EFFECT OF SOYBEAN - MAIZE CROPPING ROTATION ON SOYBEAN RHIZOBIAL POPULATION AND SOYBEAN NODULATION

BY

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DECLARATION

This thesis is my original work
and has not been presented for
a degree in any other University.

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All glory be

to Almighty God

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ABSTRACT

Experiments were conducted to determine the effects of soybean-maize cropping sequences on infectiveness of introduced Bradyrhizobium japonicum and soybean rhizobial population in the soil.

Streptomycin resistant mutant of B. japonicum strain IRj 2114 was developed, tested for ability to nodulate and fix nitrogen, and introduced on soybean seeds at the start of the field experiments. Soybean (Glycine max L.) and maize (Zea mays L.) were then grown in four cropping sequences namely: (i) soybean/soybean/soybean (SSS), (ii) soybean/soybean/maize (SSM), (iii) soybean/maize/soybean (SMS) and (iv) soybean/maize/maize (SMM). A glasshouse study was also carried out to determine the effects of soybean, maize and fallowing on the survival and establishment of rhizobia under controlled conditions.

Percentage soybean nodulation by the introduced rhizobium, and seasonal changes in soybean rhizobial populations along (AR) and between (BR) crop rows were determined using the antibiotic resistance and most probable number (MPN) methods, respectively.

Results obtained showed that the streptomycin resistant B. japonicum nodulated effectively and fixed a large quantity of nitrogen (158 mg/plant) in symbiosis with soybean.

Data on soybean nodulation showed that both the total number of nodules per plant and the proportion of nodules due to inoculum IRj 2114 rhizobium varied significantly ($P \leq 0.01$) with cropping sequences. In the first season, nodule recovery due to the introduced Rhizobium was low being only 15%. In the subsequent seasons, maize crop adversely affected soybean nodulation. In the third season, occupancy of mutant Rhizobium which was 60% for continuous soybean cropping (SSS) was only 42% for the soybean-maize rotation (SMS).

Population of soil rhizobia were similarly affected by the cropping sequences. Rhizobial numbers were significantly ($P \leq 0.05$) higher when the first two crops were soybean (SS) than when maize followed soybean (SM).

Throughout the sampling period, more rhizobia occurred along the crop rows (AR) than in the inter-row spaces (BR), indicating positive effects of rhizospheres on the rhizobial population. Pot experiment confirmed observations in the field. Greater stimulation of rhizobia was obtained for soybean than for maize.

It was concluded therefore that for successful establishment of improved strains of B. japonicum, a second soybean crop should follow the first inoculated crop.

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CHAPTER 1

INTRODUCTION

1.1 GRAIN LEGUMES AND THEIR IMPORTANCE IN AGRICULTURE

The terms "grain legumes" or "pulses" refer to leguminous plants producing dry edible seeds (Okigbo, 1976). Major grain legume species traditionally grown in the tropics include Vigna unguiculata (L.) Walp (Cowpea), V. mungo (L) Hepper (black gram), V. radiata (L.) Wilczek (green gram), Phaseolus vulgaris (L.), (Common bean), P. lunatus (Lima beans), Cajanus cajan (L.) (Millsp.) (Pigeon peas), Arachis hypogea (groundnut), Voandzeia subterranea (Bambara nuts), and Cicer arientum (L.) (Chick pea). Soybeans (Glycine max (L.) Merr.), although recently introduced into the tropics, has also gained increasing importance all over the region (Auckland, 1970).

Generally, grain legumes are grown as mixed, associated, relay and sole crops, and in crop rotations with cereals and other crops. They are utilized in several forms for food, animal feeds, soil cover and green manure (Rachie, 1977). Legumes also have special ability to grow in depleted soils and even contribute to the improvement of soil fertility through their unique symbiotic relationship with nitrogen fixing root-nodule bacteria.

Much effort has been made to improve grain legume production levels. However, average yields in most developing countries are still low when compared to those obtained in the developed countries (FAO, 1983). For instance, soybean yields are reported to average 240, 368, 553, 714 and 1167 kg/ha for Tanzania, Nigeria, Cameroon, Rwanda and Uganda respectively. In contrast, yields of the same crops recorded for United States of America (U.S.A.) and Brazil are much higher,

being in excess of 2400 kg/ha (Dunbar, 1975; Wilcox, 1987). These higher yields are attributable to the use of advanced crop production techniques including the exploitation of biological nitrogen fixation (BNF) technology. Virtanen et al. (1947), for instance, reported that 60% of nitrogen received by cultivated lands in U.S.A. is from biological nitrogen fixation.

Biological nitrogen fixation are carried out by free-living bacteria or blue-green algae which make use of nitrogen by non-symbiotic means, and by bacteria in symbiotic association with higher plants, mainly the Leguminosae.

Symbiotic association between legume plants and some bacteria of the family Rhizobiaceae has been of high significance in agriculture since 1888 (Burns and Hardy, 1975). Rhizobia (bacteria) infect legume roots and cause formation of root nodules. Nitrogen fixation takes place inside the root nodules through the action of the enzyme nitrogenase produced by the rhizobia. The rhizobia therefore provide fixed nitrogen to the plant and, in return, the plant supplies the rhizobia with carbohydrates, minerals and other nutrients.

1.2 IMPORTANCE OF NITROGEN FIXATION BY LEGUMES

When legumes are included in a cropping rotation, they fix atmospheric nitrogen and contribute to the nitrogen supply of succeeding crops (Hanson et al., 1988; Fox and Piekielek, 1988; Chapman and flyers, 1987; Hesterman et al., 1986). Estimates of the amount of nitrogen fixed by various legume species indicate that legumes have the potential to supply nitrogen for crop production (Table 1). Through nitrogen fixation, therefore, the use of costly inorganic nitrogen fertilizers can be reduced and crop as well as

protein yields could be increased. This is especially true in the tropics where nitrogen is the most limiting soil nutrient, and where subsistence farmers can not afford the cost of fertilizers.

In the tropics, the use rhizobia of has been limited mainly because effective Rhizobium strains for introduced legumes such as soybean (Glycine max L. Merrill) are lacking in the soil (Hamdi et al., 1973; Ashley, 1973; deSouza, 1969). Exploitation of symbiotic nitrogen of soybean in tropical agriculture, therefore, requires inoculation of the crop with appropriate rhizobia strains before planting (Friere, 1976). Soybean inoculation with rhizobia has been found necessary even when using promiscuous soybean varieties that are able to nodulate freely with native soil rhizobia (Pulver et al., 1982).

This is because indigenous strains are ineffective or poorly effective, and new more effective strains developed by genetic engineering and other means have to be introduced into soils.

In order for the inoculum rhizobia to be of long-term value in tropical crop production systems, they must be able to survive, colonise, live saprophytically (outside the host) and compete with indigenous rhizobial populations present in the soils. The persistence of introduced soybean inoculum rhizobia in tropical soils have, however, not been adequately investigated. Bradyrhizobium japonicum strains that nodulate soybean, have been reported to survive well in the field for more than 5 years, even in absence of the host legume (Nutman and Hearne, 1980). Crozat et al. (1982) also reported that the percentage of nodules formed by the inoculum strain increased with time indicating a permanent establishment and a high competitive ability.

On the contrary, Hiltbold et al. (1985) obtained rapid increase in soybean rhizobial populations occurring during growth of soybeans but

Table 1: **Estimates of nitrogen fixation by various legumes.**

Legumes	Nitrogen fixed kg/annum	Source
Bean (Phaseolus)	64	Lyon and Bizzel (1934)
Peas	30-140	Nutman (1965)
Cowpea	90	Wetselaar et al. (1973)
Soybean	40-140	Sundara Rao (1971)
Clovers	50-200	Nutman (1965)
Alfalfa	90-220	Bell and Nutman (1971)
Pigeon peas	133 (with P)	Sen (1958)

they realized a most rapid decline in the populations in the year when cotton was grown after soybean in a rotation. This implied that soybean-cotton rotation had detrimental effects on B. japonicum strains.

In the tropics, legumes including soybean are usually grown in rotation with non-legumes particularly cereals such as maize and rice (Sanchez, 1976). The work reported here, therefore, involved determination of the effects of various soybean-maize cropping sequences on survival, colonization and establishment of soybean rhizobia in the soil.

1.3 THE OBJECTIVES OF THE STUDY

Understanding of the variations in rhizobial population, their saprophytic competence, competitiveness and efficiency is a first step in the utilization of introduced rhizobia strains for improved production of both traditional and introduced pasture and grain legumes including soybeans in the tropics (Obaton, 1977, Alexander, 1977; Keya, 1977). In this study, therefore, efforts were made to assess the ability of introduced Bradyrhizobium japonicum IRj 2114 to survive, colonize and he established in soils where soybean was grown in various cropping sequences with maize.

