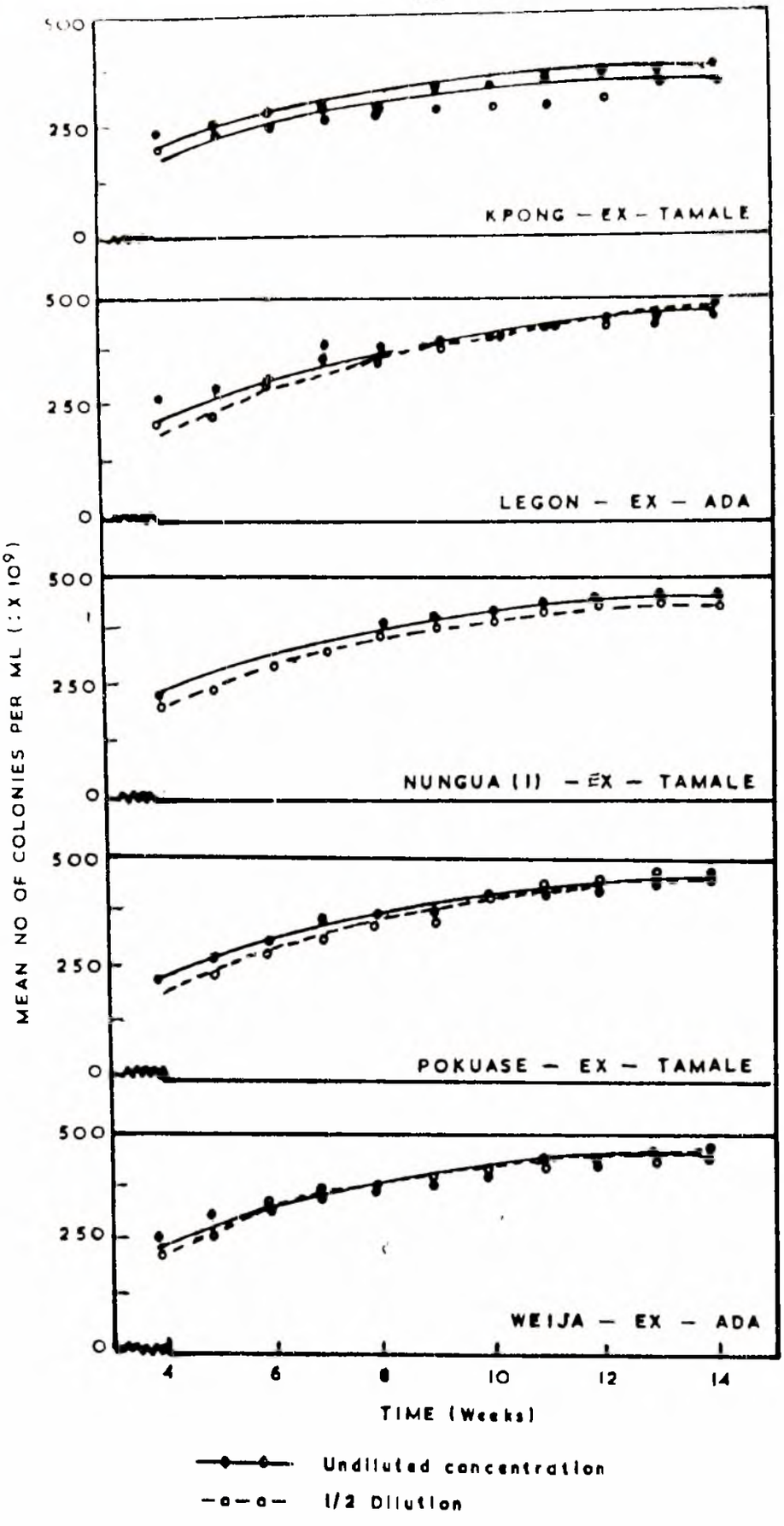

Fig. 4 Populations of five strains of Rhizobium sp. in inoculated moss carriers stored at room temperature for 14 weeks.

E. NODULATION OF PLANTS INOCULATED WITH DIFFERENT STRAINS OF RHIZOBIUM SP. IN MOSS CARRIER UNDER DIFFERENT WATER STRESS CONDITIONS

Uninoculated control plants grew poorly and did not form any nodules. They formed few leaves, only 5 - 6 in number, which were yellowish-green in colour, and the plants were generally stunted. Those receiving water each other day produced the largest leaflets of which the middle leaflet measured on the average 3.36 cm long and 1.57 cm wide. Those watered once in four days and once in six days produced smaller leaflets.

All the inoculated plants of the various watering treatments, on the other hand, nodulated. They had greater mean dry weight, they produced more leaves which were deep-green in colour (adaxial surface) and larger leaflets. The results obtained are shown in Tables 7-11.

The amount of water received by the plants greatly affected them in many ways. It affected the dry weights of the plants, the number of leaves (Plates 4 and 5) and the number of nodules formed. The values recorded for these were highest for plants watered at 2-day intervals and lowest for plants watered at 6-day intervals. Plants watered at 2-day intervals had mean dry weights from 1.00g to 1.68g, produced 10 - 15 leaves and formed 43 - 56 nodules per plant. The corresponding values for plants watered at 4-day intervals were 0.64 - 0.94g, 8 - 12 leaves and 16 - 25 nodules. And for plants watered at 6-day intervals, 0.48 - 0.96g, 7 - 10 leaves and 8 - 11 leaves.

TABLE 7a

Leaf development of Bambara groundnut, variety Ex-Tamale, inoculated with Kpong-Ex-Tamale strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following water stresses*			Mean length and width of mid-leaflet (cm) at following water stresses*		
	2	4	6	2	4	6
undiluted	15	12	9	6.13x2.29	6.00x2.10	6.32x2.08
½ Dilution	11	11	8	6.13x2.44	6.32x2.49	6.35x2.03
¼ Dilution	11	11	8	6.36x2.37	6.09x2.18	5.63x2.05
$^1/_8$ Dilution	10	8	8	5.45x2.06	6.61x2.01	6.04x1.89
Uninoculated (CONTROL)	6	6	5	3.36x1.57	2.29x1.31	1.57x0.91

* 2 = watered once in 2 days.
4 = watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 7b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Tamale, plants inoculated with Kpong-Ex-Tamale strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following water stresses*			Mean No. of Nodules per plant (to the nearest whole no.) at following water stresses*		
	2	4	6	2	4	6
Undiluted	1.44 ±0.39	0.94 ±0.25	0.70 ±0.22	55	19	9
½ Dilution	1.26 ±0.33	0.78 ±0.23	0.74 ±0.22	49	18	9
¼ Dilution	1.00 ±0.27	0.69 ±0.21	0.72 ±0.24	49	18	10
1/8 Dilution	1.10 ±0.29	0.90 ±0.28	0.62 ±0.17	43	16	10
Uninoculated (CONTROL)	0.54 ±0.16	0.32 ±0.14	0.11 ±0.03	0	0	0

* 2 = watered once in 2 days
4 = watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 8a

Leaf development of Bambara groundnut, variety Ex-Tamale, inoculated with Legon-Ex-Ada strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following water stresses*			Mean length and width of mid-leaflet (cm) at following water stresses*		
	2	4	6	2	4	6
Undiluted	12	10	8	8.18x2.76	5.45x2.31	5.55x2.05
½ Dilution	10	9	8	8.50x2.74	7.06x2.31	5.98x2.26
¼ Dilution	11	10	9	6.52x2.30	5.61x2.19	5.90x1.89
1/8 Dilution	11	9	8	6.75x2.33	6.25x2.11	5.42x2.08
Uninoculated (CONTROL)	6	6	5	3.36x1.57	2.29x1.31	1.57x0.91

* 2 = watered once in 2 days.
4 = watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 8b

Dry Weight and extent of nodulation of Bambara groundnut, variety Ex-Tamale, plants inoculated with Legon-Ex-Ada strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following water stresses*			Mean No. of Nodules per plant (to the nearest whole no.) at following water stresses*		
	2	4	6	2	4	6
Undiluted	1.59 ±0.44	0.88 ±0.28	0.64 ±0.18	44	20	11
½ Dilution	1.10 ±0.28	0.90 ±0.29	0.59 ±0.21	52	18	9
¼ Dilution	1.49 ±0.44	0.84 ±0.29	0.69 ±0.21	51	16	8
$^1/_8$ Dilution	1.36 ±0.41	0.69 ±0.24	0.68 ±0.20	50	20	11
Uninoculated (CONTROL)	0.54 ±0.16	0.32 ±0.14	0.11 ±0.03	0	0	0

* 2 = watered once in 2 days
4 = watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 9a

Leaf development of Bambara groundnut, variety Ex-Tamale, inoculated with Nungua (1) Ex-Tamale strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following water stresses*			Mean length and width of mid-leaflet (cm) at following water stresses*		
	2	4	6	2	4	6
Undiluted	12	10	8	6.98x2.79	5.61x2.14	5.27x2.05
½ Dilution	14	10	8	5.82x2.18	5.06x2.02	5.57x2.39
¼ Dilution	10	9	7	6.66x2.33	5.20x2.19	5.60x1.93
1/8 Dilution	12	9	8	6.02x2.27	6.06x2.16	6.43x2.07
Uninoculated (CONTROL)	6	6	5	3.36x1.57	2.29x1.31	1.57x0.91

* 2 watered once in 2 days.
4 watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 9b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Tamale, plants inoculated with Nungua(1)-Ex-Tamale strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following water stresses*			Mean No. of Nodules per plant (to the nearest whole no.) at following water stresses*		
	2	4	6	2	4	6
undiluted	1.27 ±0.30	0.85 ±0.24	0.72 ±0.21	47	19	10
½ Dilution	1.17 ±0.28	0.69 ±0.21	0.67 ±0.21	44	18	11
¼ Dilution	1.22 ±0.38	0.67 ±0.22	0.58 ±0.20	50	18	10
1/8 Dilution	1.25 ±0.38	0.64 ±0.21	0.70 ±0.19	49	18	9
Uninoculated (CONTROL)	0.54 ±0.16	0.32 ±0.14	0.11 ±0.03	0	0	0

* 2 = watered once in 2 days
4 = watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 10a

Leaf development of Bambara groundnut, variety Ex-Tamale, inoculated with Pokuase-Ex-Tamale strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following water stresses*			Mean length and width of mid-leaflet (cm) at following water stresses*		
	2	4	6	2	4	6
undiluted	12	11	9	6.55x2.51	6.47x2.26	4.88x1.94
½ Dilution	13	10	9	6.81x2.28	5.90x1.98	5.41x1.98
¼ Dilution	13	9	10	6.62x2.78	6.34x2.05	6.26x1.88
1/8 Dilution	10	10	9	7.19x2.59	6.18x2.55	5.30x2.09
Uninoculated (CONTROL)	6	6	5	3.36x1.57	2.29x1.31	1.57x0.91

* 2 watered once in 2 days.
4 watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 10b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Tamale, plants inoculated with Pokuase-Ex-Tamale strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following water stresses*			Mean No. of Nodules per plant (to the nearest whole no.) at following water stresses*		
	2	4	6	2	4	6
Undiluted	1.43 ±0.43	0.88 ±0.23	0.76 ±0.33	44	19	11
½ Dilution	1.39 ±0.41	0.70 ±0.19	0.57 ±0.16	51	19	9
¼ Dilution	1.29 ±0.36	0.64 ±0.17	0.61 ±0.23	56	20	9
1/8 Dilution	1.32 ±0.31	0.74 ±0.22	0.48 ±0.11	45	19	9
Uninoculated (CONTROL)	0.54 ±0.16	0.32 ±0.14	0.11 ±0.03	0	0	0

* 2 = watered once in 2 days
4 = watered once in 4 days
6 = watered once in 6 days (see Appendix A)

TABLE 11a

Leaf development of Bambara groundnut, variety Ex-Tamale, inoculated with Weija-Ex-Ada strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following water stresses*			Mean length and width of mid-leaflet (cm) at following water stresses*		
	2	4	6	2	4	6
Undiluted	13	11	9	6.13x2.48	4.76x2.03	5.63x2.21
½ Dilution	12	10	9	6.52x2.55	5.31x2.16	6.43x2.05
¼ Dilution	13	8	9	6.03x2.37	5.97x2.20	6.02x1.90
1/8 Dilution	12	11	9	7.02x2.17	5.80x2.13	5.99x2.13
Uninoculated (CONTROL)	6	6	5	3.36x1.57	2.29x1.31	1.57x0.91

* 2 = watered once in 2 days.
4 = watered once in 4 days
6 watered once in 6 days (see Appendix A)

TABLE 11b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Tamale, plants inoculated with Weija-Ex-Ada strain grown under normal day/night regime at different water stresses.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following water stresses*			Mean No. of Nodules per plant (to the nearest whole no.) at following water stresses*		
	2	4	6	2	4	6
undiluted	1.68 ±0.43	0.71 ±0.22	0.73 ±0.22	51	17	9
½ Dilution	1.65 ±0.45	0.76 ±0.23	0.51 ±0.16	53	19	11
¼ Dilution	1.24 ±0.33	0.77 ±0.20	0.68 ±0.20	53	18	10
$^1/_8$ Dilution	1.48 ±0.38	0.65 ±0.19	0.69 ±0.20	55	25	10
Uninoculated (CONTROL)	0.54 ±0.16	0.32 ±0.14	0.11 ±0.03	0	0	0

* 2 = watered once in 2 days
4 = watered once in 4 days
6 = watered once in 6 days (see Appendix A)

PLATE 4. Photograph showing bambara groundnut plants watered at different intervals after inoculation with **Rhizobium** sp. (x $^{1}/_{9}$)

Left: Watered at 2-day intervals.

Middle: Watered at 4-day intervals.

Right: Watered at 6-day intervals.

PLATE 5. Photograph showing 30 day old bambara groundnut plants watered at different intervals after inoculation with **Rhizobium** sp.
Note the differences in development of both shoot and root systems. (x $^{1}/_{6}$))

Left: Watered at 2-day intervals.

Middle: Watered at 4-day intervals.

Right: Watered at 6-day intervals.

Mean leaf number per plant

Analyses of Variance (Two-way Anova with replication) showed that there was significant effect of the water stress and inoculation on the mean leaf number for all the five strains of **Rhizobium** sp. used at both 5 per cent and 1 per cent levels of significance. There was, however, no significant effect of the interaction of the two treatments on the leaf number development and so the two treatments were acting independent of each other (Tables 12a, 13a, 14a, 15a, and 16a).

The Duncan's New Multiple Range Test of Tables 12b, 13b, 14b, 15b and 16b showed that for each strain of **Rhizobium** sp. the four initial inoculum densities fell in one group and they were not significantly different from each other at both 5 per cent and 1 per cent levels of significance, but were together different from the control.

The effect of water stress can be summarised as follows:

(a) The effects of the three water regimes were significantly different from each other at both 5 per cent and 1 per cent levels of significance in plants inoculated with Nungua(1) - Ex-Tamale strain.

(b) In plants inoculated with Kpong-Ex-Tamale strain, the effects of the three water regimes were significantly different from each other at 5 percent level of significance, but at 1 per cent level of significance effects of watering at 4 and 6-day intervals were not significantly different from each other.

(c) In plants inoculated with strains of Pokuase-Ex-Tamale and Weija-Ex-Ada, effects of different watering times were not significantly different at 5 per cent level of significance, but at 1 per cent level plants watered at 2-day intervals produced a significantly different effect from the other two treatments.

(d) In plants inoculated with strain of Legon-Ex-Ada, the effects were not significantly different at both 5 per cent and 1 per cent levels of significance.

TABLE 12a

Analysis of variance (Two-way Anova with replication) for data of Table 7a. Mean leaf number per plant.

(Plants inoculated with Kpong-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	122.75	2	61.375	8.249 **
Inoculation	292.75	4	73.188	9.837 **
Water stress & Inoculation	41.25	8	5.156	0.693 NS
Error	446.40	60	7.440	-
T O T A L	903.15	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 12b

(Data of Table 12a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
	———		5%
			1%

b) inoculation

A	B	C	D	Control	
———					5%
———					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 13a

Analysis of variance (Two-way Anova with replication) for data of Table 8a. Mean leaf number per plant.

(Plant inoculated with Legon-Ex-Ada strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	77.31	2	38.655	5.315 **
Inoculation	165.87	4	41.468	5.701 **
Water stress & Inoculation	11.09	8	1.386	0.191 NS
Error	436.40	60	7.273	-
T O T A L	690.67	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 13b

(Data of Table 13a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to

a) Water stress

	2	4	6
5%			
1%			

b) inoculation

	A	B	C	D	Control
5%					
1%					

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 14a

Analysis of variance (Two-way Anova with replication) for data of Table 9a. Mean leaf number per plant.

(Plant inoculated with Nungua(1)-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	148.88	2	74.440	21.309 **
Inoculation	219.87	4	54.968	15.735 **
Water stress & Inoculation	29.65	8	3.706	1.061 NS
Error	209.60	60	3.493	-
T O T A L	608.00	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 14b

(Data of Table 14a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to

a) Water stress

	2	4	6
5%			
1%			

b) inoculation

	A	B	C	D	Control
5%					
1%					

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 15a

Analysis of variance (Two-way Anova with replication) for data of Table 10a. Mean leaf number per plant.

(Plant inoculated with Pokuase-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	116.27	2	58.135	7.234 **
Inoculation	254.94	4	63.735	8.468 **
Water stress & Inoculation	41.86	8	5.233	0.695 NS
Error	451.60	60	7.527	-
T O T A L	864.67	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 15b

(Data of Table 15a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
_____			5%
	_____		1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 16a

Analysis of variance (Two-way Anova with replication) for data of Table 11a. Mean leaf number per plant.

(Plant inoculated with Weija-Ex-Ada strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	122.75	2	61.375	9.433 **
Inoculation	253.79	4	63.448	9.751 **
Water stress & Inoculation	45.65	8	5.706	0.877 NS
Error	390.40	60	6.507	-
T O T A L	812.59	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 16b

(Data of Table 16a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
_____			5%
	_____		1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

Mean size of middle leaflets.

It was remarkable that water stress had a great effect on the size of the leaflets of the uninoculated plants (Tables 7a, 8a, 9a, 10a, 11a), and the mean length of middle leaflets of plants watered at 2 day intervals was more than double that of plants watered at 6-day intervals. Even though the differences in the inoculated plants were not that accentuated, Analyses of Variance (Two-way Anova with replication) in Tables 17a, 18a, 19a, 20a, 21a showed there was significant effect of the water stress and inoculation on the leaflet sizes at both 5 per cent and 1 per cent levels of significance. There was a significant effect of their interaction at both 5 per cent and 1 per cent levels of significance for Legon-Ex-Ada, Nungua(1)-Ex-Tamale and Weija-Ex-Ada strains, a significant effect at 5 per cent level of significance only for Pokuase-Ex-Tamale strain, and a non-significant effect for Kpong-Ex-Tamale strain.