The objectives of the study were to determine the effects of soybean-maize cropping sequences on:

(a) the infectiveness of introduced Bradyrhizobium japonicum (strain IRj 2114),

(b) soybean rhizobial population in the soil, and hence their nitrogen fixing potential.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Nitrogen is the most limiting element in crop production. This is because atmospheric nitrogen is highly inert (Timm, 1944) and can only be transformed into usable forms such as ammonia after application of energy and reducing agents. Yet, as an essential constituent of proteins, nucleic acids and protoplasm, nitrogen is highly required by crops and when in available form it is easily lost from the soil.

It is possible to boost the nitrogen status of soils by the application of commercial fertilizers (Mughogho, 1985). However, the manufacture of chemical nitrogenous fertilizers requires large amounts of energy (Chatt, 1981) and high oil prices have increased the cost of production and distribution of these fertilizers (Pimentel, 1976). As a consequence, a relatively cheap and increasingly important source of nitrogen for the crops is that fixed by soil micro-organisms (Quispel, 1974).

2.2 BIOLOGICAL NITROGEN FIXATION (BNF)

Biological nitrogen fixation comprises of non-symbiotic and symbiotic systems. Non-symbiotic nitrogen fixation involves free-living organisms like Azotobacter, Klebsiella, Clostridium and many algae which are able to fix atmospheric nitrogen independently. Symbiotic nitrogen fixation, on the other hand, is based on very close physical and physiological associations between rhizobial bacteria

and leguminous plants. The bacteria fix atmospheric nitrogen by incorporating nitrogen gas from the atmosphere into forms utilizable by legumes for the synthesis of organic compounds.

Although non-symbiotic organisms fix nitrogen, their contribution to the nitrogen economy of the soil is not as great as those of the symbiotic ones (Hardy and Havelka, 1975). For example, Meiklejohn (1954) reported that non-symbiotic nitrogen fixation ranges from 10 to 15 kg N/ha/year. In contrast, rhizobia in symbiosis with legumes, are believed to fix nitrogen at levels varying from less than 100 kg N/ha/year to more than 600 kg N/ha/year (Graham and Hubbell, 1975). Estimates show that the symbiotic system contributes 40 million tons of nitrogen annually to grain legumes (Hardy and Havelka, 1975). Rhizobia-legume symbiosis is therefore the most important source of biologically fixed nitrogen in agricultural systems.

2.3 TAXONOMY OF RHIZOBIA

Rhizobia are rod-shaped, gram-negative and non-spore forming bacteria. They are aerobic and can be found free-living in soils, or cultured in agar (Vincent, 1982).

Systems of classification of rhizobia have undergone many changes. The earlier classifications were based on the "Serum zone" (Trinick, 1982) and "Cross inoculation group" (Jensen, 1958) concepts.

Serum zone classification was based on the characteristic reaction that many rhizobia have when they are grown in skim milk medium. The bacteria produce a superficial clear medium - the "Serum zone" - which characterizes each species by the change in pH towards acid

or alkaline. The concept has limited value in distinguishing rhizobia because even within the homogeneous group, for example rhizobia from Caragana arboroscens (Jensen, 1942) and Lucerne (Medicago sativa L.) (Trinick, 1982), it is possible to find strains with and without serum zone formation.

The cross-inoculation group classification, on the other hand, was based on the host range of the bacteria (Fred et al., 1932). Within the particular "Cross-inoculation" group, rhizobia from one plant would nodulate all other plants and vice versa. The group of rhizobia that form nodules in each member of the cross-inoculation group were then regarded as belonging to the same species.

This is illustrated in Table 2. It has however, become evident that these groups are not discrete and many reports of boundary-jumping between them are available (Trinick, 1982; Masefield, 1958; Kleczkowska et al., cited by Jensen, 1958).

Because the above systems of classification have been found to be biologically inaccurate, they have been largely discarded (Jordan, 1982). The recent concept of rhizobial classification is based on techniques designed to examine large portions of the bacterial genome. On this basis, Jordan (1984) classified root-nodule bacteria under two genera namely; (i) Rhizobium and (ii) Bradyrhizobium. Rhizobium consists of all fast growing acid producing rhizobia while Bradyrhizobium comprises of the slow growing alkali producing rhizobia. The corresponding rhizobia species, based on this classification, and their hosts are given in Table 3.

2.4 SOIL-PLANT-RHIZOBIA RELATIONSHIP

Rhizobia are known to live freely in soil in the absence of their host plants (Rovira, 1961; Vincent, 1974; Nutman and Hearne, 1980).

Table 2 **Some of the cross-inoculation groups within the Leguminosae.**

Group	Representative genera	Rhizobium species
Clover	Trifolium	R. trifolii
Pea	Pisum, Lathyrus, Lens Vicia	R. leguminosarum
Bean	Phaseolus vulgaris	R. phaseoli
Soybean	Glycine max	R. japonium
Medic	Medicago, Melilotus Trigonella	R. meliloti
Lupine	Lupinus, ornithopus	R. lupini
Cowpea	A great number of genera from three subfamilies of Leguminosae	R. spp.

Source: Trinick (1982).

Table 3 Species of the genera Rhizobium and Bradyrhizobium and their respective host.

	Bacteria	Host genera
1.	Fast-growing acid producing	
	Rhizobium meliloti	Medicago, Melilotus, Trigonella
	Rhizobium leguminosarum:	
	bivar, trifolii	Trifolium
	bivar, phaseoli	Phaseolus, Vulgaris
	bivar, viceae	Pisum, Lathyrus, Lens, Vicia
	Rhizobium loti	Lupinus, Lotus, Anthylis, Ornithopus, Leucaenae
2.	Slow-growing alkali producing	
	Bradyrhizobium japoicum	Glycine max
	Bradyrhizobium species:	
	Bradyrhizobium sp. (Vigna)	Vigna, tropical forage legumes and many others.
	Bradyrhizobium sp.(Lupinus)	Lotus pedunculatus, Lupinus sp.

Source: Krieg and Holt (1984).

However, as rhizosphere organisms, these bacteria are markedly stimulated to multiply by the root secretion of nutrients and growth factors (West, 1939), and they rapidly increase in number in rhizosperes (Vincent, 1982). This stimulation has been found to be general and not confined to leguminous roots only, although the degree of stimulation varies between non-legumes and legumes (Diatloff, 1969; Mahler and Wollum, 1982). Generally, non-legumes stimulate the rhizosphere root-nodule bacteria to a smaller degree (Lockhead, 1952; Rovira, 1961).

Multiplication and increase of rhizobia in numbers in the vicinity of legume root hairs is an essential prerequisite for the infection process and nodulation (Bergersen, 1977). In soil or cultures, the actual densities of nodule bacteria required in the rhizosphere for the successful infection are of the order of 10^6 to 10^9 organisms per ml (Purchase and Nutman, 1957).

2.4.1 Host-plant infection and nodule formation

Rhizobium forms an infection thread toward the base of the root hair and eventually penetrates the cortex of the root (Newcomb et al., 1979; Rao and Keister, 1978). The bacteria still continue to divide, then later on division stops and the bacteria grow into swollen, mostly branched cells which are called bacteriods. Bacteriods are able to fix nitrogen through nitrogenase enzyme activity. In response, the legume plant root hair undergoes rapid cell division of the meristematic tissue in the vicinity of the infection and forms a tuberous growth, the nodule (Dazzo, 1980).

Occasionally, nodule-like growths may be produced on the roots of legumes by nematodes or by crown-gall bacteria. Certain non-leguminous plants are also frequently found to possess nodule-like growths which are produced by mycorrhiza crown-gall organisms and certain nematodes. On

careful examination, however, Rhizobium induced nodules are easily distinguished from the false ones.

Individual nodules vary greatly in size and shape. For example, the cultivated annual legumes like soybean, generally have large spherical nodules, whereas those on the biennial and perennial legumes tend to be smaller, elongated, and clustered.

In the nodule, rhizobia occur in both normal vegetative rod shaped cells and as bacteriods. The bacteriods are rhizobial cells that have differentiated and are not capable of multiplication as free cells. The coexistence of both vegetative rhizobial cells and non-viable bacteriods was observed in nodules of Astragalus senicus, Medicago, Trifolium and Vicia (Date and Halliday, 1987). The vegetative cells and bacteriods show no apparent morphological differentiation (Date and Halliday, 1987).

2.4.2 Nutrient requirement for nodulation

Root nodules are rich in molybdenum, phosphorus, cobalt, iron, zinc, sulphur and nitrogen (Munns, 1977; Robson, 1978). The high concentration of these elements in the nodules is associated with high bacteroidal concentration of nuclceotides, cobalamines and proteins, including Fe-, S- and Mo-proteins, and the presence of iron in legheamoglobin (Allen and Allen, cited by Manil, 1958). Deficiencies of these elements in the soil will affect Rhizobium legume symbiosis. It is known, for example, that symbiotic plants need higher rates of phosphorus fertilization than nitrogen fed plants (Cassman, 1979), and when phosphorus requirements are not satisfied, nodule formation and functioning are adversely affected (Vincent, 1965; Olsen and Moe, 1971). Similar effects are also known for such micronutrients as molybdenum and sulphur, which are constituents of the enzyme nitrogenase and are

important nutrients for the rhizobia (Perkasen, 1977; Munns, 1978). Thus, plants that are dependent on symbiotically fixed nitrogen require greater quantities of macro- and micro-nutrients than their non-symbiotic counter-parts (Jonnes and Lutz, 1971; Burton, 1972 Robson, 1978).

2.4.3 Senescence of nodules and release of rhizobia into the soil

Nodule death occurs because of plant senescence or other factors like drought, high soil temperatures and nutritional disorders that affect nodule life. The rhizobia then die or are released into the soil, hence completing their life cycle (Sutton, 1983). Since each nodule may contain millions of rhizobia and the number of nodules that may develop on a single plant vary from a few to a thousand or more, decayed nodules release vast numbers of rhizobia into the soil, thus increasing the rhizobial population.

Bushby (1981) obtained increased number of soybean rhizobia in soils grown with the crop at 70 days after planting. He attributed the increase to nodule decay and the release of rhizobia into the soil. Where inoculation using effective Rhizobium strains has to be done in the tropics, for high yielding soybean varieties (like Bossier), therefore, rhizobia originating from nodule disintegration are likely to form an important component of the rhizobial populations which nodulate subsequent crops (Brockwell et al., 1988).

2.5 FACTORS INFLUENCING RHIZOBIAL POPULATION IN THE SOIL

Besides the presence of host and non-host plants, the persistence of free-living rhizobia in soils is generally influenced

by physical, chemical and biotic factors (Lowendorf , 1980). The major factors include soil temperature and moisture levels, pH and nutrient status of the soil, and the activity of other micro-organisms.

2.5.1 Importance of soil temperature levels

Many root-nodule, bacteria grow best under a temperature range of 25°C to 30°C (Vincent, 1970). Most strains of genus Bradyrhizobium, however, are reported to be tolerant to high soil temperatures with a maximum growth range within 30-40^{0}C (Jordan, 1984). Extremes of soil temperatures, therefore, affect the survival and persistence of rhizobia in soils.

In the tropics, high soil temperatures are a major factor limiting the activity of rhizobia, particularly exotic strains but also below 15^{0}C nodulation may not occur (Elkan, 1987). Studies in Ibadan, Nigeria, showed that soil temperatures can reach an average of 40°C at 0-15 cm depth, when soils are bare or newly planted with crops (IITA, 1972; Lal, 1975). The introduction into the tropics of temperate strains of rhizobia, as inoculum for soybeans and other crops, that are not tolerant of such high temperatures, will introduce the problem of their adaptation and persistence in these soils. The effect of high soil temperatures is made worst by the fact that such conditions occur during the dry off-season periods when crop hosts may not be growing in the field.

2.5.2 Importance of soil moisture levels

In the tropics, and else-where, soil moisture conditions may range from a state of water logging to total dryness. Because rhizobia are aerobic heterotrophs, flooding the soil reduces the gas exchange between the soil and bacteria or plant nodules and thus affect the growth and activity of rhizobia. Desiccating conditions, on the other hand, reduce available soil moisture and may lead to the death of rhizobia (Pena-Cabriales and Alexander, 1979).

Rhizobial species vary in their response to variation in soil moisture levels; while some are tolerant of a wide range of moisture conditions others are not. Osa-Afiana and Alexander (1979) compared the survival of Rhizobium trifolii and the soybean rhizobia, Bradyrhizobium japonicum, and found that higher numbers of both rhizobia species survived at 10% relative humidity than at moisture regimes ranging from 22% to 45% relative humidity. They, however, also found that while the number of surviving R. trifolii decreased with increase in soil moisture levels, the residual population of the soybean rhizobia was higher (2% of the original population) at the higher moisture level (45%) than at the lower moisture levels (0.7% to 22% relative humidity).

The variable response of the two rhizobia species was also observed under extremely high moisture levels. Thus, when soils were flooded, the population of R. trifolii was reduced by a factor of 300 (from 1.3×10^8 to 4.2×10^4 cells per gram of soil while that of soybean rhizobia was reduced by a factor of 150 (from 6.0×10^8 to 4.0×10^6 cells per gram of soil) (Osa-Afiana and Alexander, 1979).

The tolerance of soybean rhizobia of a wider range of soil moisture conditions than R. trifolii could be of importance in the

adaptability of soybean rhizobia to tropical soils that experience large variations in soil moisture levels.

2.5.3 Importance of pH and nutrient status of the soil

Both soil pH and nutrient levels have direct and interactive effects on the survival and multiplication of rhizobia in the soil, the growth of legume host, and their nodulation and nitrogen fixation. Although soil pH levels affect both plant growth and the occurrence, survival and growth of rhizobia, the rhizobia are more often affected by pH levels than the plants in as much as the host can grow in soils in which these organisms perish rapidly (Loneragan and Bowling, 1958).

Optimal pH for growth of rhizobia is between pH 6 and 7. However, the slow-growing and alkaline producing species such as Bradyrhizobium japonicum are more tolerant of lower pH levels while the fast growing and acid producing species such as R. leguminosarum are more tolerant of higher pH levels (Graham and Parker, 1964; Wilson, 1970; Jordan, 1984; Krieg and Holt, 1984). In the tropics, high temperatures together with heavy rains cause rapid decomposition of organic matter and mineral leaching especially of bases. Tropical soils are therefore mainly acidic with pH below 6 (Sanchez, 1976). Acidity directly inhibits nodule formation, and nodulation failure in acidic soils is usually attributed to poor survival of rhizobia or their failure to multiply in the rhizospheres (Vincent, 1965). Low soil pH is usually also associated with nutrient deficiency and mineral toxicity for the rhizobia. For example, molybdenum deficiency is common in acid soils (Munns, 1978). On the other hand, although iron deficiency is not common in the tropics, its high solubility under acid conditions often raises its availability

to levels toxic to both rhizobia and plants. The survival of rhizobia and legume nodulation in such tropical soils are therefore greatly affected.

2.5.4 Importance of microbial factors

The microbes that possibly regulate the number of rhizobia in tropical soils include predators like protozoa and amoebae, and parasites namely: bdellovibrios and bacteriophages. Their importance in the regulation of rhizobial populations in the soil is still unclear.

In a study of rhizobial predation by protozoans, Alexander (1975) reported that each protozoan consumes about 80 soybean rhizobia cells/day. However, the predators failed to eradicate the rhizobia because the remaining bacterial cells were able to reproduce at a rate fast enough to replace the cells that were consumed.

Danso and Alexander (1975) found amoebae to prey on root-nodule bacteria at a rate of 10^3 to 10^4 cells per replication. Despite the enormous number of rhizobia needed by the amoebae, high rhizobial population is found to survive in soils inoculated with rhizobia.

Bdellovibrios viruses are considered of little importance in lowering the populations of root-nodule bacteria in field soils. This is because the rhizobia seldomly attain population densities needed to initiate feeding of the parasites (Keya, 1974; Keya and Alexander, 1975).

Bacteriophages are also known to parasitise rhizobial cells (Vandecaveye et al., 1940). However, they do not eliminate rhizobia from the soils. The inability of bacteriophages to eliminate root-nodule bacteria from the soil is partly due to host specificity of the parasites (Hitcher, 1930). Furthermore, the existence of large numbers of bacteriophages in the soil will often lead to the development of

bacteriophage - resistant rhizobia mutants (Vandecaveye and Moodie, 1943; Kleckowska, 1957).

2.6 THE IMPORTANCE OF SOYBEAN RHIZOBIA IN TROPICAL AGRICULTURE

Soybean is an introduced crop in the tropics. Therefore, for high nitrogen fixation and better yields, the crop requires inoculation of seeds with the appropriate rhizobia strains before planting (Freire, 1976). This is because there is a lack of suitable strains of Rhizobium for soybean as has been reported on most Uganda soils (Ashley, 1973). Similar observations have been also reported from Egypt (Hamdi et al., 1973) and Kenya (deSouza, 1969).

2.6.1 Response of soybean to inoculation with rhizobia

Inoculation ensures successful symbiosis by introducing effective rhizobia strains into soils, in the proximity of seeds, thus enhancing nitrogen fixation by legume plants. Remarkable positive response of soybean to rhizobia inoculation have been obtained in many tropical countries. In Tanzania, for example, Bossier variety, which failed to nodulate without inoculation, when inoculated gave an increased yield of 300 percent (Min. Agric., Tanzania, 1978). Similarly, in experiments carried out in Nigeria using superior strain inoculates, high yielding soybean cultivars like Bossier and TGM 294-4 showed yield increases of up to 100 percent (IITA, 1978). Contribution of seed inoculation in increasing soybean yields has also been demonstrated by the use of most promising Malawian strains of rhizobia on the soybean variety Gedult. Average yields of 3148 kg seed/ha, were obtained as compared to 2703 kg/ha for the control (Anon., 1969). Studies in India by Jethmalani et

al. (1969), also showed significantly higher yields for inoculated soybean than for noninoculated crops that received 120 kgN/ha.