By the Duncan's New Multiple Range Test shown in Tables 17b, 18b, 19b, 20b and 21b, there was no effect of the initial inoculum density at both 5 per cent and 1 per cent levels of significance for Kpong-Ex-Tamale, Nungua(1)-Ex-Tamale, Pokuase-Ex-Tamale and Weija-Ex-Ada strains. For the Legon-Ex-Ada strain, the initial inoculum densities separated into two groups, undiluted and ½ dilution in one, and ¼ and 1/8 dilution in the other.

In the case of relationship between water stress and leaflet size, the Duncan's New Multiple Range Test also in Tables 17b, 18b, 19b, 20b and 21b, showed that the effects of the three watering regimes were significantly different at both 5 per cent and 1 per cent levels of significance with plants inoculated with Legon-Ex-Ada, Pokuase-Ex-Tamale, and Weija-Ex-Ada strains; the effect of 2-day watering was significantly different from the effects of 4-day and 6-day watering at both 5 per cent and 1 per cent levels of significance for plants inoculated with Nungua(1)-Ex-Tamale strain; and the effects of the three watering regimes were not significantly different at both 5 per cent and 1 per cent levels of significance for plants inoculated with Kpong-Ex-Tamale strain.

TABLE 17a

Analysis of variance (Two-way Anova with replication) for data of Table 7a. Mean size of middle leaflets

(Plant inoculated with Kpong-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	100.30	2	50.150	9.166 **
Inoculation	1666.30	4	416.575	76.141 **
Water stress & Inoculation	81.01	8	10.126	1.851 NS
Error	492.40	90	5.471	-
T O T A L	2339.75	104	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 17b

(Data of Table 17a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
_____			5%
_____			1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 18a

Analysis of variance (Two-way Anova with replication) for data of Table 8a. Mean size of middle leaflets

(Plant inoculated with Legon-Ex-Ada strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	890.05	2	445.025	55.855 **
Inoculation	2407.18	4	601.795	75.532 **
Water stress & Inoculation	386.63	8	48.329	0.066 **
Error	717.07	90	7.967	-
T O T A L	4400.93	104	-	-

** Significant at 1% level of significance

TABLE 18b

(Data of Table 18a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 19a

Analysis of variance (Two-way Anova with replication) for data of Table 9a. Mean size of middle leaflets

(Plant inoculated with Nungua(1)-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	222.11	2	111.055	35.206 **
Inoculation	1635.53	4	408.883	129.621 **
Water stress & Inoculation	235.04	8	29.38	9.314 **
Error	283.90	90	3.154	-
T O T A L	2376.58	104	-	-

** Significant at 1% level of significance

TABLE 19b

(Data of Table 19a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
	_____		5%
	_____		1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval
A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 19b

(Data of Table 19a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
	_____		5%
	_____		1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval
A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 20a

Analysis of variance (Two-way Anova with replication) for data of Table 10a. Mean size of middle leaflets

(Plant inoculated with Pokuase-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	628.65	2	314.325	71.483 **
Inoculation	1966.79	4	491.698	111.820 **
Water stress & Inoculation	93.00	8	11.625	2.644 *
Error	395.75	90	4.397	-
T O T A L	3084.19	104	-	-

** Significant at 1% level of significance

* Significant at 5% level of significance

TABLE 20b

(Data of Table 20a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 21a

Analysis of variance (Two-way Anova with replication) for data of Table 11a. Mean size of middle leaflets

(Plant inoculated with Weija-Ex-Ada strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	259.82	2	129.910	62.537 **
Inoculation	1716.07	4	429.018	206.523 **
Water stress & Inoculation	83.66	8	10.458	5.034 **
Error	186.96	90	2.077	-
T O T A L	2246.51	104	-	-

** Significant at 1% level of significance

TABLE 21b

(Data of Table 21a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

Mean dry weight of plants.

There was marked effect of inoculation on the mean dry weight of the bambara groundnut plants as there was above 30 per cent increase of all the inoculated plants over the uninoculated (control) plant.

Analyses of Variance (Two-way Anova with replication) test of Tables 22a, 23a, 24a, 25a and 26a showed significant effect of the treatments on dry weight at 5 per cent and 1 per cent levels of significance for all the inoculated plants. There was, however, no significant effect of the interaction of the two treatments on the dry weight.

There were no significant differences between the effects of the different initial inoculum densities at both 5 per cent and 1 per cent levels of significance, using the Duncan's New Multiple Range Test presented in Tables 22b, 23b, 24b, 25b and 26b.

In case of the water stress treatments, the three watering regimes had significantly different effects at 1 per cent level of significance for plants inoculated with the Kpong-Ex-Tamale strain, but at 5 per cent level of significance there was no significant difference between the effects of the 2-day and 4-day watering intervals. With plants inoculated with the remaining four strains, there was no significant difference between the effects of the 4-day and 6-day watering treatments at both 5 per cent and 1 per cent levels of significance.

TABLE 22a

Analysis of variance (Two-way Anova with replication) for data of Table 7a. Mean dry weight.

(Plant inoculated with Kpong-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	13.10	2	6.550	8.747 **
Inoculation	17.59	4	4.398	5.873 **
Water stress & Inoculation	1.92	8	0.240	0.321 NS
Error	44.93	60	0.749	-
T O T A L	77.54	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 22b

(Data of Table 22a)

Duncan's New Multiple Range Test for means of dry weight of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 23a

Analysis of variance (Two-way Anova with replication) for data of Table 8b. Mean dry weight.

(Plant inoculated with Legon-Ex-Ada strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	24.77	2	12.385	18.952 **
Inoculation	19.60	4	4.900	7.498 **
Water stress & Inoculation	3.36	8	0.420	0.643 NS
Error	39.21	60	0.654	-
T O T A L	86.94	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 23b

(Data of Table 23a)

Duncan's New Multiple Range Test for means of dry weight of Bambara groundnut plants subjected to

a) Water stress

	2	4	6
5%		———	
1%		———	

b) inoculation

	A	B	C	D	Control
5%	———				
1%	———				

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 24a

Analysis of variance (Two-way Anova with replication) for data of Table 9b. Mean dry weight.

(Plant inoculated with Nungua(1)-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	16.41	2	8.205	8.838 **
Inoculation	14.96	4	3.740	4.028 **
Water stress & Inoculation	1.11	8	0.139	0.150 NS
Error	55.71	60	0.928	-
T O T A L	88.19	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 24b

(Data of Table 24a)

Duncan's New Multiple Range Test for means of dry weight of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
	———		5%
	———		1%

b) inoculation

A	B	C	D	Control	
———					5%
———					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 25a

Analysis of variance (Two-way Anova with replication) for data of Table 10b. Mean dry weight.

(Plant inoculated with Pokuase-Ex-Tamale strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	23.75	2	11.875	27.164 **
Inoculation	19.03	4	4.758	10.883 **
Water stress & Inoculation	2.42	8	0.303	0.692 NS
Error	26.23	60	0.437	-
T O T A L	71.43	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 25b

(Data of Table 25a)

Duncan's New Multiple Range Test for means of dry weight of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
	———		5%
	———		1%

b) inoculation

A	B	C	D	Control	
———					5%
———					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

TABLE 26a

Analysis of variance (Two-way Anova with replication) for data of Table 11b. Mean dry weight.

(Plant inoculated with Weija-Ex-Ada strain).

Source of Variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	36.47	2	18.235	52.100 **
Inoculation	20.25	4	5.063	14.464 **
Water stress & Inoculation	5.25	8	0.656	1.875 NS
Error	21.00	60	0.350	-
T O T A L	82.97	74	-	-

** Significant at 1% level of significance

NS Non-Significant

TABLE 26b

(Data of Table 26a)

Duncan's New Multiple Range Test for means of dry weight of Bambara groundnut plants subjected to

a) Water stress

2	4	6	
	_____		5%
	_____		1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: 1/8 dilution

Mean number of nodules per plant

Finally the result of Analyses of Variance Tests (Two-way Anova with replication) presented in Tables 27a, 28a, 29a, 30a and 31a showed that there was significant effect at both 5 per cent and 1 per cent levels of significance of the two treatments, that is water stress and inoculation, as well as their interaction on the mean number of nodules formed by the bambara groundnut plants.

Tables 27b, 28b, 29b, 30b and 31b show the results of Duncan's New Multiple Range Test. Inoculation had a clear effect, and the effect of all of the inoculations were not significantly different from each other but different from effects of the controls at both 5 per cent and 1 per cent levels of significance.

The effects of the three watering regimes were significantly different from each other at both 5 per cent and 1 per cent levels for plants inoculated with Nungua(1)-Ex-Tamale and Pokuase-Ex-Tamale strains. The effects of the three levels of watering were also different from each other at 1 per cent level of significance for plants inoculated with Kpong-Ex-Tamale and Legon-Ex-Ada strains, but at 5 per cent level of significance there was no significant differences between the effects of 4-day and 6-day watering regimes. Plants inoculated with Weija-Ex-Ada strain showed non-significant differences between the effect of 4-day and 6-day watering regimes at both 5 per cent and 1 per cent levels of significance.

TABLE 27a

Analysis of variance (Two-way Anova with replication) for data of Table 7b. Mean number of nodules.

(Plants inoculated with Kpong-Ex-Tamale strain).

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	13904.35	2	6952.175	120.725 * *
Inoculation	7918.59	4	1979.648	34.377 * *
Water stress & Inoculation	3664.85	8	458.106	7.955 * *
Error	3455.20	60	57.587	-
TOTAL	28942.99	74	-	-

* * Significant at 1% level of significance.

TABLE 27b

(Data of Table 27a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) water stress

2	4	6	
	_____		5%
			1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: $^1/_8$ dilution.

TABLE 28a

Analysis of variance (Two-way Anova with replication) for data of Table 8b. Mean number of nodules.

(Plants inoculated with Legon-Ex-Ada strain).

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	13911.15	2	6955.575	120.785 * *
Inoculation	8015.66	4	2003.915	34.798 * *
Water stress & Inoculation	3716.18	8	464.523	8.067 * *
Error	3200.80	60	53.347	-
TOTAL	28843.79	74	-	-

* * Significant at 1% level of significance.

TABLE 28b

(Data of Table 28a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) water stress

2	4	6	
	_____		5%
			1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval

4: 4-day watering interval

6: 6-day watering interval

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 29a

Analysis of variance (Two-way Anova with replication) for data of Table 9b. Mean number of nodules.

(Plants inoculated with Nungua(1)-Ex-Tamale strain).

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	12346.16	2	6173.080	106.986 * *
Inoculation	7672.53	4	1918.133	33.243 * *
Water stress & Inoculation	3179.39	8	397.424	6.888 * *
Error	3462.00	60	57.700	-
TOTAL	26660.00	74	-	-

* * Significant at 1% level of significance.

TABLE 29b

(Data of Table 29a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) water stress

2	4	6	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval

4: 4-day watering interval

6: 6-day watering interval

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 30a

Analysis of variance (Two-way Anova with replication) for data of Table 10b. Mean number of nodules.

(Plants inoculated with Pokuase-Ex-Tamale strain).

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	13830.32	2	6915.160	107.959 * *
Inoculation	8154.99	4	2038.748	31.829 * *
Water stress & Inoculation	3771.81	8	471.476	7.361 * *
Error	3843.21	60	64.054	-
TOTAL	29600.33	74	-	-

* * Significant at 1% level of significance.

TABLE 30b

(Data of Table 30a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) water stress

2	4	6	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval
4: 4-day watering interval
6: 6-day watering interval

A: Undiluted concentration
B: ½ dilution
C: ¼ dilution
D: $^1/_8$ dilution.

TABLE 31a

Analysis of variance (Two-way Anova with replication) for data of Table 11b. Mean number of nodules.

(PLants inoculated with Weija-Ex-Ada strain).

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Water stress	16000.88	2	8000.440	74.250 * *
Inoculation	9208.13	4	2302.033	21.365 * *
Water stress & Inoculation	4095.39	8	511.924	4.751 * *
Error	6465.00	60	107.750	-
TOTAL	35769.40	74	-	-

* * Significant at 1% level of significance.

TABLE 31b

(Data of Table 31a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) water stress

2	4	6	
	_____		5%
	_____		1%

b) inoculation

A	B	C	D	Control	
_____					5%
_____					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

2: 2-day watering interval

4: 4-day watering interval

6: 6-day watering interval

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

Size of nodules

Measurements of the diameters of the nodules showed that in all the treatments nodule size was affected by the amount of water the plants received. The data in Table 32 and the histograms in Figs. 5 - 9 show that plants watered at 2-day intervals formed the largest nodules and those watered at 6-day intervals had the smallest nodules.

TABLE 32

Mean diameters of nodules formed by Bambara groundnut plants grown under different watering regimes for 30 days.

Rhizobium Inoculum	Initial density of Inoculum	Mean diameters (cm) of nodules of plants watered once in 2 days	4 days	6 days
Kpong-Ex-Tamale strain	Undiluted	2.62	1.48	0.69
	½ dilution	2.68	0.79	0.79
	¼ dilution	2.38	1.84	0.77
	1/8 dilution	2.56	0.88	0.83
Legon-Ex-Ada strain	Undiluted	2.11	1.90	0.87
	½ dilution	2.30	0.99	0.85
	¼ dilution	2.26	1.04	0.64
	1/8 dilution	2.42	1.05	0.58
Nungua(1)-Ex-Tamale strain	Undiluted	2.79	2.13	0.59
	½ dilution	2.38	0.96	0.71
	¼ dilution	2.48	0.73	0.74
	1/8 dilution	2.31	0.92	0.67
Pokuase-Ex-Tamale strain	Undiluted	3.01	0.99	0.85
	½ dilution	2.64	0.87	0.77
	¼ dilution	2.24	1.02	0.69
	1/8 dilution	2.23	0.97	0.62
Weija-Ex-Ada strain	Undiluted	2.75	0.88	0.82
	½ dilution	2.39	1.06	0.81
	¼ dilution	2.89	1.12	0.61
	1/8 dilution	2.14	0.85	0.74

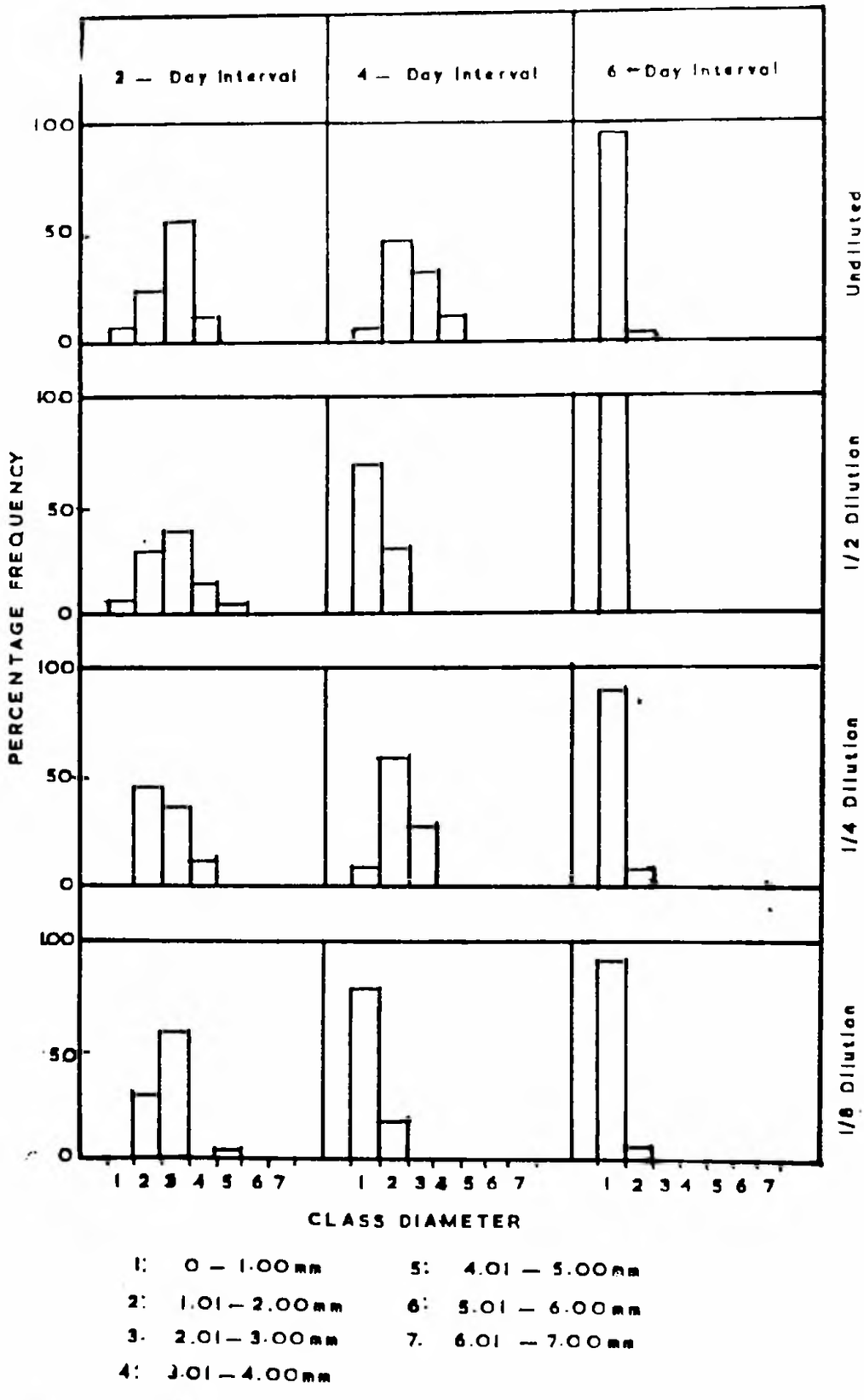

Fig 5 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Tamale variety, inoculated with Kpong-Ex-Tamale strain of Rhizobium sp. and grown under different water stress regimes for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