Rhizobium-soybean symbiosis is of comparable importance to other Rhizobium-legume associations with respect to nitrogen economy of the succeeding crop. Gomez (1968) studied soybean(s)-maize(m) cropping sequences namely m-m, s-s, m-s and s-m. He found that maize in rotation with soybean maintained high yields similar to those of sequential maize fertilized with nitrogen. Similarly, Caldwell (1982) obtained 14 percent yield increase above nitrogen treatments for maize following soybean and attributed this to nitrogen fixed by the soybean.

The contribution of soybean rhizobia to soil nitrogen economy was also demonstrated in an intercrop system by Searle et al. (1981). They showed that nitrogen uptake by wheat following an intercrop of maize and soybean was about twice that following maize alone without nitrogen and was equivalent to that following maize fertilized with 100 kgN/ha. This could be of great significance in the tropics where intercropping and crop rotations are major crop production systems (Okigbo, 1978). The long term value of rhizobial strains introduced into soils through inoculation will, however, only be realized if the production of soybean crops is substantially supported by nitrogen fixed by these rhizobia.

2.6.2 The survival of introduced rhizobia in soybean-cereal rotations

Fields in which soybeans have been groan frequently have populations of bradyrhizobia which are normally adequate for effective nodulation of subsequent soybean crops (Crozat et al., 1982; Weaver et al., 1972). However, there is evidence of very low field recovery of nodules from inoculum rhizobia.

Johnson et al. (1965) obtained 5% of nodules from the inoculum applied at standard rate (approximately $1x10^5$ cells/seed). Cardwell and Grant (1970) reported a range of 5 to 10% nodulation due to inoculum rhizobia while Ham et al. (1971) realized 0-17% nodule recovery from inoculum strains. These observations indicate poor survival, colonization and establishment of inoculum rhizobia in these soils.

Under tropical conditions, non-indigenous legume species, such as soybean, have to be inoculated with appropriate rhizobial strains in order for them to successfully fix nitrogen (Freire, 1976). Because such legumes are commonly grown in rotations with cereals namely: maize (Zea mays L.), rice (Oryza sativa L.), wheat (Triticum aestivum L.) and sorghum (Sorghum bicolor L.) (Okigbo, 1978; Sanchez, 1976) a major problem is the ability of the introduced rhizobia to survive during the non-legume cropping season and so sustain high yields of a subsequent legume crop without reinoculation.

CHAPTER 3

GENERAL MATERIALS AND METHODS

Materials and methods outlined in this chapter are of a general nature; those specific to particular experiments are given in the appropriate sections.

3.1 LOCATION OF EXPERIMENTS

Field and pot experiments were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, between March 1984 and June 1985. The Institute is located in the rain forest savanna transition zone of South-western Nigeria at latitude 7° 30'N and longitude 30° 54'E, and occupies about 1000 ha. The topography of the site is rolling with dominant slopes between 3 and 10%. The landform is that of an eroded pediment plain, with well incised valleys forming a trellis pattern.

Moorman et al. (1975) gives a detailed description of the climate and soils of IITA. Annual rainfall at the station is bimodal, with peaks in June and September and a major dry season between December and February. Total rainfall ranges from 788 mm to 1884 mm. Annual average temperatures range from 21.30°C to 31.20°C with extreme daily minimum and maximum temperatures of 8.30°C and 38.00°C, respectively.

Soils of IITA have been classified under 8 series, namely Ekiti Lwo, Egbeda, Ibadan, Gambari, Apomu, Iregun and Matako (Moorman et al., 1975). On the basis of the FAO classification,

these soil types can be grouped as follows: Lwo, Egbeda, Ibadan and Iregun are Ferric Luvisols, Ekiti is an Eutric Cambisol, Gambari is Plinthic Luvisol, Matako is Mollic Gleysol and Apomu is an Albic Arenosol.

Field experiments were carried out on Block A10 in an area of about 0.1 hectare in size. The soil of this block is of the Apomu series, consisting of sandy upland and slopes which are especially drought susceptible, with high leaching losses of applied nutrients (Moorman et al., 1975). Because the area was partly flat and had not been grown with soybean for over three years, it was considered suitable for the study on soybean-maize rotation involving inoculation of soybean with Bradyrhizobium.

3.2 SOIL TYPES AND FERTILIZERS USED

When carrying out the first pot experiments, two soil types namely IITA and Fashola soils were used. IITA soil was collected from Block A10 while the other soil was collected from Fashola, located in a savanna grassland region 70 km North of IITA. IITA soil has a high population of native rhizobia as compared to Fashola soil that had hardly any rhizobia (Ayanaba et al., 1981). The use of these soils, therefore, provided contrasting ecological environments (presence or absence of native rhizobia) for evaluation of symbiosis of the rhizobia strain used.

The soils were collected at 0-15 cm depth. Each soil type was mixed thoroughly and a composite sample of 1 kg taken for determination of physical, chemical and microbial characteristics. Soil texture was determined using the hydrometer method (IITA, 1979). Chemical analysis included determination of pH, organic carbon, total nitrogen and such minerals as phosphorus, exchangeable potassium, calcium, manganese and aluminium (IITA, 1979; Bremner, 1960; Walkley, 1947).

Determination was also made of the population of indigenous soybean rhizobia found in these sails. The results of the soil analysis are presented in Appendix 1. Fashola soil had no soybean rhizobia and low nitrogen content and hence the soil was suitable for evaluation of infectivity and effectiveness of introduced mutant strain.

Based on chemical characteristics of the soils the fertility level was low, in both field and pot experiments, nutrients were added to the soils to raise soil fertility to levels considered ideal for rhizobial activity (Vincent, 197O). Phosphorus was supplied as single superphosphate at the rate of 100 kg P_2O_5/ha. The single superphosphate applied was assumed to supply about 12% sulphur. Molybdenum was applied in form of sodium molybdate at the rate of 1 kg Mo per hectare. Potassium was supplied as muriate of potash at 6O kg K_2O per hectare.

The same applications of K and Mo were done before every subsequent planting. Phosphorus was not applied in subsequent seasons because single superphosphate has a good residual property (Sanchez, 1976) and substantial quantities of P are available to subsequent crops. Application of the fertilizers was done before planting and the fertilizers worked into the soil.

3.3 SOYBEAN AND MAIZE CULTIVARS USED

Soybean (Glycine max L.; cultivar TGx-17-2Ge) and maize (Zea mays L.; variety Gusau-82) were used as test crops for both field and pot experiments. Both maize and soybean varieties are local cultivars developed at IITA.

TGx-17-2GE is a moderately promiscuous soybean cultivar as it is able to nodulate fairly well with rhizobia indigenous to IITA soils (IITA, 1982). The cultivar also has high germination percentage.

The maize variety Gusau-82 was preferred because it has almost equal maturity period and equal inter-row spacing requirements as the soybean cultivar. This made soil sampling and other agronomic operations easy.

3.4 THE SOYBEAN RHIZOBIA USED

Rhizobium bank of IITA microbiology laboratory had two strains of soybean rhizobia which exhibited differing resistance to antibiotics. One was resistant to streptomycin and another to spectomycin. Bradyrhizobium japonicum strain IRj 2114, resistant to streptomycin (aminoglycoside), was preferred to the spectomycin resistant mutant. This was because mutants which are resistant to streptomycin are more stable and frequently do not lose their symbiotic capacity (Somasegaran and Hoben, 1985).

3.4.1 Development of a Culture of Spontaneous mutant of Irj 2114

To recognize the inoculum strain after it had been introduced into soil, a mutant of IRj 2114 was developed for spontaneous resistance level of 1000 micro(µ)g/ml streptomycin sulphate as described by Hagedorn (1979). Samples of IRj 2114 strain from selected slant were aseptically cultured in a flask containing 50 ml of yeast mannitol broth (YMB).

The broth was prepared by dissolving 10.0g of mannitol; 0.5g potassium hypophosphate (K_2HPO); 0.2g crystalline magnesium sulphate

($MgSO \cdot 7H_2O$); 0.1g sodium chloride (Nacl) and 1g yeast extract in 1 litre of distilled water. The solution was then adjusted to pH 6.8 and autoclaved at 121^0C and 718.50 pascals pressure for 30 minutes, before cooling. The broth culture of the rhizobium was grown on a rotary shaker (100 revolutions/min) under normal laboratory conditions for 7 days (Vincent, 1970). Samples of the IRj 2114 strain from this culture were then aseptically transferred onto yeast mannitol agar (YMA) plates.

Yeast mannitol agar (YMA) was prepared by adding 3g of potato dextrose agar to 200 ml of yeast mannitol broth (YMB) in a 500 ml Erlenmeyer flask and autoclaving as above. Ten millilitres of a solution of 400 mg of streptomycin sulphate dissolved in 20 ml of distilled water filtered through a sterile millipore filter of 0.2 µm pore size was then added to the YMA maintained at 60^0C. The mixture was shaken carefully to avoid the formation of air bubbles, after which the flasks were returned to the water bath maintained at 60^0C for 10 minutes to re-equilibrate and allow the air bubbles to dissipate from the agar. Approximately 200 ml of this mixture were then poured onto a sterile petri dish to form an agar plate of 1000 µg streptomycin/ml agar.

Inoculum of the IRj 2114 streptomycin resistant strain, cultured in YMB, was aseptically spread on the YMA plates using a sterile loop. The plates were then incubated at 28°C for seven days in an inverted position, there after the plates were examined for rhizobial growth.

Yeast mannitol broth containing streptomycin (YMB-Str.) at a concentration of 1000 µg/ml was prepared as for YMA of the plates above except that no agar was added. Two hundred ml of the YMB str. was put into each flask.

Distinct colonies from the plates with rhizobial growth were selected and, using a sterile loop, part of the colony was aseptically transferred into the flasks. The inoculated YMB-Str, was then placed on a rotary shaker for 7 days. The resulting culture contained IRj 2114 cells with spontaneous resistance to 1000 µg streptomycin/ml broth. Authentication of the culture was done using procedures described by Vincent (1970), before use as inoculum.

CHAPTER 4

GLASSHOUSE EVALUATION OF MUTANT IRj 2114 *RHIZOBIUM* FOR NODULATION AND NITROGEN FIXATION

4.1 INTRODUCTION

During the process of marking rhizobial strains with antibiotics, such as streptomycin, they may lose their ability to establish viable symbiotic relationships with the host plants (Josey *et al.*, 1979). On this basis, an experiment was carried out to ascertain the ability of the IRj 2114 spontaneous mutant rhizobium to nodulate and fix nitrogen.

4.2 MATERIALS AND METHODS

The activity of the IRj 21I4 mutant was assessed in Fashola soil which had no soybean rhizobia, and was low in nitrogen and in IITA soil, the main experimental soil that had native soybean rhizobia. The soils were collected and sieved through a 2 mm sieve before pot filling. Eighteen medium sized plastic pots (top diameter 20 cm, depth 18 cm) cure each filled with 3 kg of the soils. Nine pots were allocated to each soil type.

Based on chemical analyses of the soils (Appendix 1), nutrients were added to boost their fertility levels. Phosphorus, potassium and molybdenum were applied as described in Chapter 3, Section 3.2. As part of the treatments, nitrogen fertilizer was applied to soils in three pots, for each soil type at the rate of 80 g of urea per pot. This was equivalent to 120 kg N/ha.

Good soybean seeds were selected and surface sterilized by immersing in 0.2% HgCl for 3 minutes followed by rinsing with 95%

ethanol. The seeds were then washed in 8 changes of sterilized distilled water before inoculation with rhizobia (Vincent, 1970). Inoculation was done by applying a heavy suspension of IRj 2114 mutant, rhizobium, cultured in yeast mannitol broth as described in Chapter 3, Sub-Section 3.4.1 on the sterile seeds. To improve the survival of the rhizobia pre-sterilized peat was added to the Bradyrhizobium broth suspension at the rate of 25 g of peat to 100 ml of broth suspension (Vincent, 1970). A boiled solution of 100 g gum arabic in 230 ml of sterile water was also added to seeds as an adhesive at a rate of 4 ml for about 100 seeds. The seeds were then mixed thoroughly for 5 minutes. This process gave an inoculation rate of approximately 10^7 rhizobia per seed.

The inoculated and uninoculated soybean seeds were planted in pots at the rate of 6 seeds per pot. The treatment combinations were as below:

(i) Inoculation with IRj 2114 mutant Rhizobium, resistant to 1000 µg streptomycin/ml of broth.

(ii) No inoculation but inorganic nitrogen was applied at the rate of 120 kg N/ha.

(iii) Control- with no inoculation and no nitrogen application. Each combination was replicated three times.

Fourteen days after germination, when healthy plants could be selected, seedlings were thinned to 3 plants per pot. This was the maximum number of plants that could be maintained in each pot on 3 kg of soil up to the time of sampling, seven weeks after planting. Immediately after thinning, supplemental nutrients required for rhizobial and plant growth (Appendix 2) were added in solution form

by uniformly spraying and working it into the soil. All the pots were daily watered using tap water throughout plant growth period,

4.2.1 Evaluation of nodulation, dry matter production and nitrogen contents of inoculated and uninoculated soybean plants

To determine nodulation, plant dry matter production and nitrogen accumulation in plant shoots, the soybean plants were harvested 49 days after planting. Plants from each pot were carefully uprooted using a hand trowel, and plant tops excised at crown level, placed in paper bags, oven dried at 70^0C to constant weight, and the above soil surface dry matter production obtained. The shoots were then ground to fine texture and total nitrogen content of the shoots determined using the micro-Kjeldahl method. Nodulation was assessed by examining the roots of individual plants, Nodules were picked from the roots, and those that dropped off into the soil were also collected. The nodules were washed and counted and the total number of nodules collected from each pot recorded. The nodules were then oven dried to constant weight as above, and their oven dry weights determined.

Data collected were subjected to analysis of variance (ANOVA) and means compared using the least significant difference (LSD) test, at the level of $P \leq 0.01$ (Steel and Torrie, 1960). When analyzing for nodule per plant and dry weight of individual nodules, data for plants that received nitrogen were excluded because of the depressive effects that nitrogen application has on soybean nodulation (Diatloff 1967; McNeil 1982; Herridge et al., 1984).

4.3 RESULTS

4.3.1 Nodulation of inoculated and uninoculated soybean

Data on the nodulation of soybeans under the different, treatment conditions are presented in Table 5. Analysis of variance of tire number of nodules on soybean plants showed that nodulation was significantly ($P \leq 0.01$) influenced by soil type and seed inoculation with the mutant rhizobia (Table 4a). There were also significant ($P \leq 0.01$) interactions between the two factors.

A high number of nodules were formed by soybean inoculated with mutant. IRj 2114 in both Fashola and IITA soils. The number of nodules on plants grown in Fashola soil were substantially higher than that on plants grown in IITA soils. There were on average 82.0 and 74.0 nodules per plants for the two soils, respectively. For IITA soil, nodulation of plants inoculated with mutant rhizobia were 50% higher than uninoculated plants (Table 4b).

Mean dry weights of individual nodules were significantly ($P \leq 0.01$) influenced by the simple and interaction effects of soil type and soil treatment (Table 5a). Nodules formed by the IRj 2114 mutant were smaller than those formed by the indigenous soybean rhizobia. Thus the average weight of nodules obtained from plants inoculated with rhizobia and grown in Fashola soil was 6.9 mg while that for uninoculated plants grown in IITA, soil was 10.4 mg (Table 5b). Observations on soybean nodulation showed that IRj 2114 mutant rhizobia formed numerous nodules with soybeans. The nodules formed were, however, smaller than those formed by the native rhizobia strains in IITA soil.

Table 4: Analysis of variance (a) and means (b) on nodules from test plants grown under 2 growth conditions, 7 weeks after planting

(a) Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Soil types (A)	1	1323.000	1323.000	13.40**
Soil treatments (B)	1	8533.330	8533.330	86.70**
A x B	1	2465.330	2465.330	25.10**
Error	8	877.330	98.420	
Total	11	13108.990		

**Significant ($P \leq 0.01$); C.V. = 19.30%

(b) Mean number of nodules per plant

Treatments	Soil types	
	IITA	Fashola
Inoculation with IRj 2114 mutant	74.00	82.00
No inoculation	49.00	0.00

LSD (1%) = 23 nodules.

4.3.2 Shoot dry matter yields and nitrogen content in soybean shoots

Analyses of dry weight of soybean shoots and their nitrogen contents are presented in Tables 5 and 7 respectively. Both dry matter yields and nitrogen content of plants were highest when soybeans received fertilizer nitrogen. However, inoculation of soybean with IRJ 2114 mutant rhizobia also effectively increased shoot dry weight and nitrogen content of the crop. Dry matter yields were significantly ($P \leq 0.01$) influenced by soil type, seed inoculation and by their interaction effects (Table 6a),

In IITA soil, plants that received fertilizer nitrogen had significantly higher dry matter yields than those inoculated with IRj 2114 mutant. This was not the case in Fashola soil, where dry matter yield of inoculated plants was comparable to that of N-fertilized plants (Table 6b). Data presented, further showed that in both IITA and Fashola soils, inoculated plants produced more dry matter (6.3 g/plant IITA soil; 8.0 g/plant Fashola), than the uninoculated plants (4.6 g/plant IITA soil; 3.9 g/plant Fashola soil).

Nitrogen contents in shoots followed the pattern of city matter yields. However, soil type did not significantly influence the N-content of plants (Table 7a). Highest nitrogen content of shoots was recorded for plants inoculated with mutant IRj 2114 and grown in Fashola soil (228 mgN/plant) although the amount was not significantly different from those of nitrogen fed plants (Table 7b).

Results of plant growth and nitrogen accumulation in shoots showed therefore that IRj 2114 mutant rhizobium had a high level of activity.