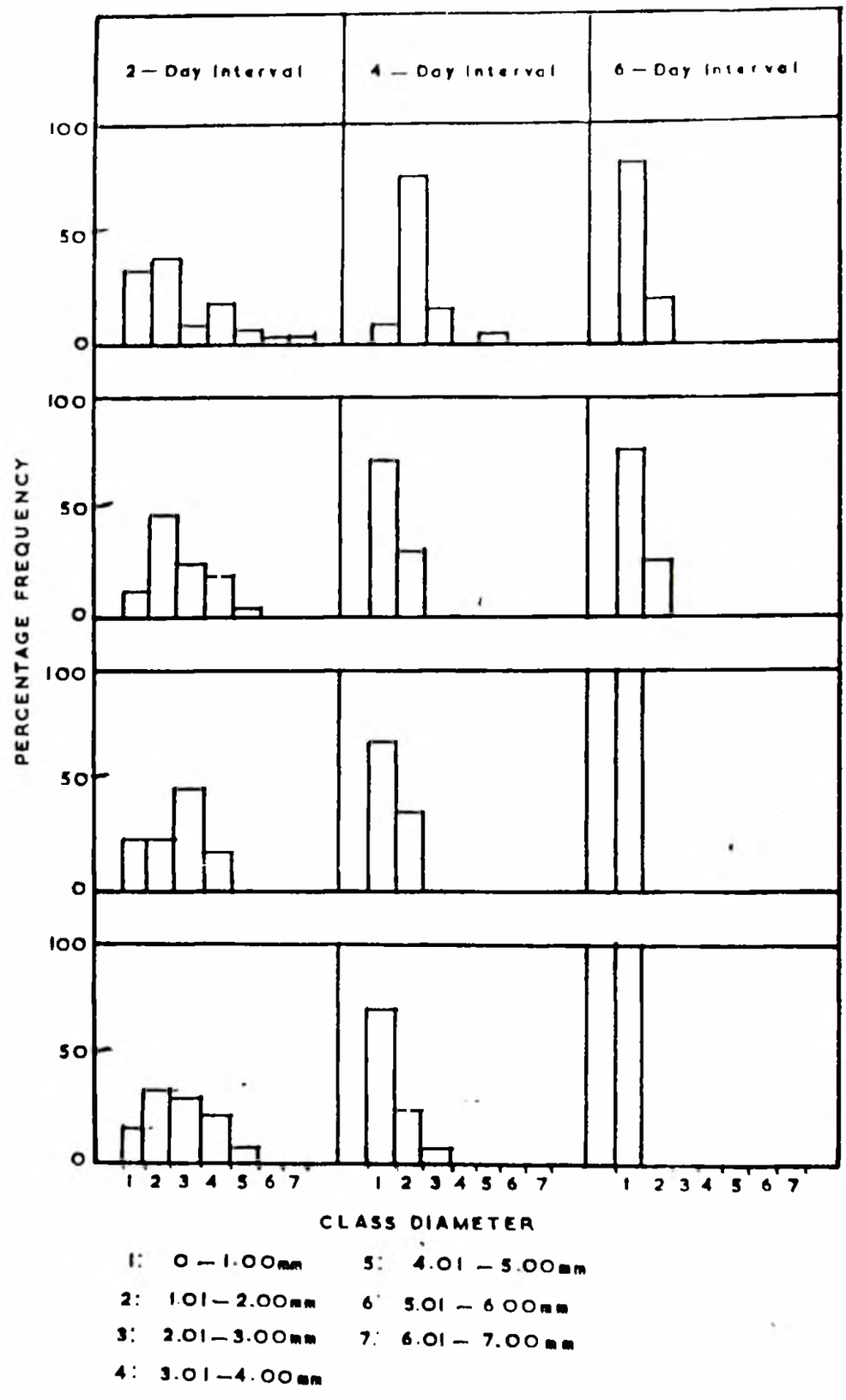

Fig. 6 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Tamale variety, inoculated with Legon-Ex-Ada strain of Rhizobium sp. and grown under different water stress regimes for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

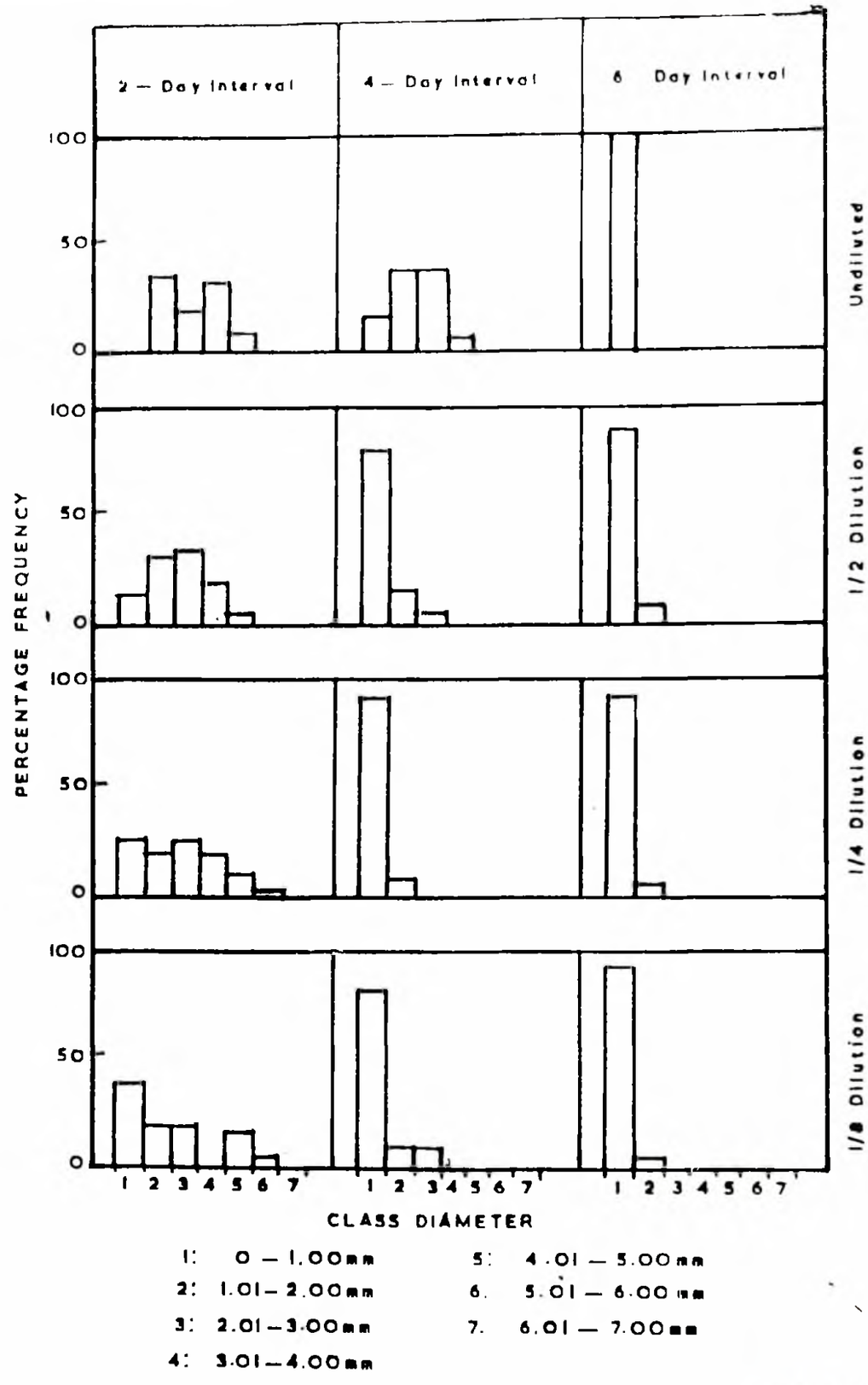

Fig 7 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Tamale variety, inoculated with Nungua(1)-Ex-Tamale strain of Rhizobium sp. and grown under different water stress regimes for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

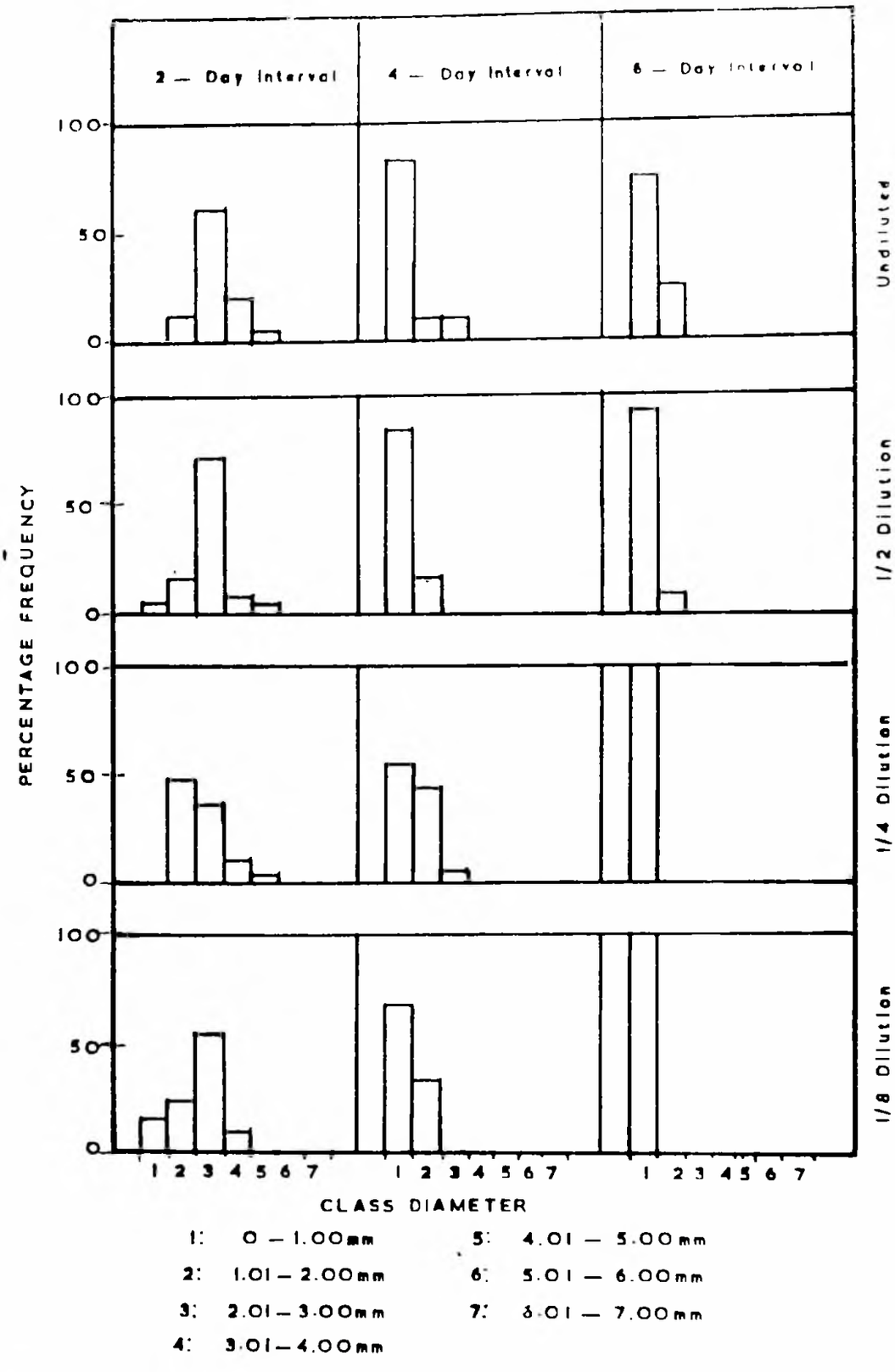

Fig. 8 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Tamale variety, inoculated with Pokuase-Ex-Tamale strain of Rhizobium sp. and grown under different water stress regimes for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

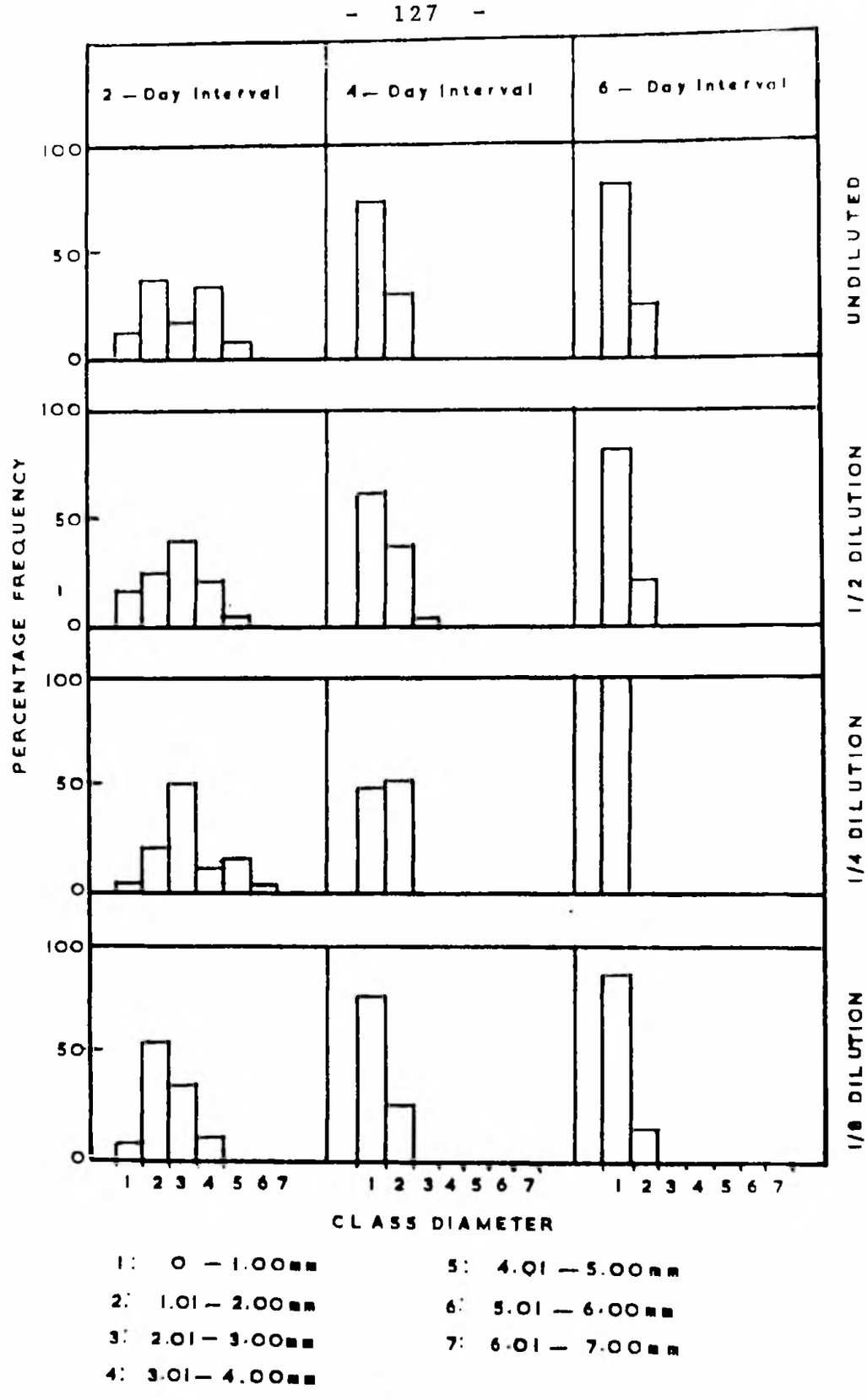

Fig. 9 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Tamale variety, inoculated with Weija-Ex-Ada strain of Rhizobium sp, and grown under different water stress regumes for 30 days. Seeds inoculated wtih moss carrier with different initial Broth culture

F. NODULATION OF PLANTS INOCULATED WITH DIFFERENT STRAINS OF RHIZOBIUM SP. IN MOSS CARRIER UNDER DIFFERENT LIGHT INTENSITIES

Using daylight, a fluctuation in light intensity should be expected each day with the highest intensity occurring around mid-day. It will be erroneous, therefore, to present a single value of light intensity for each of the three treatments. The graphs of light intensities at 9.00 am, 12.00 noon and 3.00 pm in Fig. 10 show that for Shed 1, providing the lowest light intensity, intensity at 9.00 am was between 1100 and 2200 lux, at 12.00 noon, it was between 4030 and 6200 lux, and it was between 1600 and 1900 lux at 3.00 pm. The corresponding values for Shed 2 were 1400 to 2800, 6400 to 9100 and 2900 to 3500 lux, respectively. And for Shed 3, 5300 to 6200, 8800 to 10000, and 4600 to 6400 lux. The three treatments will be referred to in the text as low light intensity, medium light intensity and high light intensity, for conditions under Shed 1, 2, and 3 respectively (Plate 6).

Inoculation of the bambara groundnut plants with the moss carrier was very effective under all the three different light intensities under which the plants were grown. The inoculated plants grew well and those under the two lower light intensities, Sheds 1 and 2, had green foliage. The plants raised under the highest light intensity, Shed 3, showed some yellowing of the leaves. The uninoculated (control) plants, also, had yellowish-green foliage (Plate 7).

Light intensity affected both inoculated and uninoculated plants. In the control set of plants, the mean numbers of leaves per plant, the size of the middle leaflets, and the mean dry weights of the plants decreased with increase in light intensity. The number of leaves in the inoculated plants was apparently not affected by light intensity within the range used in this investigation. The size of the middle leaflets, the dry weights of the plants and the number of nodules, however, decreased with increasing light intensity. The values recorded are presented in Tables 33 - 37.

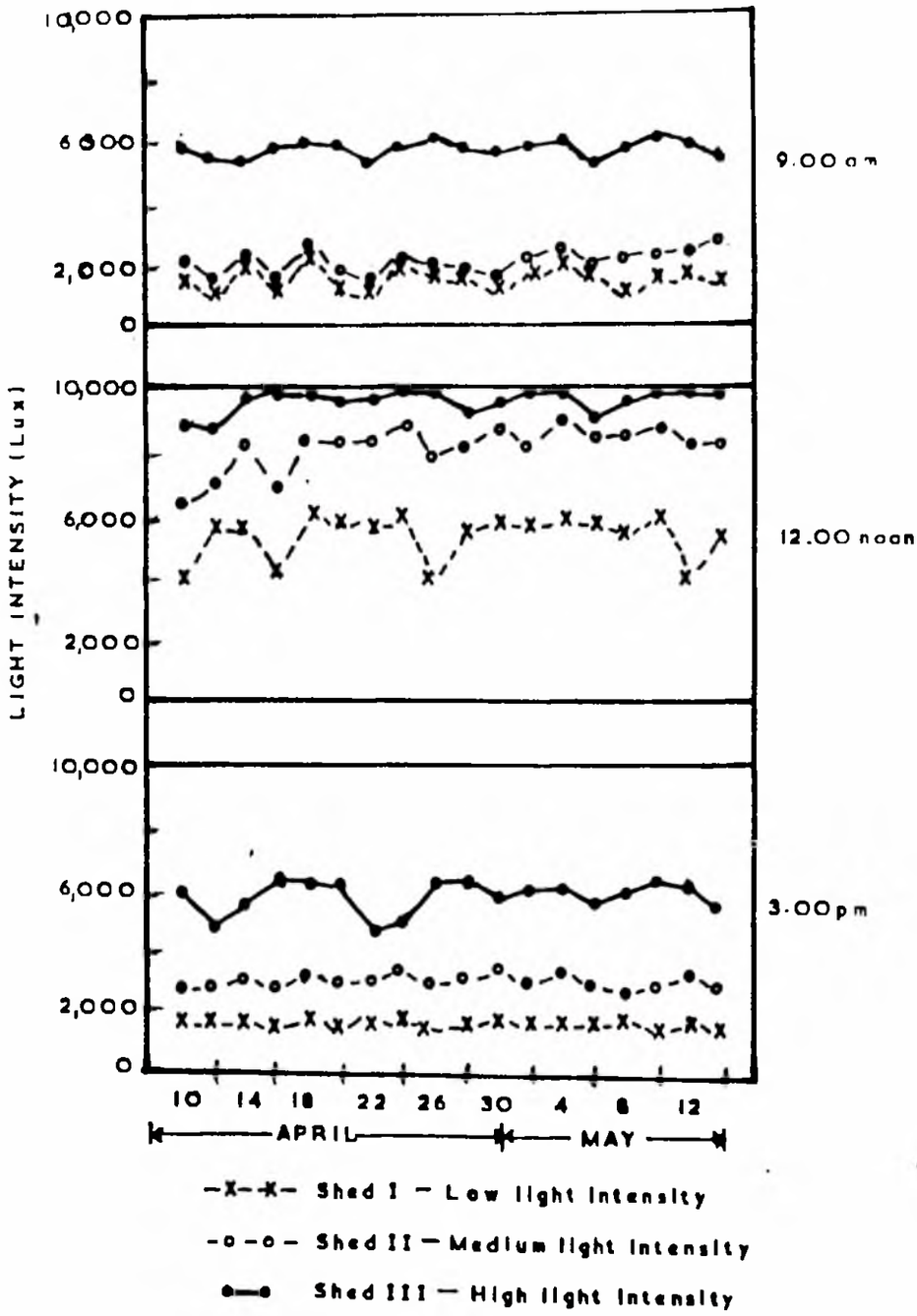

Fig. 10 Recording of light intensities under sheds 1, 2 and 3 at 9.00 a.m., 12.00 noon and 3.00 p.m. during period of growth of Bambara groundnut plants, Ex-Ada variety, inoculated with different strains of Rhizobium sp.

PLATE 6: Photograph showing the sheds of three light intensities with the removable sides of the two sheds with battens taken off to show the growing bambara groundnut plants. (x1/48)

Left: High light intensity (Shed 3)

Middle: Medium light intensity (Shed 2)

Right: Low light intensity (Shed 1)

PLATE 7: Photograph showing bambara groundnut plants raised under different light intensities after inoculation with **Rhizobium** sp. ($x\ ^{1}/_{10}$)

Left: Low light intensity (Shed 1)

Middle: Medium light intensity (Shed 2)

Right: High light intensity (Shed 3)

TABLE 33a

Leaf development of bambara groundnut, variety Ex-Ada, inoculated with Kpong-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following light intensities*			Mean length and width of mid-leaflet (cm) at following light intensities.*		
	L	M	H	L	M	H
Undiluted	25	25	26	6.09x2.70	5.47x2.27	4.90x1.74
½ Dilution	28	27	26	6.14x2.31	5.70x2.31	5.07x1.79
¼ Dilution	28	27	27	6.89x2.84	5.59x2.33	5.26x1.96
1/8 Dilution	27	26	28	5.60x2.34	5.79x2.49	5.16x1.64
Uninoculated (CONTROL)	21	19	11	3.00x1.40	2.20x1.19	2.00x0.98

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 33b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Ada, plants inoculated with Kpong-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following light intensities*			Mean No. of Nodules per plant (to the nearest whole no.) at following light intensities.		
	L	M	H	L	M	H
Undiluted	1.91 ±0.26	1.49 ±0.35	1.21 ±0.26	63	47	16
½ Dilution	1.77 ±0.30	1.50 ±0.33	1.18 ±0.37	65	48	18
¼ Dilution	1.62 ±0.30	1.35 ±0.29	1.17 ±0.22	66	48	20
1/8 Dilution	1.64 ±0.34	1.40 ±0.38	1.15 ±0.21	59	43	21
Uninoculated (CONTROL)	0.67 ±0.14	0.53 ±0.13	0.29 ±0.06	0	0	0

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 34a

Leaf development of Bambara groundnut, variety Ex-Ada, inoculated with Legon-Ex-Ada strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following light intensities*			Mean length and width of mid-leaflet (cm) at following light intensities.*		
	L	M	H	L	M	H
Undiluted	26	23	26	6.21x2.36	5.90x2.16	4.80x1.64
½ Dilution	25	22	26	5.83x2.50	5.67x2.21	4.93x1.53
¼ Dilution	25	24	26	6.10x2.36	5.46x2.00	4.77x1.56
$1/_8$ Dilution	27	25	26	5.70x2.28	5.66x2.14	4.84x1.56
Uninoculated (CONTROL)	21	19	11	3.00x1.4	2.20x1.19	2.00x0.98

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 34b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Ada, plants inoculated with Legon-Ex-Ada strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following light intensities*			Mean No. of Nodules per plant (to the nearest whole no.) at following light intensities.		
	L	M	H	L	M	H
Undiluted	1.73 ±0.28	1.46 ±0.32	1.17 ±0.29	65	43	18
½ Dilution	1.82 ±0.35	1.43 ±0.21	1.41 ±0.38	60	42	21
¼ Dilution	1.77 ±0.30	1.46 ±0.24	1.06 ±0.21	58	49	17
$^1/_8$ Dilution	1.69 ±0.31	1.50 ±0.31	1.10 ±0.21	67	43	20
Uninoculated (CONTROL)	0.67 ±0.14	0.53 ±0.13	0.29 ±0.06	0	0	0

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 35a

Leaf development of Bambara groundnut, variety Ex-Ada, inoculated with Nungua(1)-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following light intensities*			Mean length and width of mid-leaflet (cm) at following light intensities.*		
	L	M	H	L	M	H
Undiluted	26	25	27	7.83x2.79	5.41x2.36	4.57x1.60
½ Dilution	26	26	28	6.21x2.49	4.91x1.94	5.24x1.76
¼ Dilution	28	27	28	6.31x2.40	5.46x2.13	4.96x1.59
1/8 Dilution	27	25	26	6.51x2.66	5.41x2.16	5.08x2.00
Uninoculated (CONTROL)	21	19	11	3.00x1.40	2.20x1.19	2.00x0.98

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 35b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Ada, plants inoculated with Nungua(1)-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following light intensities*			Mean No. of Nodules per plant (to the nearest whole no.) at following light intensities.		
	L	M	H	L	M	H
Undiluted	1.93 ±0.47	1.46 ±0.28	1.24 ±0.22	68	45	17
½ Dilution	1.75 ±0.38	1.43 ±0.44	1.17 ±0.24	67	43	19
¼ Dilution	1.84 ±0.28	1.82 ±0.35	1.12 ±0.24	66	44	17
1/8 Dilution	2.91 ±0.61	1.58 ±0.26	0.91 ±0.19	64	43	19
Uninoculated (CONTROL)	0.67 ±0.14	0.53 ±0.13	0.29 ±0.06	0	0	0

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 36a

Leaf development of bambara groundnut, variety Ex-Ada, inoculated with Pokuase-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following light intensities*			Mean length and width of mid-leaflet (cm) at following light intensities.*		
	L	M	H	L	M	H
Undiluted	25	29	25	6.87x2.57	5.77x2.36	5.01x1.77
½ Dilution	28	26	27	6.47x2.43	5.51x2.17	5.63x1.79
¼ Dilution	27	28	27	5.67x2.27	5.86x2.30	5.71x2.16
$1/_8$ Dilution	27	27	27	6.01x2.49	5.37x2.01	5.11x1.77
Uninoculated (CONTROL)	21	19	11	3.00x1.40	2.20x1.19	2.00x0.98

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 36b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Ada, plants inoculated with Pokuase-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following light intensities*			Mean No. of Nodules per plant (to the nearest whole no.) at following light intensities.		
	L	M	H	L	M	H
Undiluted	2.70 ±0.28	1.48 ±0.30	1.18 ±0.23	70	49	18
½ Dilution	1.62 ±0.33	1.38 ±0.34	1.19 ±0.24	78	50	18
¼ Dilution	1.72 ±0.29	1.33 ±0.29	1.05 ±0.22	66	51	19
1/8 Dilution	1.79 ±0.33	1.52 ±0.35	1.02 ±0.26	64	45	20
Uninoculated (CONTROL)	0.67 ±0.14	0.53 ±0.13	0.29 ±0.06	0	0	0

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 37a

Leaf development of bambara groundnut, variety Ex-Ada, inoculated with Weija-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean No. of leaves per plant (to the nearest whole no.) at following light intensities*			Mean length and width of mid-leaflet (cm) at following light intensities.*		
	L	M	H	L	M	H
Undiluted	26	26	26	7.49x3.00	5.81x2.56	5.00x1.64
½ Dilution	28	26	27	6.33x2.73	6.03x2.19	5.06x1.75
¼ Dilution	26	28	26	6.30x2.50	6.07x2.66	5.24x1.89
$\frac{1}{8}$ Dilution	27	26	26	8.56x2.93	5.63x1.90	5.53x1.94
Uninoculated (CONTROL)	21	19	11	3.00x1.40	2.20x1.19	2.00x0.98

* L: Low light intensity

M: Medium light intensity

H: High light intensity

TABLE 37b

Dry weight and extent of nodulation of Bambara groundnut, variety Ex-Ada, plants inoculated with Weija-Ex-Tamale strain grown under normal day/night regime at different light intensities.

Concentration of Initial Broth Culture Inoculum of moss Carrier	Mean Dry wt. per plant (g) at following light intensities*			Mean No. of Nodules per plant (to the nearest whole no.) at following light intensities.		
	L	M	H	L	M	H
Undiluted	1.94 ±0.48	1.48 ±0.35	0.98 ±0.27	67	44	18
½ Dilution	1.81 ±0.43	1.46 ±0.33	1.06 ±0.30	60	46	20
¼ Dilution	1.73 ±0.26	1.48 ±0.29	1.23 ±0.26	62	44	19
⅛ Dilution	1.81 ±0.25	1.52 ±0.38	1.09 ±0.20	54	44	19
Uninoculated (CONTROL)	0.67 ±0.14	0.53 ±0.13	0.29 ±0.06	0	0	0

* L: Low light intensity

M: Medium light intensity

H: High light intensity

The data were analysed statistically and the results can be summarised as follows:

Mean number of leaves per plant

The pertinent data appear in Tables 33a, 34a, 35a, 36a and 37a. The Analyses of Variance (Two-way Anova with replication) in Tables 38a, 39a, 40a, 41a and 42a indicate that there was no significant effect of light intensity on mean leaf number produced by the inoculated plants. There was significant effect of inoculation on mean leaf number at both 5 per cent and 1 per cent levels of significance. The effect of the interaction of light intensities and inoculation on mean leaf number was significant at only the 5 per cent level of significance.

Results of the Duncan's New Multiple Range Test for means of leaf number in Tables 38b, 39b, 40b, 41b and 42b showed that the mean numbers of leaves of inoculated plants with different initial inoculum densities were not significantly different from each other, but all were different at both 5 per cent and 1 per cent levels of significance from the mean number of leaves of the uninoculated plants.

TABLE 38a

Analysis of variance (Two-way Anova with replication) for data of Table 33a. Mean leaf number.
(Plant inoculated with Kpong-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	65.31	2	32.655	2.705 NS
Inoculation	1163.39	4	290.848	24.090 **
Light intensity & Inoculation	245.09	8	30.636	2.538 *
Error	724.40	60	12.073	-
TOTAL	2198.19	74	-	-

** Significant at 1% level of significance.

* Significant at 5% level of significance.

NS Non-significant.

TABLE 38b

(Data of Table 38a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to

inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^{1}/_{8}$ dilution.

TABLE 39a

Analysis of variance (Two-way Anova with replication) for data of Table 34a. Mean leaf number.
(Plants inoculated with Legon-Ex-Ada strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	79.04	2	39.52	2.662 NS
Inoculation	849.28	4	212.32	14.301 **
Light intensity & oculation	291.36	8	36.42	2.453 *
Error	890.80	60	14.847	-
TOTAL	2110.48	74	-	-

** Significant at 1% level of significance.

* Significant at 5% level of significance.

NS Non-significant.

TABLE 39b

(Data of Table 39a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 40a

Analysis of variance (Two-way Anova with replication) for data of Table 35a. Mean leaf number.
(Plant inoculated with Nungua(1)-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	27.23	2	13.615	0.917 NS
Inoculation	1158.72	4	289.680	19.512 **
Light intensity & Inoculation	286.64	8	35.830	2.413 *
Error	643.60	60	10.727	-
TOTAL	2116.19	74	-	-

** Significant at 1% level of significance.

* Significant at 5% level of significance.

NS Non-significant.

TABLE 39b

(Data of Table 40a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 41a

Analysis of variance (Two-way Anova with replication) for data of Table 36a. Mean leaf number.
(Plant inoculated with Pokuase-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	90.72	2	45.360	2.966 NS
Inoculation	1246.99	4	311.748	20.385 **
Light intensity & Inoculation	262.21	8	32.776	2.143 *
Error	917.60	60	15.293	-
TOTAL	2523.52	74	-	-

** Significant at 1% level of significance.

* Significant at 5% level of significance.

NS Non-significant.

TABLE 41b

(Data of Table 41a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^{1}/_{8}$ dilution.

TABLE 42a

Analysis of variance (Two-way Anova with replication) for data of Table 37a. Mean leaf number.
(Plant inoculated with Weija-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	52.16	2	26.080	2.192 NS
Inoculation	1085.25	4	271.313	22.799 **
Light intensity & Inoculation	266.91	8	33.364	2.804 *
Error	714.00	60	11.900	-
TOTAL	2118.32	74	-	-

** Significant at 1% level of significance.

* Significant at 5% level of significance.

NS Non-significant.

TABLE 42b

(Data of Table 42a)

Duncan's New Multiple Range Test for means of leaf number of Bambara groundnut plants subjected to

inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

Mean length and width of mid-leaflets

Analyses of Variance (Two-way Anova with replication) as presented in Tables 43a, 44a, 45a, 46a and 47a for the means of leaflet sizes showed there was significant effect of all the treatments applied and their interaction on mean leaflet size for all the five strains at both 5 per cent and 1 per cent levels of significance.

Duncan's New Multiple Range Test of Tables 43b, 44b, 45b, 46b and 47b show that sizes of leaflets of all inoculated plants were significantly larger than those of uninoculated plants at both 5 per cent and 1 per cent levels of significance.

Also plants under low, medium and high light intensities produced leaves which differed significantly in size from each other at both 5 per cent and 1 per cent levels of significance.

TABLE 43a

Analysis of variance (Two-way Anova with replication) for data of Table 33b. Mean size of mid-leaflet.
(Plant inoculated with Kpong-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	623.38	2	311.690	112.406 **
Inoculation	1663.46	4	415.865	149.975 **
Light intensity & Inoculation	180.15	8	22.519	8.121 **
Error	249.56	90	2.773	-
TOTAL	2716.55	104	-	-

** Significant at 1% level of significance.

TABLE 43b

(Data of Table 43a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) different light intensities

	1	2	3
5%			
1%			

b) inoculation

	A	B	C	D	Control
5%					
1%					

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 44a

Analysis of variance (Two-way Anova with replication) for data of Table 34b. Mean size of mid-leaflet.
(Plant inoculated with Kpong-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	597.04	2	298.52	112.113 **
Inoculation	1168.55	4	292.138	109.716 **
Light intensity & Inoculation	88.14	8	11.018	4.138 **
Error	239.64	90	2.663	-
TOTAL	2093.37	104	-	-

** Significant at 1% level of significance.

TABLE 44b

(Data of Table 44a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 45a

Analysis of variance (Two-way Anova with replication) for data of Table 35b. Mean size of mid-leaflet.
(Plant inoculated with Nungua(1)-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	1025.10	2	512.550	108.430 **
Inoculation	1626.62	4	406.655	86.028 **
Light intensity & Inoculation	310.83	8	38.854	8.220 **
Error	425.43	90	4.727	-
TOTAL	3387.98	104	-	-

** Significant at 1% level of significance.

TABLE 45b

(Data of Table 45a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 46a

Analysis of variance (Two-way Anova with replication) for data of Table 36b. Mean size of mid-leaflet.
(Plant inoculated with Pokuase-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	353.88	2	176.940	45.590 **
Inoculation	1604.80	4	401.200	103.372 **
Light intensity & Inoculation	165.47	8	20.684	5.329 **
Error	349.30	90	3.881	-
TOTAL	2473.45	104	-	-

** Significant at 1% level of significance.

TABLE 46b

(Data of Table 46a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 47a

Analysis of variance (Two-way Anova with replication) for data of Table 37b. Mean size of mid-leaflet.
(Plant inoculated with Weija-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	1386.66	2	693.330	178.642 **
Inoculation	2296.21	4	574.053	147.909 **
Light intensity & Inoculation	655.92	8	81.990	21.125 **
Error	583.60	90	6.484	-
TOTAL	4922.39	104	-	-

** Significant at 1% level of significance.

TABLE 47b

(Data of Table 47a)

Duncan's New Multiple Range Test for means of leaflet size of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

Mean dry weight of plants

Results of Analyses of Variance test (Two-way Anova with replication) of Tables 48a, 49a, 50a, 51a and 52a show that there was significant effect for the two treatments, light intensity and inoculation, on the mean dry weights at both 5 per cent and 1 per cent levels of significance. There was, however, no significant effect of their interaction.

Tables 48b, 49b, 50b, 51b and 52b are the Duncan's New Multiple Range Test carried out for the mean dry weights. The values for plants of the various initial inoculum densities of all the five strains were different at both 5 per cent and 1 per cent levels of significance from that of the control plants.

The effects of light intensity were:

a. The effects of the three light intensities on plants inoculated with Legon-Ex-Ada, Pokuase-Ex-Tamale, and Weija-Ex-Ada strains were significantly different from each other at 5 per cent level of significance, but at the 1 per cent level of significance effects of medium and high light intensities were not significantly different.

b. Effects of the three light intensities were significantly different from each other at both 5 per cent and 1 per cent levels of significance for plants inoculated with Kpong-Ex-Tamale and Nunugua(1)-Ex-Tamale strains.

TABLE 48a

Analysis of variance (Two-way Anova with replication) for data of Table 33a. Mean dry weight of plants.
(Plant inoculated with Kpong-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	14.23	2	7.115	7.974 **
Inoculation	44.54	4	11.315	12.479 **
Light intensity & Inoculation	0.69	8	0.086	0.097 NS
Error	53.54	60	0.892	-
TOTAL	113.00	74	-	-

** Significant at 1% level of significance.

NS Non-significant

TABLE 48b

(Data of Table 48a)

Duncan's New Multiple Range Test for means of of dry weights of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 49a

Analysis of variance (Two-way Anova with replication) for data of Table 34a. Mean dry weight of plants.
(Plant inoculated with Legon-Ex-Ada strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	14.05	2	7.025	8.982 **
Inoculation	43.36	4	10.840	13.859 **
Light intensity & Inoculation	3.92	8	0.490	0.627 NS
Error	46.93	60	0.782	-
TOTAL	108.26	74	-	-

** Significant at 1% level of significance.

NS Non-significant

TABLE 49b

(Data of Table 49a)

Duncan's New Multiple Range Test for means of of dry weights of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
	———		1%

b) inoculation

A	B	C	D	Control	
———					5%
———					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 50a

Analysis of variance (Two-way Anova with replication) for data of Table 35a. Mean dry weight of plants.
(Plant inoculated with Nungua(1)-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	23.44	2	11.720	7.850 **
Inoculation	45.24	4	11.310	7.575 **
Light intensity & Inoculation	2.11	8	0.264	0.177 NS
Error	89.58	60	1.493	-
TOTAL	160.37	74	-	-

** Significant at 1% level of significance.

NS Non-significant

TABLE 50b

(Data of Table 50a)

Duncan's New Multiple Range Test for means of of dry weights of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 51a

Analysis of variance (Two-way Anova with replication) for data of Table 36a. Mean dry weight of plants.
(Plant inoculated with Kpong-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	18.98	2	9.490	10.477 **
Inoculation	42.50	4	10.625	11.730 **
Light intensity & Inoculation	2.28	8	0.285	0.315 NS
Error	54.35	60	0.906	-
TOTAL	118.11	74	-	-

** Significant at 1% level of significance.

NS Non-significant

TABLE 51b

(Data of Table 51a)

Duncan's New Multiple Range Test for means of of dry weights of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 52a

Analysis of variance (Two-way Anova with replication) for data of Table 37a. Mean dry weight of plants.
(Plant inoculated with Weija-Ex-Ada strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	22.04	2	11.020	10.196 **
Inoculation	45.14	4	11.285	10.441 **
Light intensity & Inoculation	2.09	8	0.261	0.242 NS
Error	64.85	60	1.081	-
TOTAL	134.12	74	-	-

** Significant at 1% level of significance.

NS Non-significant

TABLE 52b

(Data of Table 52a)

Duncan's New Multiple Range Test for means of of dry weights of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
	———		1%

b) inoculation

A	B	C	D	Control	
———					5%
———					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

Mean number of nodules per plant

Nodulation did not take place in the uninoculated plants exposed to all three light intensities.