Table 5: Analysis of variance (a) and means (b) of dry weight of individual nodule (mg) from test plants grown under 2 growth conditions, 7 weeks after planting

(a) Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Soil types (A)	1	117.190	117.190	340.50**
Soil treatments (B)	1	23.240	23.240	67.50**
A x B	1	50.840	50.840	147.70**
Error	8	2.750	0.340	
Total	11	194.020		

**Significant ($P \leq 0.01$); C.V = 8.90%

(b) Mean dry weight of individual nodules (mg)

Treatments	Soil types	
	IITA	Fashola
Inoculation	9.00	6.90
No inoculation	10.40	0.00

LSD (1%) = 1.60 mg

Table 6: **Analysis of variance (a) and means (b) of dry shoot weight (g) from test plants grown under 3 growth conditions, 7 weeks after planting**

(a) Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Soil types (A)	1	2.233	2.233	7.50**
Soil treatments (B)	2	58.410	29.205	97.90**
A x B	2	4.604	2.302	7.70**
Error	12	3.581	0.298	
Total	17	68.829		

**Significant ($P \leq 0.01$)

C.V = 8.20%

(B) Mean dry shoot weight (g) per plant

Treatments	Soil types	
	IITA	Fashola
Application of 120 kgN/ha	8.0	9.0
Inoculation with IRj 2114 mutant	6.3	8.0
No inoculation	4.6	3.9

LSD (1%) = 0.96 g

Table 7: Analysis of variance (a) and means (b) of nitrogen harvest (mg/plant) from plants grown under 3 growth conditions, 7 weeks after planting

(a) Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Soil types (A)	1	93.389	93.389	0.24
Soil treatments (B)	2	52629.778	26310.389	66.90**
A x B	2	6263.444	3131.772	8.00**
Total	12	4721.333	393.444	
Total	17	63698.944		

**Significant ($P \leq 0.01$)

C.V = 11.30%

(b) Means of nitrogen harvest (mg/plant)

Treatments	Soil types	
	IITA	Fashola
Application of 120 kgN/ha	203	220
Inoculation with IRj 2114 mutant	201	228
No inoculation	127	70

LSD (1%) = 49 mgN

4.4 DISCUSSION

Results obtained showed that mutant IRj 2114 rhizobium nodulated effectively with soybean and also contributed significantly to the nitrogen economy of the plant.

Nodulation of soybean in Fashola soil, that had no native soybean rhizobia, was higher than in IITA soil that had indigenous rhizobia (Table 4b). However, inoculation also led to a near doubling of the number of nodules on soybean grown in IITA soil. Inoculation of leguminous crops grown in soils free of specific rhizobia causes good nodulation provided favorable conditions prevail (Graham and Harris, 1982; Chowdhurry 1975). This perhaps explains the high nodulation obtained in Fashola soil. The good response of soybean to inoculation also obtained in IITA soil was attributable to the good nodulating qualities and high competitive ability of the mutant IRj 2114 strain used.

Results obtained also showed that the many nodules formed by IRj 2114 mutant rhizobium were small and of low weights (Table 5b). This observation was similar to that obtained by Rosendhal (1984). Singleton and Stockinger (1983) consider that for rhizobia strains that form small nodules, compensation occurs by increased nodulation. This ensures high active nodule mass and a high capacity of the rhizobia to fix nitrogen.

Observations on crop performance showed that inoculated plants developed vigorously, and their shoot dry matter yields were comparable to those that received fertilizer nitrogen (Table 6b) and showed a level of about 84% symbiotic effectiveness (Gibson, 1987). Shoot dry matter weights are, however, usually insensitive measures of the development of symbiosis (Brockwell et al., 1985) and instead symbiotic, effectiveness was used. Based on the levels of nitrogen in plant shoots, it was

evident that the symbiotic effectiveness was high when IRj 2114 mutant was used as an inoculum for soybean. For plants grown in Fashola soil, the amount of nitrogen in shoots attributable to biological nitrogen fixation was 158 mgN/plant. This was 69% of the total plant nitrogen (Table 7b) which is equivalent to at least 227 kgN/ha fixed per hectare, the minimum calculated by Neves et al. (1985) Patterson and Larue (1983).

Results of this study show clearly that IRj 2114 mutant rhizobium was very active in terms of nodulation and nitrogen fixation and was therefore an effective microsymbiont of soybean.

CHAPTER 5

EFFECT OF SOYBEAN-MAIZE CROPPING SEQUENCES ON THE INFECTIVENESS OF INTRODUCED B. JAPONICUM AND ON POPULATION OF SOYBEAN RHIZOBIA

5.1 INTRODUCTION

There is evidence that rhizobia grow in the soil in the absence of their host (Pena-Cabriales and Alexander, 1983; Tuzimura and Watanabe, 1962; Rovira, 1961). However, the establishment of introduced rhizobial population in soil has always been difficult; more so in soils under crop rotations when the rhizobia have to live saprophytically (Hiltbold et al., 1985).

In fields where legumes such as soybeans have been frequently grown, populations of bradyrhizobia are usually in excess of $1x10^4$/g soil (Weaver et al., 1972; Crozat et al., 1982; Mahler and Wollum, 1982). If such high rhizobial population were of the inoculum strain, and was maintained following a non-legume crop in a rotation, it would act as a source of inoculum for subsequent legume crops (Brockwell et al., 1984; Brockwell et al., 1985), and so eliminate the need for seed inoculation.

Little is known about the effects of soybean/cereal cropping rotation on the establishment and infectiveness of inoculum rhizobial strains. The absence of such information hinders the utilization of biological nitrogen fixation in crop production. In this study, therefore, the effects of soybean/maize cropping sequences on population of IRj 2114 inoculum strain of soybean rhizobia were assessed in the field and in the glasshouse.

5.2 MATERIALS AND METHODS

5.2.1 Effect of cropping sequences on soybean nodulation and on population of soybean rhizobia in the field

In this study, assessment was made of nodulation of soybean by the indigenous and introduced IRj 2114 mutant rhizobial strain, and of populations of soybean rhizobia in the soil, for four soybean/maize cropping sequences over three seasons.

5.2.1.1 Establishment of field experiments

The experiments commenced in June 1984. In the first season (June - September, 1984), population of mutant IRj 2114 was established in the field by planting soybean seeds inoculated with the mutant Rhizobium. Three plots, each measuring 27m by 9m, were used for planting.

Based on studies reported elsewhere (Abd-el Ghafter, 1976; Sayed, 1979), seven day old broth culture of the mutant rhizobia diluted ten times was used to inoculate soybean seeds before planting. 100 ml of the diluent were mixed with 15.0g of peat and 4.0ml of gum arabic solution (as an adhesive). The resulting slurry was then used to inoculate 100.0g of soybean seeds. Analysis, using the most probable number (MPN) method (Sub-Section 5.2.1.3.2) showed that the rate of inoculation was approximately 10^8 rhizobia per seed.

Inoculated soybean seeds were planted in the plots, on 13th June 1984, at a spacing of 75 by 10 cm. Two seeds were planted her hole and, 14 days after planting, seedlings were thinned to one plant per hill. This gave a population of about 3.24×10^5 soybean plants per hectare.

In the second season (October 1984 - January 1985), each of the three plots was divided into two sub-plots, each measuring 13m by 9m. After random assignment, one sub-plot was planted with soybean and the other with maize. Planting was done on 4th October, 1984. Uninoculated soybean (or maize) seeds were used for planting, and the crop established as described above. Maize was planted at a spacing of 75 cm by 25 cm, and a rate of two seeds per hole. It was later thinned to one plant per hill, 2 weeks after planting, thus giving a maize population of about 5.3 x 10^4 plants per hectare.

In the third season (February - May 1985), each sub-plot was again sub-divided into two equal portions measuring 6m by 9m. Maize and soybean were then assigned randomly to each portion, uninoculated seeds planted on 13th February 1985 and the crops established as described above.

The four different cropping sequences obtained from the three plantings were as follows:

(i) soybean - soybean - soybean (SSS),

(ii) soybean - soybean - maize (SSM),

(iii) soybean - maize – soybean (SMS),

(iv) soybean - maize – maize (SMM).

The development of the cropping sequences in the three is illustrated by Figure 1.

In all seasons, crops were kept free of weeds by regular weeding using hand hoes. The crops received uniform application of potassium (60kg K_2O/ha), phosphorus (100kg P_2O_5/ha) and molybdenum (1kg Mo/ha) fertilizers as described in Chapter 3, Section 3.2.

During dry spells, crops were irrigated using over-head sprinklers. Leaf pests on soybeans were controlled with nuvacron (dichlorovos), applied, only when necessary, using an electrodyn

sprayer that minimized insecticide drift (Singh, 1981). This, and the fact that nuvacron is rapidly decomposed by the plant, minimized any possible effects of pesticide residues on soil rhizobia.

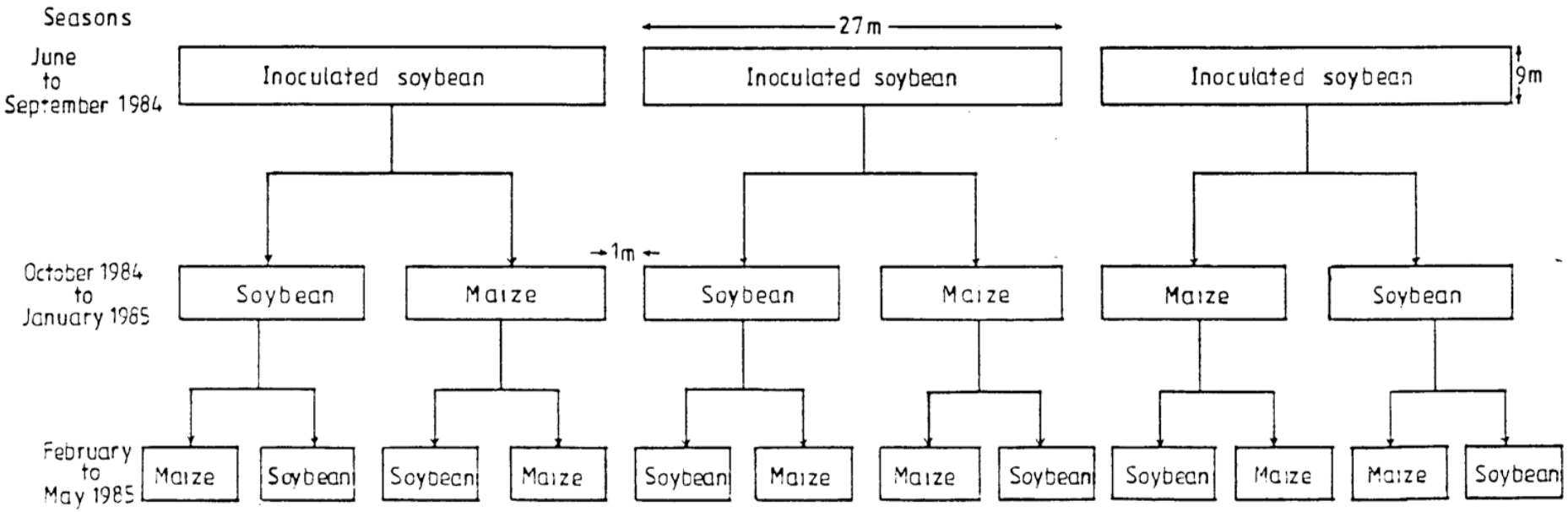
At the end of each season, mature crops were cut at ground-level using cutlasses. Crop trash was raked off the plots and soil samples then taken before preparing the land for the next planting.

5.2.1.2 Assessment of soybean nodulation

Each season, sampling was carried out 49 days after crop planting and assessment made of (i) total nodulation per soybean plant and (ii) percentage modulation due to the introduced IRj 2114 mutant rhizobia. Twenty soybean plants were randomly selected from middle rows of each plot, sub-plot or sub-subplot, and carefully uprooted using a hand shovel. For each plant, nodules on the roots and those that dropped off the roots at harvest were collected, washed and counted. The nodules were stored in a refrigerator for a maximum period of 14 days before completion of typing for antibiotic resistance to assess the level of soybean nodulation due to introduced mutant rhizobium.

Nodule typing was carried out using the procedure described by Obaton (1973). Thirty nodules were randomly selected for each cropping sequence and singly surface sterilized using mercuric chloride (Vincent, 1970). Each nodule was gently squeezed between the tips of ethanol-flamed forceps and immersed into 1 ml of sterile yeast mannitol broth (YMB). Inoculated YMB was thoroughly mixed, after which a sterile loop was, used to aseptically transfer samples from the YHB to plates containing either plain yeast mannitol agar (YMA), or YMA to which was added streptomycin at 1,000 µg/ml of YMA.

Fig. 1 Field layout showing the development of 4 soybean/maize cropping sequences during three seasons.



One column of grids of each plate (6 squares) was allocated to IRj 2114 mutant culture as a check. Thirty of the remaining grids were then typed with nodule samples from the inoculated YMB. The typing was replicated three times.

Plates were incubated at 28˚C for seven days and the rhizobial growth scored. Percentage nodulation caused by the IRj 2114 mutant Rhizobium was calculated using the formula:

$$X = y/m \times 100$$

where X = percent nodulation due to mutant rhizobium,

y = positive scores on plate with streptomycin,

and m = positive scores on plate with plain YMA.

5.2.1.3 Enumeration of soil rhizobia using the "Most Probable Number" (MPN) technique

When enumerating rhizobia in the soil or on seeds, the "most probable number" (MPN) technique (Tuzimura and Watanabe, 1961) as modified by Weaver and Fredrick (1982) was used. The technique is based on plant infection and determines the number of viable rhizobia in the presence of other organisms. Sterile plastic plant growth pouches, obtained from Scientific Products, Evanston, Illinois, (U.S.A.), were used. Rhizobial counts were made for soil samples collected. The test was conducted in a small screen-house.

5.2.1.3.1 Sampling of field soil

Assessment of populations of soybean rhizobia in the different experimental plots was carried out by sampling soil at the end of each cropping season. After clearing crop residues from the plots, soil samples, taken at a depth of 0-15 cm, were randomly obtained from two

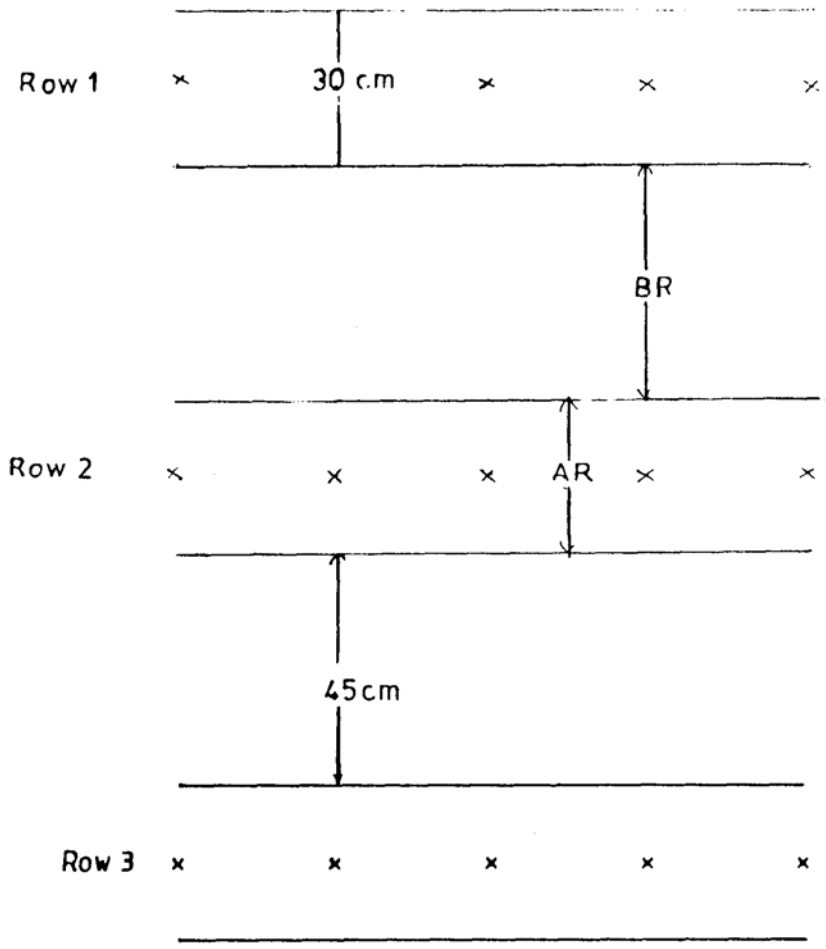
sampling position (i) along the crop rows (AR) and (ii) between the rows (BR) as shown in Figure 2.

For each sampling position, 20 samples were collected, using a soil auger of 6.0 cm diameter, and a composite sample made for each plot. Sub-samples taken from these composite samples were then used to enumerate soybean rhizobia using the MPN technique.

5.2.1.3.2 Preparation of growth pouches and establishment of test plants

Twenty pouches per soil sample were used for the test. Each pouch was divided into two equal compartments using a plastic heat sealer. Paper wick for each pouch was also cut into two and inserted into each pouch compartment. This procedure ensured that the few available pouches were enough for the test, and the pouches occupied limited space in the screenhouse. Pouches were packed in handling racks and 20 ml of nitrogen-free plant nutrient solution (Appendix 3) added to each compartment. The openings of pouches were wrapped with a sheet of aluminum foil and the pouches then sterilized by autoclaving at 120°C for 30 minutes. They were left to cool to room temperature before use.

Sorted soybean seeds used in the test were surface sterilized with mercuric chloride (Vincent, 1970) and pre-germinated by incubating in sterile moist cotton wool in petri-dishes at 28°C for 3-5 days. Seedlings with clean radicles and free of fungal growth were selected and planted in pouches under aseptic conditions. Two seedlings were established in each pouch compartment. The pouches were kept in the screen-house for one week after which well established seedlings were inoculated with rhizobial suspensions being tested.



AR = along crop row BR = between crop row

Fig. 2 Positions from which soil samples were taken for the enumeration of soybean rhizobia obtained in plots of differing soybean / maize cropping sequences.