The mean number of nodules of inoculated plants ranged between 54 and 78, 42 and 51, and 16 and 21 for plants raised under low, medium and high light intensities, respectively. The mean number of nodules formed by the inoculated plants appear in Tables 33b, 34b, 35b, 36b and 37b.

The Two-way Anova with replication method was again used to find the significant effect of light intensity, and inoculation, and the interaction of the two on nodulation. Tables 53a, 54a, 55a, 56a and 57a indicate that there was significant effect of all light intensities and inoculation and their interactions for plants inoculated with the five strains, at both 5 per cent and 1 per cent levels of significance.

The Duncan's New Multiple Range Test for means of nodule number presented in Tables 53b, 54b, 55b, 56b and 57b showed significant effect of inoculation at both 5 per cent and 1 per cent levels of significance.

Mean numbers of nodules formed under the three light intensities were all significantly different from each other at both 5 per cent and 1 per cent levels of significance.

TABLE 53a

Analysis of variance (Two-way Anova with replication) for data of Table 33b. Mean number of nodules per plant. (Plant inoculated with Kpong-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	16032.2	2	8016.10	87.132 **
Inoculation	22038.9	4	5509.72	59.888 **
Light intensity & Inoculation	4212.3	8	526.54	5.723 **
Error	5520.0	60	92.00	-
TOTAL	47803.4	74	-	-

** Significant at 1% level of significance.

TABLE 53b

(Data of Table 43a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 54a

Analysis of variance (Two-way Anova with replication) for data of Table 34b. Mean number of nodules per plant. (Plant inoculated with Legon-Ex-Ada strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	15097.0	2	7548.52	85.571 **
Inoculation	21065.8	4	5266.45	59.701 **
Light intensity & Inoculation	4173.1	8	521.64	5.913 **
Error	5292.8	60	88.21	-
TOTAL	45628.7	74	-	-

** Significant at 1% level of significance.

TABLE 54b

(Data of Table 54a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 55a

Analysis of variance (Two-way Anova with replication) for data of Table 35b. Mean number of nodules per plant. (Plant inoculated with Nungua(1)-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	18466.6	2	9233.3	118.32 **
Inoculation	21895.7	4	5473.9	70.15 **
Light intensity & Inoculation	4677.8	8	584.7	7.493 **
Error	4682.0	60	78.0	-
TOTAL	49722.1	74	-	-

** Significant at 1% level of significance.

TABLE 55b

(Data of Table 55a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 56a

Analysis of variance (Two-way Anova with replication) for data of Table 36b. Mean number of nodules per plant. (Plant inoculated with Pokuase-Ex-Tamale strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	20670.6	2	10335.3	102.57 **
Inoculation	25274.1	4	6318.5	62.71 **
Light intensity & Inoculation	5576.3	8	697.0	6.918 **
Error	6045.6	60	100.7	-
TOTAL	57566.7	74	-	-

** Significant at 1% level of significance.

TABLE 56b

(Data of Table 56a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

TABLE 57a

Analysis of variance (Two-way Anova with replication) for data of Table 37b. Mean number of nodules per plant.

(Plant inoculated with Weija-Ex-Ada strain)

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value
Light intensity	13871.2	2	6935.6	63.05 **
Inoculation	20682.9	4	5170.7	47.006 **
Light intensity & Inoculation	3812.2	8	476.5	4.332 **
Error	6600.8	60	110.0	-
TOTAL	44967.2	74	-	-

-

** Significant at 1% level of significance.

TABLE 57b

(Data of Table 57a)

Duncan's New Multiple Range Test for means of nodule number of Bambara groundnut plants subjected to

a) different light intensities

1	2	3	
			5%
			1%

b) inoculation

A	B	C	D	Control	
					5%
					1%

Note: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

1: Under Shed 1

2: Under Shed 2

3: Under Shed 3

A: Undiluted concentration

B: ½ dilution

C: ¼ dilution

D: $^1/_8$ dilution.

Size of nodules

Light intensity also affected the sizes of the nodules, as can be seen from the mean diameters of nodules of the various treatments in Table 58, and the histograms of class - diameters of the nodules in Figs. 11 - 15.

Nodules formed by plants raised under Low light intensity had class-diameters ranging from 2.51 to 4.21cm. Those formed by plants raised under medium and high light intensities ranged from 1.79 to 2.63cm, and 0.87 to 1.95cm, respectively.

TABLE 58

Mean diameters of nodules formed by Bambara groundnut plants grown under different light intensities for 30 days.

Rhizobium Inoculum	Initial density of Inoculum	Mean diameters (cm) of nodules of plants grown under Shed 1	Shed 2	Shed 3
Kpong-Ex-Tamale strain	Undiluted	3.20	2.06	1.84
	½ dilution	2.51	2.31	0.89
	¼ dilution	2.84	2.27	1.82
	1/8 dilution	2.63	1.96	1.89
Legon-Ex-Ada strain	Undiluted	3.32	2.63	1.62
	½ dilution	2.96	2.36	1.63
	¼ dilution	3.12	2.13	1.74
	1/8 dilution	3.56	2.32	1.12
Nungua(1)-Ex-Tamale strain	Undiluted	3.22	2.25	1.78
	½ dilution	2.74	1.35	0.99
	¼ dilution	2.67	2.35	1.10
	1/8 dilution	3.67	1.79	1.00
Pokuase-Ex-Tamale strain	Undiluted	2.91	1.97	0.87
	½ dilution	2.79	2.33	1.89
	¼ dilution	2.59	1.88	1.89
	1/8 dilution	2.67	2.42	1.67
Weija-Ex-Ada strain	Undiluted	3.60	1.67	1.65
	½ dilution	2.74	2.04	1.25
	¼ dilution	4.12	2.38	1.36
	1/8 dilution	2.71	2.36	1.95

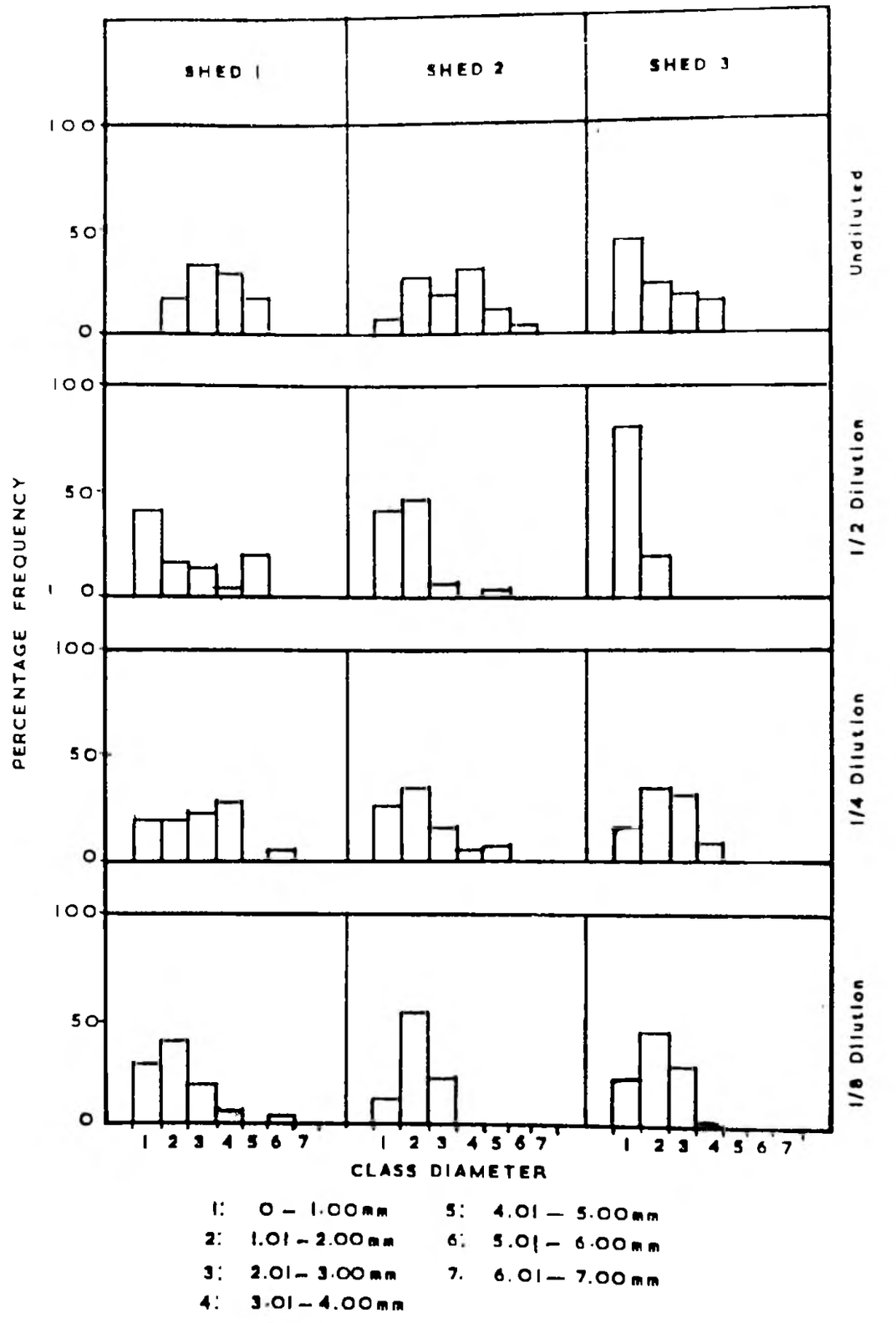

Fig. 11 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Ada variety, inoculated with Kpong-Ex-Tamale strain of Rhizobium sp. and grown under different light intensities for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

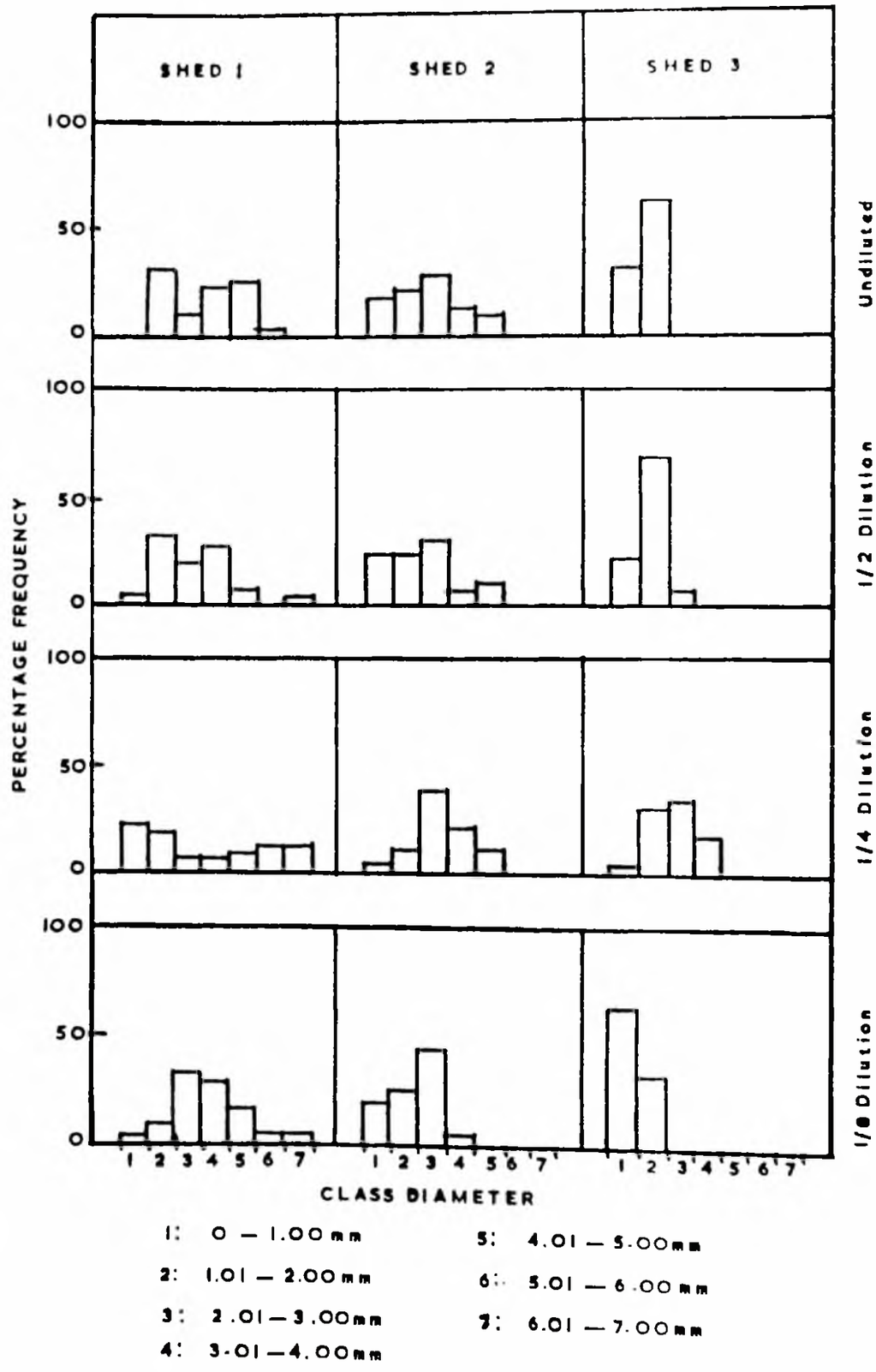

Fig. 12 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Ada variety, inoculated with Legon-Ex-Ada strain of Rhizobium sp and grown under different light intensities for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

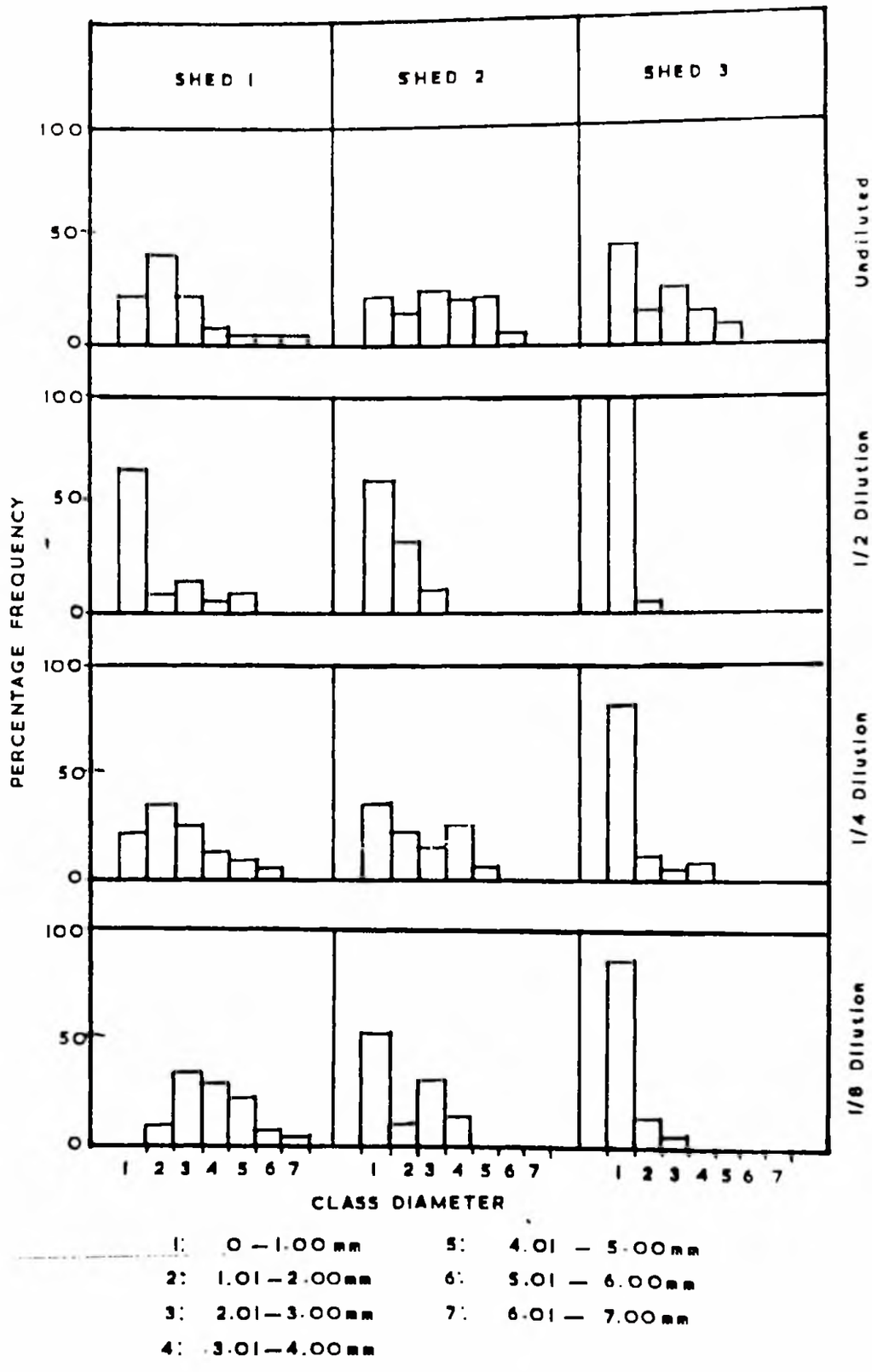

Fig. 13 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Ada variety, inoculated with Nungua(1)-Ex-Tamale strain of Rhizobium sp and grown under different light intensities for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

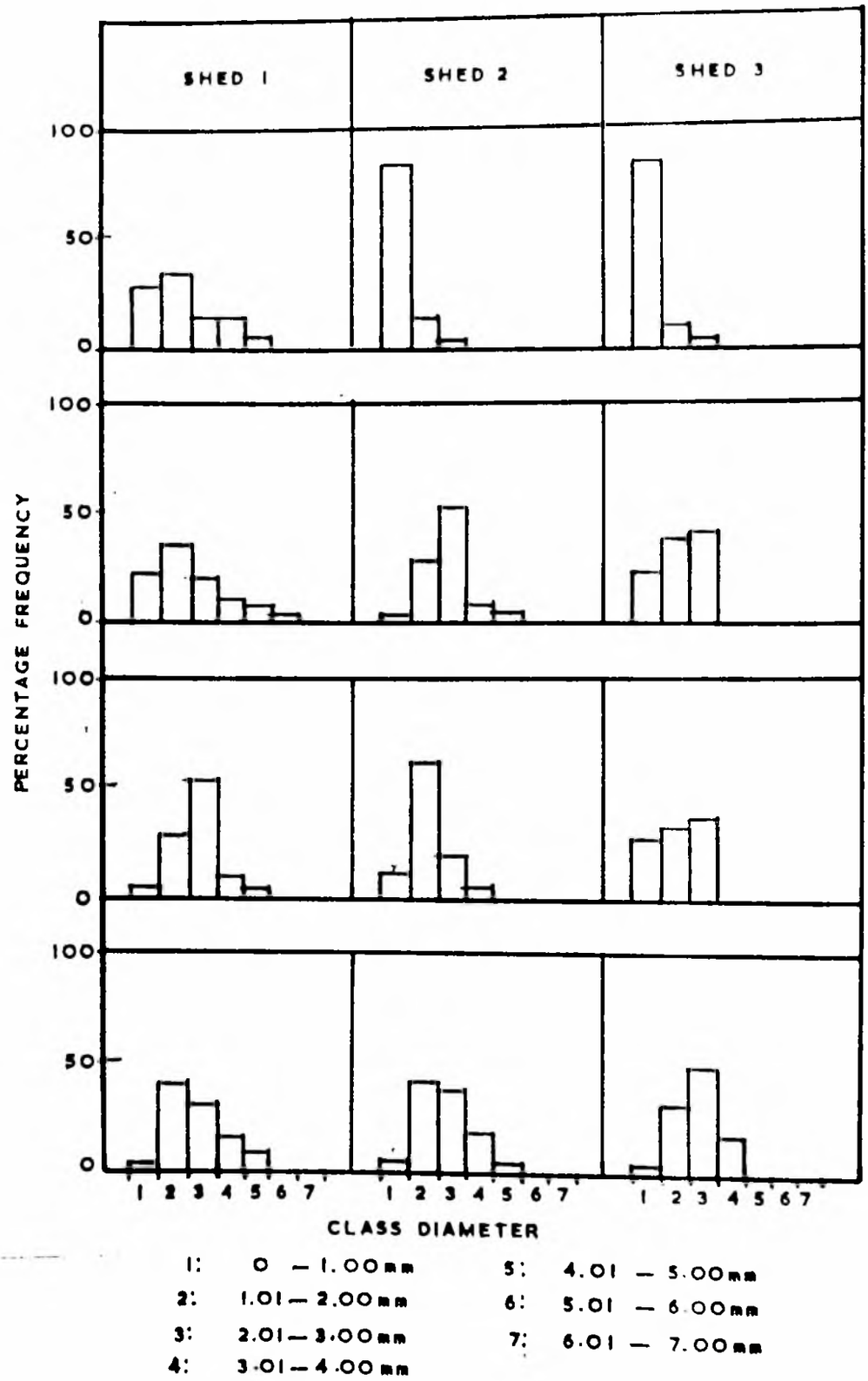

Fig. 14 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Ada variety, inoculated with Pokuase-Ex-Tamale strain of Rhizobium sp and growth under different light intensities for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

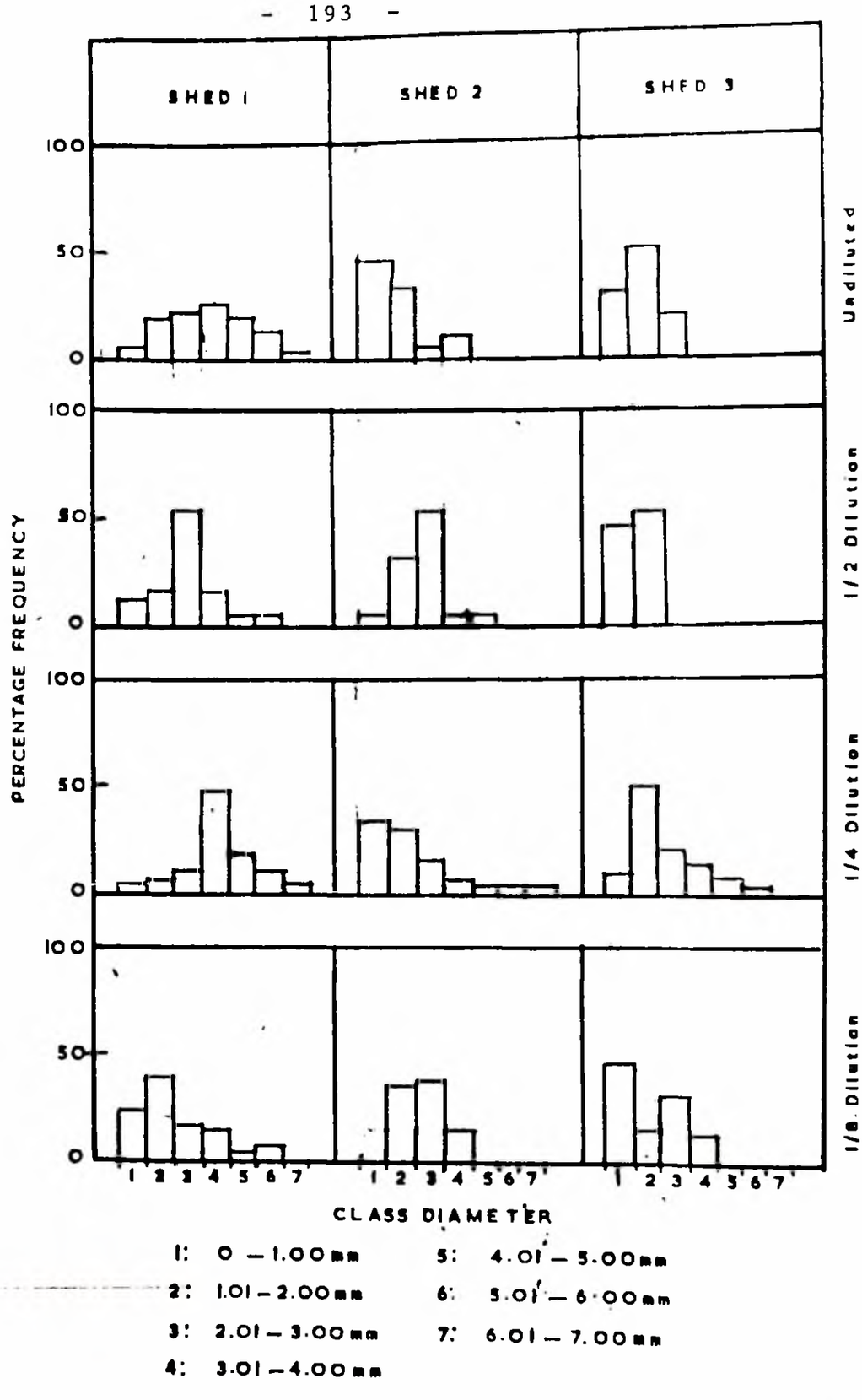

Fig.15 Distribution of class-diameters of nodules of Bambara groundnut plants, Ex-Ada variety inoculated with Weija-Ex-Ada strain of Rhizobium sp and grown under different light intensities for 30 days. Seeds inoculated with moss carrier with different initial Broth culture.

V. GENERAL DISCUSSION

Bambara groundnut is an important legume crop in Tropical Africa and comes only third after cowpea and groundnut. It is a savanna plant and there can be no limit to available land for its cultivation. It is drought resistant and yields a reasonably good crop even when grown on poor soils. In spite of these attributes, very little research has been carried out on the crop to support the industry, neither has the acreage under cultivation increased over the years. Indeed, an FAO report (FAO, 1988) pointed out that bambara groundnut production has declined in recent years and it is being replaced by an expanding groundnut production. May be if the different dietary preparations of the product in the individual African countries diffuse throughout the producing countries, its usage in each country will broaden and encourage increased production.

The range of usage is striking. For example, in Ghana, the beans are soaked, boiled and made into a type of porridge which is sweetened with sugar. In Cameroun, they are used raw or, after cooking, ground and added to soups and stews. In Tanzania, the boiled seeds are crushed, groundnut paste added, and the mixture eaten with other diets. In Malagasy, they are often added to stew with rice or eaten with a green leafy vegetable, and in Southern Africa the seed flour is mixed with oil or butter and eaten with meat, or made into cakes.

Cultivation of bambara groundnut on very large scale will obviously face certain problems. The plant is attacked by few diseases, the most serious ones are **Fusarium** wilt and leaf spot by **Cercospora canescens**. However, the plant is attacked by many pests. Attack by root knot nematodes (**Meloidogyne** sp.) is widespread. Leaf hoppers for example, **Empoasca facialis** and **Hilda**

potruclis are important in Natal and Tanzanian, respectively. In Ghana, the plant is attacked by larvae of the butterfly, **Dracrisia maculosa**, and in Uganda by larvae of **Lamprosema indicata** (FAO, 1988).

Climatic and edaphic factors can also affect productivity; the major factors being nutrients, water and light. A search through the relevant literature recorded no studies on the nutritional requirements of bambara groundnut.

Nitrogen was present in all the soils from the eight localities. The highest recorded mean percentage was 0.132 per cent (soil from Pokuase) and the lowest mean percentage of 0.025 per cent (Ashiaman soil) (see Table 1a). Mean total nitrogen content in soils recommended for good plant growth (Bremner and Edwards, 1965) ranges from 0.05 per cent to 0.25 per cent. The soil samples which were used with nitrogen content within this range were those from Kpong, Legon, Nungua(1), Pokuase and Weija. The plants raised in these soils coincidentally grew better, with mean dry weights ranging from 0.46g to 2.74g, than those raised in the nitrogen-poor soils of Ashiaman, Nungua(2) and Shiashie with only 0.50 and 0.69g; 0.42 and 0.44g; and 0.41 and 0.72g mean dry weights (Ex-Ada and Ex-Tamale varieties), respectively (see Table 3).

It was found in this investigation that severe water stress naturally greatly affected growth and nodulation of the bambara groundnut plant. What is important in the present observation is the relationship between the degree of the effect and the interval of watering. Plant dry weights and number of nodules formed are two features which showed a uniform relationship. The data in Tables 7 to 11 indicated that the mean dry weight of plants watered once in two days was approximately two times that of plants watered once in six days. The ratio of mean number of nodules of bambara groundnut

plants watered once in two, four and six days was approximately 1:2:4-5.

Water affects plant growth by affecting internal physiological processes and conditions. Growth of plants is controlled by rates of cell division and enlargement and by the supply of organic and inorganic compounds required for the synthesis of new protoplasm and cell walls. Cell enlargement is particularly dependent on at least a minimum degree of cell tugor, and stem and leaf elongation are quickly checked or stopped by water deficits (Miller, 1965). This is well illustrated by the sizes of the middle leaflets of bambara groundnut plants recorded in Tables 7 to 11.

Decrease in water content invariably reduces the rate of photosynthesis (Brix, 1962) and this leads to decrease in dry weight. Consequently, dry weights of the bambara groundnut plants decreased with an increase in the interval of watering. Values of mean dry weights for plants watered once in two, four and six days, respectively, were 0.54 - 1.68g, 0.32 - 0.94g and 0.11 - 0.76g (values for both inoculated and uninoculated plants) (see Table 7 - 11).

Moisture deficits have been found to prevent the synthesis of proteins by many observers (eg. Barnett and Naylor, 1966; Petrie and Wood, 1938; Yarooh, 1958). Studies on the ability of Arizona Common and Coastal Bermuda grass (**Cynodon dactylon** (L) Pers) to synthesize amino acids and proteins during water stress by Barnett and Naylor (1966) showed that amino acids were continually synthesized during the water stress treatments, but protein synthesis was inhibited and protein levels decreased. Water stress induced a 10- to 100-fold accumulation of free asparagine.

Gates and Bonner (1960) showed that the amount of both DNA and RNA per leaf decreased under water deficits, although they were similar in the water - deficient and control treatments of tomatoes on a dry weight basis. The decrease in nucleic acids per leaf is intimately related to the slower growth rate per leaf. Gates and Bonner (1960) attempted to verify whether this decrease was due to decreased synthesis in response to water deficits. Their results showed that the rate of P^{32} incorporation into nucleic acids under water deficits is probably due to accelerated destruction rather than decreased synthesis. Since protein synthesis is related to RNA, the effects of a moisture deficit on nucleic acids will reduce the rate of protein synthesis indirectly. Bambara groundnut plants watered once in 4 days and 6 days which grew less vigorously might have suffered impairment of protein synthesis.

Water shortage also impairs auxin production so that cell enlargement and growth is suppressed (Treshow, 1970). Limited cell enlargement leads to reduced leaf areas, shortened internodes, and stunted and rosetted plants.

There was marked effect of light intensity on the growth and development of the bambara groundnut plants (see Tables 33 - 37). Different levels of light intensity within the range used in this investigation supported development of the plants to different degrees.

It is well known that in the undergrowth of forests where the light intensity is low, the shrubs and grasses develop broad leaves which trap maximum light in the dim light. In this work the largest leaflets were produced by plants exposed

to the lowest light intensity. The mean leaflet dimensions of 5.60 x 2.27 - 8.56 x 3.00 cm were reduced to 4.91 x 1.90 - 6.07 x 2.66 cm under the medium light intensity and still further to 4.57 x 1.53 - 5.71 x 2.16 cm under the highest light intensity (see Tables 33 - 37).

However, the lowest light intensity provided in this work seems apparently to be the best for the growth of bambara groundnut plants. For, the mean dry weights of the plants, of lowest light intensity through the medium to the highest light intensity were 1.62 - 2.91g, 1.33 - 1.82g and 0.91 - 1.41g, respectively. The corresponding mean number of nodules per plant were also 54 - 78, 42 - 51 and 16 - 21, respectively.

Light intensity directly affect photosynthesis. Many studies (eg. Hall and Rao, 1986) have shown that photosynthesis becomes less efficient under high light intensity. Judging from the productivity of the bambara groundnut plants, light intensities between 8800 and 10000 lux have proved to be unfavourable to the plants. Too high a light intensity depresses photosynthetic rate through a rapid photo-oxidation of chlorophyll.

The low light intensity 1100 to 6000 lux which has supported the best growth in the growth of bambara groundnut plants, may be quite close to the light intensity the crop encounters under mixed farming conditions in Ghana. The findings also point out that in Northern parts of Ghana where it is grown as a monocrop fully exposed to sunlight, the productivity of the plants falls short of their potential. Further work must be carried out to determine the lowest light intensity capable of supporting good productivity. This knowledge is important in deciding spacing in mixed farms and acceptable levels of shading of bambara groundnut plants.

Even though this experiment was not carried out long enough to include fruiting it is reasonable to assume that the larger the plant the greater will be the number of flowers and, therefore, the higher the yield. Plants at the low light intensity which produced the largest plants (see Plate 7) are most likely to give the best yield.

Legumes stand apart from other crops because of their need for mutual bacterial partners, the **Rhizobium** sp. in a very special relationship. The symbiotic association involving legumes and **Rhizobium** sp. leads to the fixation of atmospheric nitrogen in a high energy - consuming process catalysed by the enzyme nitrogenase and which uses ATP of respiration. The mode of infection of clover by the nodule bacteria has been closely studied by Nutman (1958). A later account of the actual cell entry was later presented by Jordan, Grinyer and Coulter (1963).

The nodule-root complex formation involves three phases: (a) nodule development, (b) bacteroid formation, and (c) leghaemoglobin synthesis.

Legume roots exude vitamin B which stimulates growth of **Rhizobium** sp. in their rhizosphere. The root produces in addition tryptophan which the bacterium converts into Indole Acetic Acid (IAA). The IAA causes characteristic curling and sometimes branching of the root hairs. Polysaccharides of **Rhizobium** slime at the same time induces the production of the wall attacking enzyme, polygalacturonase (PG) by the legume root. IAA and PG combine to soften the apex of the root hair causing invagination of the wall at the apex to start the formation of an infection thread - a cellulose tube filled with mucilage.

The **Rhizobium** cells multiply rapidly at this stage to produce small spherical flagellated cells referred to as swarmer cells, which swim into the mucilage of the infection thread. The infection thread begins to grow towards the base of the root hair led by the root hair nucleus. When the infection thread establishes contact with the wall between the epidermal cell of the root hair and the adjacent cortical wall, the same process of wall invagination takes place admitting the infection thread into the cortical cell.

The legume cortex, remarkably contains tetraploid cells among the diploid cells which are the destination of the infection threads. When the infection thread eventually enters a tetraploid cell, it branches profusely, and numerous vesicles appear on its external surface. The swarmer cells migrate into these vesicles, which are in turn blebbed off into the cell. The cell also encloses the detached vesicles in its cytoplasm with its own synthesized membrane. After this the tetraploid cells are stimulated to divide repeatedly to form the primodium nodule. The nodule grows considerably and pushes out of the root assuming the characteristic nodule shape.

The swarmer cells are then modified to make them functional nitrogen fixers. They lose their flagella and grow into swollen mishapen and even branching forms - the bacteroids. The bacteroids contain the nitrogenase and fix nitrogen.

Paradoxically, the oxygen which will be required for respiration to generate ATP for nitrogen fixation inactivates nitrogenase. Nitrogenase contains two proteins; Protein 1 which consists of Molybdenum, non-heme iron and labile sulphur.

It is called Molybdoferredoxin, and is not particularly sensitive to oxygen; and Protein 2 which consists of no Molybdenum, non-heme iron and less labile sulphur. It is called Azoferredoxin and is very sensitive to oxygen.

The tetraploid cells of the nodule play a crucial role of protecting nitrogenase from oxygen injury. They contain a haemoglobin - leghaemoglobin - which has strong affinity to oxygen and binds the oxygen in the nodule, releasing it gradually to the bacteroid for respiration and ATP generation. The nodule thus provides the proper environment to protect nitrogenase from oxygen.

Association of a bacterium with a legume happens because part of the genetic information for synthesizing the necessary haemoglobin is present in the bacterium, and part is coded by genes in the plant. This explains the specificity of the relationship between the **Rhizobium** species. Presently, the recognised Legume group - **Rhizobium** sp. associations are as shown in Table 59.

TABLE 59 CROSS - INOCULATION GROUPS* OF RHIZOBIUM

Rhizobium sp.	Cross-inoculation groupings.	Legume types
R. Leguminosarum	Pea group	**Pisum, Vicia,,** Lens
R. phaseoli	Bean group	**Phaseolus**
R. trifolii	Clover group	**Trifolium**
R. meliloti	Alfalfa group	**Melilotus, Medicago, Trigonella**
R. lupini	Lupini group	**Lupini, Orinthopus**
R. japonicum	Soybean group	**Glycine**
Rhizobium sp.	Cowpea group	**Vigna, Arachis**

* The principle of cross-inoculation grouping is based on the ability of an isolate of **Rhizobium** sp. to form nodules in a limited genera of species of legumes related to one another (Subba Rao, 1977).

A single infective species of **Rhizobium** is generally able to infect several different plant species. This specificity appears to reside in a single bacterial gene or in a number of closely linked genes, because the ability to infect a particular plant group can be transferred from one species of **Rhizobium** to another by the process of DNA - mediated transformation.

The bambara groundnut has been reported to be nodulated in various geographical areas (Allen and Allen, 1981) but comparatively little attention has been given to its **Rhizobium** affinities or its nitrogen - fixing capacity. Studies by Somasegaran, Abaidoo and Kumaga (1990) showed that strain TAL 169 which was derived from nodules of **Vigna unguiculata** was consistently effective on all genotypes of bambara groundnut.

The genus **Rhizobium** comprises of two main groups, the fast and slow growers, distinguished by their relative speed of growth and change of pH induced in YMA. The 'fast growers' have a Mean Generation Time under the most favourable conditions of about 2 - 4 hours and produce detectable colonies on YMA in 2 - 3 days, and large, gummy colonies up to 5mm diameter, or more, by 5 days at 25°C. the Mean Generation Time of the slow growers is more likely to be 6 - 8 hour and colonies seldom exceed 1 - 2mm diameter after 10 days (Subba Rao, 1977). This work showed that all the five strains used produced detectable colonies in 2 - 3 days suggesting they were all fast growers.

Because of the specificity of the association, the differences in the nodulation ability of bambara groundnut plants in the different soil samples shown in Table 3, ranging from a mean of three nodules per plant in Shiashie soil to as high as 44 per plant in Weija soil; can be attributed to differences in level of populations of the appropriate strain of **Rhizobium** sp. Naturally, there could be some other contributing factors such as soil nutrient levels, pH, etc.

The variability of **Rhizobium** sp. population has made the inoculation of legumes with their associate strains a routine agricultural practice in many countries as has been mentioned in the Introduction and Literature Review. Attention is usually given to (a) the number of viable rhizobia supplied, (b) purity of the culture, and (c) ability of the rhizobia to nodulate effectively the particular legume plant. Major consideration is given to the last, because the only distinction between rhizobia and certain related bacteria is the ability to produce nodules (Burton, Martinez and Curley, 1972).

The production of rhizobial cultures on a large scale is prepared in stainless steel fermenter vessels with adequate facilities for aeration and agitation of the culture medium. A medium of yeast extract mannitol broth is commonly used and the organisms are grown for 96 hours. The fermenter is sterilized with pressurized steam and the air used for aeration of the culture medium is sterilized by filters before it is introduced into the culture medium.

In India, in the absence of traditional fermenters, the rhizobia are raised in shake cultures using 1L or 2L glass bottles or flasks (Sahni, 1976).

Unavailability of a steel fermenter should, therefore, not be a constraint in the inoculation practice. What matters is an effective carrier by which the inoculum will be applied to the seed with the aid of a good adhesive. Essentially, a suitable material for use as a carrier must be able to retain the viability of the rhizobia over long periods. It must also decompose in a relatively short time, have a high water holding capacity and should not contain substances that are inhibitory to the rhizobia.

Peat possesses these important characteristics and has been the traditional material used as a carrier. Efficient substitutes have been adopted in many areas which lack peat. These include bagasse, bagassilo, coffee husk, coir dust, filter mud (a by-product from sugar cane factories), lignite and sawdust. By the present investigation, decomposed moss (**Brachymenium** sp.) has been added to the list of substitutes.

The moss compost discovered in this investigation took only 15 days to decompose. It apparently contained no inhibitory compounds as the rhizobia did not only survive in it

for 14 weeks at normal atmospheric temperature but also multiplied in it (see Fig. 4). A Stationary Phase of growth was attained by the tenth week, but there was no sign of autolysis by the end of the storage period of 14 weeks.

The rhizobial populations in inoculated moss compost at room temperature reached a level of 1×10^{11} cells per gram of compost in 2 weeks (see Table 6). This compares very well with populations achieved with other carriers. Populations of 1×10^{10} cells per gram after 12 days has been reported using bagasillo (Ryder, 1984), of 1×10^{8} cells per gram for filter mud in 14 days (Anyango, 1984), and 1×10^{9} cells per gram for lignite (Subba Rao, 1977).

The extensive results in Tables 12 - 58 of experiments in which the inoculated bambara groundnut plants either received different amounts of water or were exposed to different light intensities showed that inocula in the moss carrier were very effective. For example, whereas the number of nodules formed by Ex-Ada variety plants in Legon soil was 41 and the mean nodule diameter was 1.81mm (see Table 3), plants inoculated with strain of **Rhizobium** sp. isolated from these nodules and incorporated into the moss carrier formed averagely 67 nodules per plant (see Table 34b) with a mean diameter of 3.56mm (see Table 58).

Similarly, the mean number of nodules per plant formed by Ex-Tamale variety of bambara groundnut growing in Kpong soil was 44 (see Table 3) and the mean nodule diameter was 2.47mm. Using moss carrier containing Kpong-Ex-Tamale strain of **Rhizobium** sp. to inoculate Ex-Tamale variety of bambara plants, the mean number of nodules per plant was 55 (see Table 7B) and the mean nodule diameter was 2.68mm (see Table 32).

Inoculation of new plots for raising bambara groundnut has been tried by Dadson, Brooks and Wutoh (1987) and by Stanton, Doughty, Orracca-Tetteh and Steele (1966) with success. Inoculation of the seeds with moss carrier should be regarded as a much more convenient practice.

It is worthy of note that the two bambara groundnut varieties, Ex-Ada and Ex-Tamale, showed significantly different responses when they were planted in the same sample of soil (see Table 3). This stresses the idea of legume - **Rhizobium** sp. genetical compatibility. It is, therefore, important to take into account the interaction of host variety and strain of **Rhizobium** sp. in any future attempts at commercial production of moss carrier for bambara groundnut.

Since **Brachymenium** sp. has turned out to be a good carrier, a survey of its habitats must be carried out. The best among them can consequently be conserved and properly managed as sources for moss material.

Finally, it has been clearly shown that there are many different strains of **Rhizobium** sp. associated with bambara groundnut in Ghana. The most efficient among those isolated during the course of this work were tested under different watering regimes and light intensities. They may not necessary be the best of all the available strains present in Ghanaian soils, as the collection of soils for the experiment was limited to an area within a radius of only 180 km from Legon. This leaves unsampled the vast areas of the Northern part of the country where bambara groundnut is intensively grown. Isolation from all bambara growing regions in Ghana should be an essential future exercise. It is only when the best strains have been identified for each plant variety that the greatest benefit can be derived from bambara groundnut seed inoculation.

It may be concluded that compost of **Brachymenium** sp. is a good **Rhizobium** carrier and can be used for routine inoculation of bambara groundnut seeds before planting to enhance nodulation. Commercial preparation of the inoculum carrier can be embarked upon after:

(a) the best strain of **Rhizobium** sp. for each bambara groundnut variety has been identified, and

(b) the best habitats of **Brachymenium** sp. have been identified, and plans for conservation and controlled utilization of the species have been worked out.

VI SUMMARY

1. Strains of **Rhizobium** sp. were obtained from nodules of bambara groundnut plants which were grown in soils collected from legume plots at Ashiaman, Kpong, Legon, Nungua, Pokuase, Shiashie and Weija.

2. The pH of soils ranged from pH 6.48 to 6.88.

3. Soils from Legon and Weija were sandy-clay loamy soils; those from Ashiaman, Nungua, Pokuase and Shiashie were sandy loam soils; and soil from Kpong, sandy clay soil.

4. The percentage nitrogen content of the soils was, in ascending order: Ashiaman, 0.025; Shiashie, 0.028; Nungua (plot 2), 0.039; Nungua (plot 1), 0.049; Weija, 0.055; Kpong, 0.067; Legon, 0.069; and Pokuase, 0.132 per cent.

5. The mean percentage organic matter of the soils was, in ascending order: Ashiaman, 0.468; Shiashie, 0.578; Nungua (plot 2). 0.977; Nungua (plot 1), 1.472; Legon, 1.733; Kpong, 2.009; Weija, 2.064; Pokuase, 4.513 per cent.

6. Total viable cell count studies using Congo Red YMA indicated the presence of **Rhizobium** sp in all the eight soil samples. Soils from Kpong, Legon, Nungua(plot 1), Pokuase and Weija had high populations of 180 x 104, 138 x 10^4, 112 x 10^4, 111 x 10^4 and 165 x 10^4 cells per gram of soil, respectively. Soils from Ashiaman, Nungua(plot 2) and Shiashie had very low populations, not exceeding 20 x 10^4 cells per gram of soil.

7. Plants of the two varieties of bambara groundnut, Ex-Ada and Ex-Tamale, growing in the eight soil samples could be separated into three categories on the basis of extent of growth and nodulation.

Group 1: Luxuriantly growing plants with deep-green foliage and most abundant nodules. The plant variety and **Rhizobium** sp. strain association were:

i. Ex-Ada variety and Legon strain
ii. Ex-Ada variety and Weija strain
iii. Ex-Tamale variety and Kpong strain
iv. Ex-Tamale variety and Nungua(plot 1) strain
v. Ex-Tamale variety and Pokuase strain.

Group 2: Plants which grew moderately well and nodulation was intermediate. The plant variety - **Rhizobium** sp. strain association were:

i. Ex-Ada variety and Kpong strain
ii. Ex-Ada variety and Nungua(1) strain
iii. Ex-Ada variety and Pokuase strain
iv. Ex-Tamale variety and Legon strain
v. Ex-Tamale variety and Weija strain.

Group 3: Stunted plants with yellowish-green foliage and the lowest mean number of nodules per plant. The plant variety - **Rhizobium** sp. strain association were:

i. Ex-Ada variety and Ashiaman strain
ii. Ex-Ada variety and Nungua(plot 2) strain
iii. Ex-Ada variety and Shiashie strain
iv. Ex-Tamale variety and Ashiaman strain
v. Ex-Tamale variety and Nungua(2) strain
vi. Ex-Tamale variety and Shiashie strain.

8. The five strains of **Rhizobium** sp. of Group 1 infected Ex-Ada variety plants grown in 'Seedling Agar', and caused nodulation. The plants formed between 28 and 34 nodules per plant, and had between 0.41 and 0.49g mean shoot dry weights.

9. Undiluted and ½, ¼ and $^1/_8$ dilutions of the broth cultures of the five selected strains of **Rhizobium** sp. were used in inoculating, individually, samples of the ground moss compost at a ratio of 20ml broth to 10g compost which brought the moisture content to 50 per cent.

10. All the five strains multiplied rapidly in the moss carrier and cell count of 1×10^{11} cells per gram of compost was recorded after incubation at $30^{o}C$ for two weeks. Secondly the initial inocula of the four different concentrations produced almost similar population levels at the end of 14 days.

11. Shelf life studies on the moss carrier inoculated with the undiluted and ½-diluted broth cultures only, showed gradual increase in population counts with all the strains of **Rhizobium** sp. in the moss carrier over 10 weeks. The maximum population level was thereafter maintained until the 14th week without autolysis setting in.

12. Moss carriers containing the five strains of **Rhizobium** sp. and gum arabic as adhesive were used to inoculate Ex-Tamale variety of bambara groundnut plants which were then watered either once in two days or once in four days or once in six days.

13. The plants were raised in sterile loamy soil and control plants were not inoculated. Observations made can be summarised as follows:

    (a) Control plants did not nodulate and were generally stunted with yellowish-green leaves.
    (b) All the inoculated plants nodulated.
    (c) Inoculated plants watered once in two days had mean dry weights between 1.00 and 1.68g; produced 10 to 15 leaves per plant and formed 43 to 56 nodules per plant, after 30 days. The middle leaflets measured averagely 5.45 - 8.50 x 2.06 2.79cm.

    (d) Inoculated plants watered once in four days had mean dry weights between 0.64 and 0.94g; produced 8 to 12 leaves per plant; and formed 16 to 25 nodules per plant, after 30 days. The middle leaflets measured averagely 4.76 - 7.06 x 1.98 - 2.55cm.

(e) Inoculated plants watered once in six days had mean dry weights between 0.48 and 0.96g; produced 7 to 10 leaves per plant; and formed 8 to 11 nodules per plant, after 30 days. The middle leaflets measured averagely 4.88 - 6.43 x 1.88 - 2.39cm.

(f) Uninoculated control plants also responded to watering treatment.

i. Those watered once in two days had mean dry weight of 0.54g; and produced 6 leaves per plant, after 30 days. The middle leaflets measured averagely 3.36 x 1.57cm.

ii. Those watered once in four days had mean dry weight of 0.32g and produced 6 leaves per plant, after 30 days. The middle leaflet measured averagely 2.29 x 1.31cm.

iii. Those watered once in six days had mean dry weight of 0.11g; and produced 5 leaves per plant, after 30 days. The middle leaflets measured averagely 1.57 x 0.91cm.

14. Moss carriers containing the five strains of **Rhizobium** sp. and gum arabic as adhesive were used to inoculate Ex-Ada variety of bambara groundnut plants which were then exposed to light of different intensities, namely, low, medium and high light intensities.

15. The low light intensity treatment was 1100-2200 lux at 9.00am, 4030 6200 lux at noon and 1600-1900 lux at 3.00pm; the medium light intensity treatment was 1400-2800 lux at 9.00am, 6400-9100 lux at noon and 2900-3500 lux at 3.00pm; and the high light intensity treatment was 5300-6200 lux at 9.00am, 8800-10000 lux at noon and 4600-6400 lux at 3.00pm.

16. Approximately the same number of leaves were formed by the inoculated plants under the three light intensities. Other features of the plants, however, differed with light intensity. To summarise:

(a) Inoculated plants under low light intensity had mean dry weights between 1.62 and 2.91g; and formed 54 to 78 nodules per plant, after 30 days. The middle leaflets measured averagely 5.60 - 8.56 x 2.27 - 3.00cm.

(b) Inoculated plants under medium light intensity had mean dry weights between 1.33 and 1.82g; and formed 42 to 51 nodules per plant, after 30 days. The middle leaflets measured averagely 4.91 - 6.07 x 1.90-2.66cm.

(c) Inoculated plants under high light intensity had mean dry weights between 0.91 and 1.54g; and formed 16 to 21 nodules per plant, after 30 days. The middle leaflets measured averagely 4.57 - 5.71 x 1.53 - 2.16cm.

17. The uninoculated control plants which did not nodulate, however, showed an effect of different light intensities and number of leaves formed. Briefly, in the uninoculated plants:

(a) those under low light intensity had mean dry weight of 0.67g; and produced 21 leaves per plant, after 30 days. The middle leaflets measured averagely 3.00 x 1.40 cm.

(b) those under medium light intensity had mean dry weight of 0.53g; and produced 19 leaves per plant, after 30 days. The middle leaflets measured averagely 2.20 x 1.19 cm.

(c) those under high light intensity had mean dry weight of 0.29g; and produced 11 leaves per plant, after 30 days. The middle leaflets measured averagely 2.00 x 0.98 cm.

18. With both light intensity and soil moisture content tests, the size of nodules was directly related to the number of nodules formed. The larger the number of nodules per plant the larger the nodule size.

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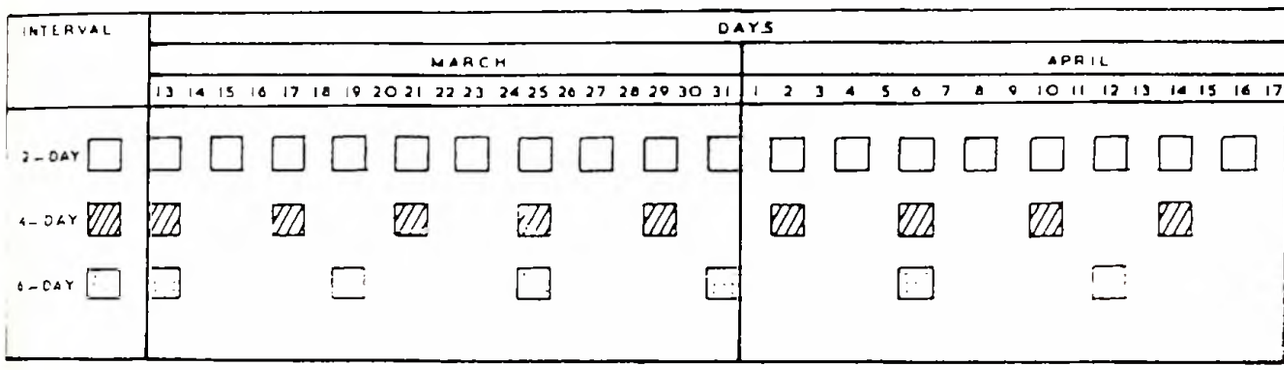
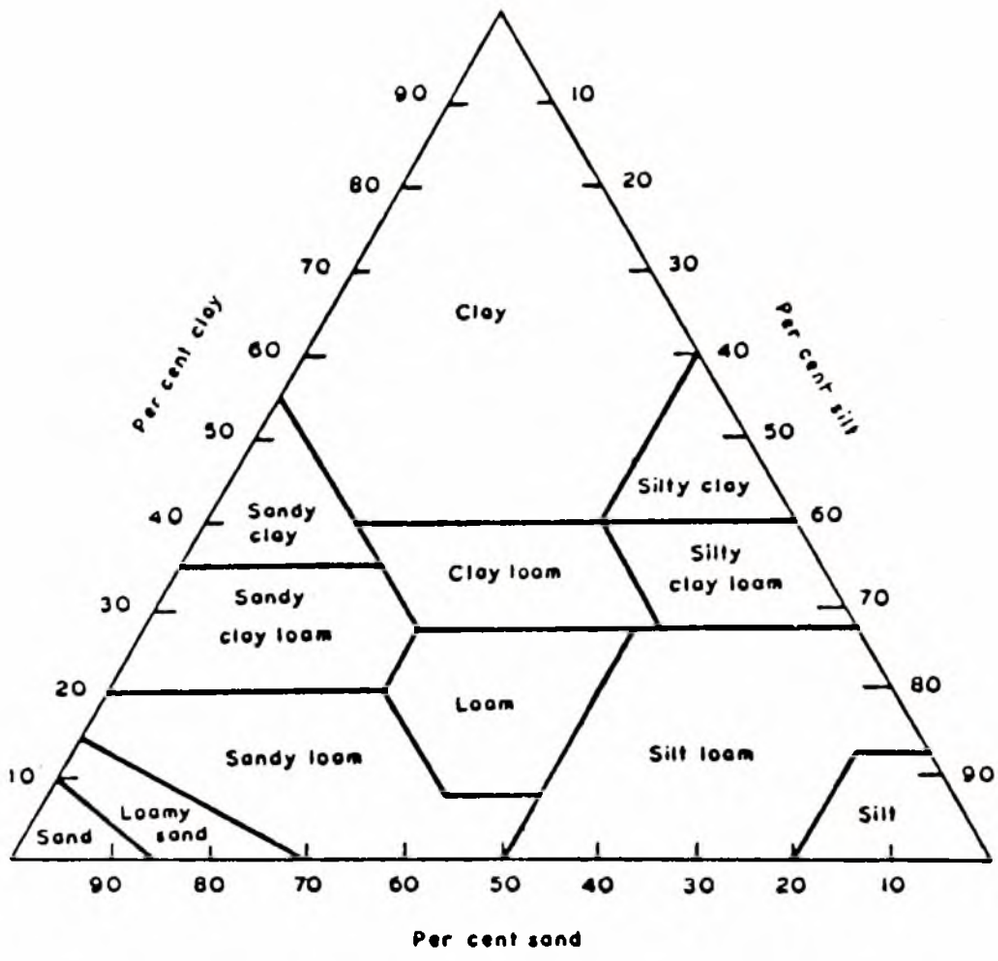
APPENDIX A

DAYS FOR WATERING – BAMBARA GROUNDNUT VARIETY Ex – TAMALE



APPENDIX B



The Prescott Triangle showing the relationship between contents of clay, silt and sand in determining the different kinds of soil.

APPENDIX C

Distribution of nodule size in two varieties of Bambara groundnut plants raised in soils from legume plots from different localities for 30 days under normal day/night regime.

(Data for Fig. 2).

Source of Soil	Plant variety	Percentage frequency of nodule in class-diameter (mm)					
		0.56.1.05	1.06-1.55	1.56-2.05	2.06-2.55	2.56-3.05	3.06-3.55
Kpong	Ex-Tamale	8.7	0	10.9	28.3	32.6	19.6
Legon	Ex-Ada	0	13.0	71.7	15.2	0	0
Nungua (1)	Ex-Tamale	17.4	39.1	30.4	10.9	2.2	0
Pokuase	Ex-Tamale	0	39.1	34.8	17.4	8.7	0
Weija	Ex-Ada	2.2	73.9	19.6	4.4	0	0

APPENDIX D

Distribution of nodule size of Bambara groundnut, Ex-Ada variety, raised on 'Seedling Agar' for 20 days at 28°C.

(Data for Fig. 3)

Strain of *Rhizobium* sp. from nodules of	Percentage frequency of nodule in class-diameter (mm)				
	0.26-0.65	0.66-1.05	1.06-1.45	1.46-1.85	1.86-2.25
Ex-Ada in Legon soil	7.1	46.4	32.1	14.3	0
Ex-Ada in Weija soil	7.1	17.9	39.3	35.7	0
Ex-Tamale in Kpong soil	28.6	17.9	32.1	21.4	0
Ex-Tamale in Nungua(1) soil	0	10.7	21.4	60.7	7.1
Ex-Tamale in Pokuase soil	0	21.4	21.4	46.4	10.7

APPENDIX E_1

Growth of five strains of **Rhizobium** sp. in inoculated moss carrier stored at room temperature for 14 weeks.

(Data for Fig. 4)

Strains of **Rhizobium** sp. from nodules of	Inoculum concentration	Population of the various strains of **Rhizobium** sp. after the following number of weeks: ($x10^9$)					
		4	5	6	7	8	9
Ex-Ada in Legon soil	Undiluted	252	269	300	380	380	381
	½ Dilution	200	208	273	342	348	365
Ex-Ada in Weija soil	Undiluted	246	292	311	337	341	368
	½ Dilution	220	232	312	356	366	379
Ex-Tamale in Kpong soil	Undiluted	246	250	279	280	280	316
	½ Dilution	220	241	247	261	270	270
Ex-Tamale in Nungua(1) soil	Undiluted	224	224	272	301	377	381
	½ Dilution	206	225	280	317	325	365
Ex-Tamale in Pokuase soil	Undiluted	226	255	289	331	355	356
	½ Dilution	210	229	270	297	340	349

APPENDIX E_2

Growth of five strains of **Rhizobium** sp. in inoculated moss carrier stored at room temperature for 14 weeks.

(Data for Fig. 4) (Cont'd).

Strains of **Rhizobium** sp. from nodules of	Inoculum concentration	Population of the various strains of **Rhizobium** sp. after the following number of weeks: ($x10^0$)				
		10	11	12	13	14
Ex-Ada in Legon soil	Undiluted	387	392	421	429	429
	½ Dilution	389	413	417	417	432
Ex-Ada in Weija soil	Undiluted	383	416	417	433	435
	½ Dilution	385	392	420	430	442
Ex-Tamale in Kpong soil	Undiluted	318	332	364	365	382
	½ Dilution	271	287	293	335	336
E Tamale in Nungua(1) soil	Undiluted	400	405	423	425	427
	½ Dilution	373	384	395	408	422
Ex-Tamale in Pokuase soil	Undiluted	394	396	408	415	425
	½ Dilution	380	403	416	418	418

APPENDIX F_1

Distribution of nodule size of Bambara groundnut plants grown under 2-day watering intervals for 30 days.
(Data for Figs 5 - 9)

Rhizobium Inoculum	Initial density of Inoculum	Percentage frequency of nodule in class-diameters (cm)						
		0-1.00	1.01-2.00	2.01-3.00	3.01-4.00	4.01-5.00	5.01-6.00	6.01-7.00
Kpong-Ex-Tamale strain	Undiluted	7.1	23.2	57.1	12.5	0	0	0
	½ Dilution	6.8	30.5	40.7	15.3	5.1	1.7	0
	¼ Dilution	3.5	45.6	38.6	12.3	0	0	0
	$^1/_8$ Dilution	1.7	31.0	60.3	1.7	5.2	0	0
Legon-Ex-Ada strain	Undiluted	32.2	37.3	6.8	16.9	3.4	1.7	1.7
	½ Dilution	10.2	45.8	23.7	18.6	1.7	0	0
	¼ Dilution	20.3	20.3	44.1	15.3	0	0	0
	$^1/_8$ Dilution	14.0	31.6	28.1	21.1	5.3	0	0
Nungua(1)-Ex-Tamale strain	Undiluted	3.8	35.8	18.9	32.1	9.4	0	0
	½ Dilution	12.3	13.6	33.3	19.3	3.5	0	0
	¼ Dilution	25.9	19.0	25.9	19.0	8.6	1.7	0
	$^1/_8$ Dilution	39.0	18.6	18.6	3.4	15.3	5.1	0
Pokuase-Ex-Tamale strain	Undiluted	5.4	10.7	60.7	19.6	3.6	0	0
	½ Dilution	3.4	15.5	70.7	6.9	3.4	0	0
	¼ Dilution	5.0	48.3	35.0	10.0	1.7	0	0
	$^1/_8$ Dilution	14.5	22.6	54.8	8.1	0	0	0
Weija-Ex-Ada strain	Undiluted	10.2	35.6	15.3	3.22	6.8	0	0
	½ Dilution	15.0	23.3	38.3	20.0	3.3	0	0
	¼ Dilution	3.6	20.0	50.9	10.9	12.7	1.8	0
	$^1/_8$ Dilution	5.5	52.7	32.7	9.1	0	0	0

APPENDIX F_7.

Distribution of nodule size of Bambara groundnut plants grown under 4-day watering intervals for 30 days.

(Data for Figs 5 - 9)

Rhizobium Inoculum	Initial density of Inoculum	Percentage frequency of nodule in class-diameters (cm)						
		0-1.00	1.01-2.00	2.01-3.00	3.01-4.00	4.01-5.00	5.01-6.00	6.01-7.00
Kpong-Ex-Tamale strain	Undiluted	6.5	48.4	32.3	12.9	0	0	0
	½ Dilution	68.2	31.8	0	0	0	0	0
	¼ Dilution	10.3	61.5	28.2	0	0	0	0
	1/8 Dilution	81.8	18.2	0	0	0	0	0
Legon-Ex-Ada strain	Undiluted	7.4	74.1	14.8	0	3.7	0	0
	½ Dilution	70.6	29.4	0	0	0	0	0
	¼ Dilution	66.7	33.3	0	0	0	0	0
	1/8 Dilution	70.6	23.5	5.9	0	0	0	0
Nungua(1)-Ex-Tamale strain	Undiluted	15.4	38.5	38.5	7.7	0	0	0
	½ Dilution	77.8	16.7	5.6	0	0	0	0
	¼ Dilution	91.7	8.3	0	0	0	0	0
	1/8 Dilution	80.0	10.0	10.0	0	0	0	0
Pokuase-Ex-Tamale strain	Undiluted	84.0	8.0	8.0	0	0	0	0
	½ Dilution	83.3	16.7	0	0	0	0	0
	¼ Dilution	53.6	42.9	3.6	0	0	0	0
	1/8 Dilution	67.6	32.4	0	0	0	0	0
Weija-Ex-Ada strain	Undiluted	72.4	27.6	0	0	0	0	0
	½ Dilution	61.0	36.6	2.4	0	0	0	0
	¼ Dilution	47.7	50.8	1.5	0	0	0	0
	1/8 Dilution	75.0	25.0	0	0	0	0	0

APPENDIX F_3.

Distribution of nodule size of Bambara groundnut plants grown under 6-day watering intervals for 30 days.

(Data for Figs 5 - 9)

Rhizobium Inoculum	Initial density of Inoculum	Percentage frequency of nodule in class-diameters (cm)						
		0-1.00	1.01-2.00	2.01-3.00	3.01-4.00	4.01-5.00	5.01-6.00	6.01-7.00
Kpong-Ex-Tamale strain	Undiluted	96.3	3.7	0	0	0	0	0
	½ Dilution	100	0	0	0	0	0	0
	¼ Dilution	91.7	8.3	0	0	0	0	0
	$^1/_8$ Dilution	91.7	8.3	0	0	0	0	0
Legon-Ex-Ada strain	Undiluted	81.8	8.2	0	0	0	0	0
	½ Dilution	75.0	25.0	0	0	0	0	0
	¼ Dilution	100	0	0	0	0	0	0
	$^1/_8$ Dilution	100	0	0	0	0	0	0
Nungua(1)-Ex-Tamale strain	Undiluted	100	0	0	0	0	0	0
	½ Dilution	90.7	9.3	0	0	0	0	0
	¼ Dilution	93.3	6.7	0	0	0	0	0
	$^1/_8$ Dilution	93.8	6.2	0	0	0	0	0
Pokuase-Ex-Tamale strain	Undiluted	47.4	42.1	10.5	0	0	0	0
	½ Dilution	53.8	38.5	7.7	0	0	0	0
	¼ Dilution	100	0	0	0	0	0	0
	$^1/_8$ Dilution	100	0	0	0	0	0	0
Weija-Ex-Ada strain	Undiluted	77.8	22.2	0	0	0	0	0
	½ Dilution	80.0	20.0	0	0	0	0	0
	¼ Dilution	100	0	0	0	0	0	0
	$^1/_8$ Dilution	85.7	14.3	0	0	0	0	0

APPENDIX G.

Recordings of light intensities during growth of Bambara groundnut plants, Ex-Ada variety, under Sheds 1, 2 and 3 on the following dates: (X10) Lux.

(Data for Fig. 10)

Date	9.00 am			12.00 noon			3.00 pm		
	SHED			SHED			SHED		
	1	2	3	1	2	3	1	2	3
10/4/91	160	230	600	410	640	893	183	290	593
12/4/91	129	190	573	573	690	880	190	290	473
14/4/91	213	260	553	556	846	966	183	303	550
16/4/91	129	180	590	410	686	1000	176	290	620
18/4/91	220	280	600	620	853	1000	190	303	620
20/4/91	129	190	590	573	846	966	176	300	610
22/4/91	110	140	530	543	846	966	180	303	460
24/4/91	213	240	600	573	900	1000	180	350	500
26/4/91	160	223	620	403	810	1000	170	300	630
28/4/91	190	210	590	543	846	920	176	310	630
30/4/91	129	190	580	593	900	966	190	350	570
2/5/91	175	230	590	573	830	1000	180	310	600
4/5/91	203	273	600	593	910	1000	190	350	610
6/5/91	200	270	530	580	846	920	180	310	550
8/5/91	110	240	580	550	890	966	190	290	593
10/5/91	160	250	610	600	900	1000	160	300	640
12/5/91	168	260	600	410	846	1000	190	340	610
14/5/91	160	280	550	550	853	893	180	310	550

APPENDIX H_1.

Distribution of nodule size of Bambara groundnut plants grown under Low light intensity for 30 days.
(Data for Figs 11 - 15)

Rhizobium Inoculum	Initial density of Inoculum	Percentage frequency of nodule in class-diameters (cm)						
		0-1.00	1.01-2.00	2.01-3.00	3.01-4.00	4.01-5.00	5.01-6.00	6.01-7.00
Kpong-Ex-Tamale strain	Undiluted	2.9	17.1	34.3	28.6	15.7	0	1.4
	½ Dilution	41.4	15.7	14.3	5.7	20.0	2.9	0
	¼ Dilution	20.0	20.0	22.9	28.6	2.9	5.7	0
	$^1/_8$ Dilution	30.0	41.4	20.0	7.1	0	1.4	0
Legon-Ex-Ada strain	Undiluted	2.9	31.4	11.4	24.3	25.7	4.3	0
	½ Dilution	5.7	32.9	21.4	27.1	8.6	1.4	2.9
	¼ Dilution	24.3	21.4	8.6	8.6	11.4	12.9	12.9
	$^1/_8$ Dilution	2.9	8.6	37.1	28.6	14.3	4.3	4.3
Nungua(1)-Ex-Tamale strain	Undiluted	22.9	40.0	22.9	5.7	2.9	2.9	2.9
	½ Dilution	68.6	5.7	17.1	2.9	5.7	0	0
	¼ Dilution	22.9	32.9	24.3	11.4	7.1	1.4	0
	$^1/_8$ Dilution	0	7.1	37.1	27.1	20.0	5.7	2.9
Pokuase-Ex-Tamale strain	Undiluted	27.1	38.6	14.3	14.3	5.7	0	0
	½ Dilution	22.9	35.7	20.0	10.0	8.6	1.4	0
	¼ Dilution	4.3	28.6	54.3	10.0	2.9	0	0
	$^1/_8$ Dilution	1.4	40.0	31.4	15.7	8.6	0	0
Weija-Ex-Ada strain	Undiluted	4.3	18.6	21.4	24.3	18.6	11.4	1.4
	½ Dilution	11.4	15.7	54.3	12.9	2.9	2.9	0
	¼ Dilution	2.9	5.7	11.4	47.1	18.6	10.0	4.3
	$^1/_8$ Dilution	22.9	38.6	15.7	14.3	2.9	5.7	0

APPENDIX H$_2$.

Distribution of nodule size of Bambara groundnut plants grown under Median light intensity for 30 days.

(Data for Figs 11 - 15)

Rhizobium Inoculum	Initial density of Inoculum	Percentage frequency of nodule in class-diameters (cm)						
		0-1.00	1.01-2.00	2.01-3.00	3.01-4.00	4.01-5.00	5.01-6.00	6.01-7.00
Kpong-Ex-Tamale strain	Undiluted	7.1	27.1	18.6	31.4	11.4	4.3	0
	½ Dilution	41.4	45.7	7.1	0	4.3	1.4	0
	¼ Dilution	27.1	35.7	17.1	7.1	8.6	2.9	0
	$^1/_8$ Dilution	12.9	54.3	22.9	5.7	0	0	0
Legon-Ex-Ada strain	Undiluted	18.6	21.4	25.6	14.3	11.4	0	0
	½ Dilution	24.3	24.3	31.4	8.6	11.4	0	0
	¼ Dilution	5.7	12.9	41.4	22.9	12.9	1.4	0
	$^1/_8$ Dilution	20.0	25.7	45.7	5.7	2.9	0	0
Nungua(1)-Ex-Tamale strain	Undiluted	20.0	14.3	22.9	18.6	21.4	2.9	0
	½ Dilution	60.0	31.4	8.6	0	0	0	0
	¼ Dilution	34.3	22.9	14.3	24.3	4.3	0	0
	$^1/_8$ Dilution	51.4	8.6	28.6	11.4	0	0	0
Pokuase-Ex-Tamale strain	Undiluted	82.9	14.3	2.9	0	0	0	0
	½ Dilution	2.9	28.6	54.3	8.6	5.7	0	0
	¼ Dilution	11.4	61.4	20.0	5.7	0	0	0
	$^1/_8$ Dilution	2.9	40.0	37.1	17.1	2.9	0	0
Weija-Ex-Ada strain	Undiluted	45.7	37.1	5.7	11.4	0	0	0
	½ Dilution	5.7	28.6	54.3	5.7	5.7	0	0
	¼ Dilution	37.1	34.3	14.3	5.7	2.9	2.9	2.9
	$^1/_8$ Dilution	14.3	34.3	37.1	14.3	0	0	0

APPENDIX H_3.

Distribution of nodule size of Bambara groundnut plants grown under High light intensity for 30 days.

(Data for Figs 11 - 15)

Rhizobium Inoculum	Initial density of Inoculum	Percentage frequency of nodule in class-diameters (cm)						
		0-1.00	1.01-2.00	2.01-3.00	3.01-4.00	4.01-5.00	5.01-6.00	6.01-7.00
Kpong-Ex-Tamale strain	Undiluted	44.3	24.3	17.1	14.3	0	0	0
	½ Dilution	80.0	20.0	0	0	0	0	0
	¼ Dilution	17.1	35.7	32.9	11.4	2.9	0	0
	$^1/_8$ Dilution	22.9	45.7	28.6	2.9	0	0	0
Legon-Ex-Ada strain	Undiluted	37.1	62.9	0	0	0	0	0
	½ Dilution	22.9	68.6	8.6	0	0	0	0
	¼ Dilution	5.7	34.3	37.1	20.0	0	2.9	0
	$^1/_8$ Dilution	65.7	34.3	0	0	0	0	0
Nungua(1)-Ex-Tamale strain	Undiluted	42.9	14.3	22.9	14.3	5.7	0	0
	½ Dilution	97.1	2.9	0	0	0	0	0
	¼ Dilution	81.4	10.0	2.9	5.7	0	0	0
	$^1/_8$ Dilution	85.7	11.4	2.9	0	0	0	0
Pokuase-Ex-Tamale strain	Undiluted	88.6	8.6	2.8	0	0	0	0
	½ Dilution	22.9	37.1	40.0	0	0	0	0
	¼ Dilution	27.1	32.9	40.00	0	0	0	0
	$^1/_8$ Dilution	2.9	31.4	48.6	17.1	0	0	0
Weija-Ex-Ada strain	Undiluted	30.0	50.0	20.0	0	0	0	0
	½ Dilution	45.7	52.9	1.4	0	0	0	0
	¼ Dilution	8.6	50.0	18.6	12.9	7.1	2.9	0
	$^1/_8$ Dilution	45.7	14.3	28.6	11.4	0	0	0