5.2.1.3.3 Inoculation of plants and enumeration of rhizobia

Seedlings in each pouch compartment were inoculated using 1 ml of soil rhizobial suspensions. The suspensions were obtained by shaking 10.0g soil samples in 90 ml of sterilized tap water. The resulting suspension was of 10^{-1} dilution. Using 10 ml of this suspension, a 10-fold dilution of the suspension was made with the resultant dilution levels ranging from 10^{-1} to 10^{-10}.

Plants in four replicate pouch compartments were used per dilution. Apart from these pouches, negative and positive control pouches were also established. Negative control pouches contained plants not inoculated with soil or rhizobia suspensions; these indicated whether cross contamination occurred during growth. Positive control plants were inoculated with mutant rhizobium, and they helped to monitor the suitability of plant growth conditions for nodulation.

Pouches were supported in groups of 60 in wire racks, each rack contained test pouches and the positive and negative controls. The racks were placed in a screen-house maintained at 28˚C, with filtered air flow. About 1.7×10^4 lux of light intensity was provided from over-head fluorescent bulbs. Throughout the study period, 20 ml of sterile dilute nitrogen-free nutrient solution and distilled water were added in each pouch compartment on alternate days. Although positive controls showed nodulation within three weeks, test plants were kept for four weeks to ensure that adequate time was given for nodulation.

At the end of four weeks, roots of all plants were examined for nodulation. All replicate compartments with one or more nodules on the plants were scored as positive. For each dilution level, the total

number of positive replicate compartments were obtained. A grand total of the positive scores was obtained for each soil sample tested by adding total positive scores for the 10 dilution series. From the Most Probable Number table (Appendix 4), the most likely number of rhizobia corresponding to a particular number of positive scores was obtained for the least dilute number of the series. The estimated number of rhizobia occurring per gram of soil was calculated, in accordance with procedure given by Vincent (1970), using the formula:

$$X = \frac{(m \times d)}{(v \times g)} \times 100$$

Where X = number of rhizobia per gram of soil,

m = most likely number from the MPN table for the lowest dilution of the series,

d = lowest dilution,

v = volume of aliquot applied to plants,

and g = weight of soil sample.

The most probable numbers (MPN) of soybean rhizobia per gram of soils were transformed to $\log_{10}$ MPN or $\log_{10}$ (MPN + 1), to ensure detection of differences in populations of the rhizobia (Russek and Caldwell, 1983; Crozat et al., 1982). Data collected were then subjected to analysis of variance (ANOVA) and means for the first and second seasons compared using least significant difference (LSD). Single degree of freedom comparisons in the three orthogonal sets (SSS vs SSM, SMS vs SMM and SSM vs SMS and SMM) and their interactions with two sampling positions along (AR) and between (BR) the crop rows were made on means obtained in the third season.

5.2.2 Glasshouse evaluation of the establishment of soybean rhizobia in fallow soil and in soils planted with soybean or maize in the soils

The aim of this study was to assess, under controlled conditions, the survival, colonization and establishment of, both the introduced and indigenous soybean rhizobia in the presence of soybean host and non-host rotational maize and in fallow soil. The experiment was set up in a glasshouse during March 1985 using IITA soil.

Nine medium sized pots (top diameter 20 cm, depth 18 cm) were each filled with 3 kg of soil as described in Chapter 4 (Section 4.2). Soils in the pots were wetted with distilled water before inoculation with IRj 2114 mutant rhizobium.

Ninety millilitres of seven day old culture of mutant rhizobium were put to dilution of 10^{-1}. Soil in each pot was inoculated with the diluent at a rate of 100 ml per pot, poured on the surface of the soil. The inoculum was then thoroughly mixed into the soil using clean hand shovels. Enumeration of soybean rhizobia from soils based on composite samples showed that inoculation resulted in approximate populations of 10^{8} rhizobia per gram of soil.

After soil inoculation, three sets of pots were planted with maize, soybean or left fallow [without any plant growth, Jensen and Sorensen (1987)]. Five seeds of each crop were planted per pot and the seedlings thinned to three per pot, two weeks after planting. Because soybean nodules are known to start decaying and releasing rhizobia into the soil after 70 days from planting (Bushby, 1981), the soils were sampled 70 days after crop planting. A second crop of soybean and maize was then established in the same pots as described above, but without re-inoculated with the rhizobium. The double planting ensured

that meaningful data were obtained that covered the 120 days of crop development in the field.

The replicate pots were arranged on a bench in a randomized complete block design. They were separated from each other by 20 cm wide space. For both plantings, all pots were watered regularly using tap water. Composite nitrogen-free nutrient solution (Appendix 3) was also added at the rate of 20 ml per pot every week. However, phosphorus was not reapplied for the second crop since the single superphosphate provided residual phosphorus in adequate amounts.

5.2.2.1 Enumeration of soil rhizobia using the MPN technique

Sixty days after planting, maize and soybean shoots were trimmed off. To allow for the roots and nodules to decay, soils in the pots were regularly mixed and watered for a further ten days before soil sampling was done. After the interval, 10.0g composite samples of soils were obtained for each treatment combination and used in the enumeration of soybean rhizobia.

Most probable numbers (MPN) of soybean rhizobia per gram of soil were transformed using $\log_{10}$, and the data subjected to analysis of variance (ANOVA). The means compared using the least significant difference (LSD) test at 1% level.

5.3 RESULTS

5.3.1 Nodulation of soybean under different soybean-maize cropping sequences in field

Data on soybean nodulation under continuous soybean cropping (SSS) are presented in Table 8. Both the total number of nodules

per plant and percentage of nodules formed by mutant rhizobium varied significantly ($P \leq 0.05$) with the cropping season (Table 8a).

Nodulation increased with cropping seasons (Table 8). In the first season, the average number of nodules per plant was 29. This increased significantly in the second season to an average of 39 nodules per plant, an increase of 34%. Further increase occurred in the third season, however, it was only 15% over the level obtained in the second season and the increase was not significant.

Nodule occupancy by IRj 2114 mutant rhizobium also increased with the cropping seasons (Table 8b). Fifteen percent of nodules of the first season crop were due to the mutant rhizobium. During the second and third seasons, nodule occupancy by the introduced rhizobium increased approximately 2.5 and 4.0 times respectively over the level obtained during the first season.

When soybean was grown in rotation with maize (SMS), the maize grown during second season adversely affected nodulation of subsequent soybean crop (Table 9). Analyses of variance of data collected showed that types of cropping sequences significantly ($P \leq 0.01$) influenced nodulation of soybean in the third season (Table 9a).

Mean number of nodules per plant ranged from 45 for continuous soybean (SSS) to 26 for the soybean-maize rotation (SMS) (Table 9b). Although the percentages of nodules due to the mutant rhizobium obtained under the SSS and SMS cropping sequences were not significantly different (Table 9a), lower recovery of nodules of the introduced rhizobium was obtained under soybean-maize rotation (SMS) (42%) as compared to continuous soybean cropping (SSS) (60%). Generally, soybean-maize rotation (SMS) did not favor infectiveness of soybean rhizobia.

Table 8: **Summary of analyses of variance (a) and means (b) of total number of nodules on soybean plants and percentage of nodules formed by IRj 2114 mutant rhizobium in continuous soybean cropping for three seasons**

(a) Summary of analyses of variance

Source of variation	Degrees of freedom	Mean square	
		Number of nodules/ plant	% nodules due to IRj 2114
Replication	2	0.100 n.s	144.445 n.s
Seasons	2	0.032*	1519.445*
Error	4	0.003	19.445
C.V. (%)		4.4	72.0

n.s = not significant

*Significant at $P \leq 0.05$.

(b) Means

Season	Mean number nodules/plant	% nodules due to IRj 2114
1	29	15
2	39	38
3	45	60
LSD (5%)	7	10

Table 9: Summary of analyses of variance (a) and means (b) of total number of nodules on soybean plant and percentage of nodules formed by IRj 2114 mutant rhizobium under continuous soybean cropping (SSS) and soybean in rotation with maize (SMS) assessed during third season

(a) Summary of analyses of variance

Source of variation	Degrees of freedom	Mean squares	
		Total number of nodules/ plant	% nodules due to IRj 2114
Replication	2	22.333 n.s	29.166 n.s
Types of cropping sequence	1	522.666**	204.116 n.s
Error	2	2.083	229.000
C.V (%)		24.20	35.00

(b) Means

Types of cropping sequence	Mean number of nodules/ plant	% nodules due to plantIRj 2114
SSS	45	60
SMS	26	42
LSD (%)	8	n.s

n.s = not significant

** = Significant ($P \leq 0.01$)

5.3.2 Population of soybean rhizobia under different soybean-maize cropping sequences in field

Observations on the effects of four soybean-maize cropping sequences (SSS, SSM, SMS and SMM) on field populations of soybean rhizobia at two sampling positions are presented in Tables 10, 11 and 12.

Throughout the study, cropping sequence significantly ($P \leq 0.05$ and 0.01) influenced soybean rhizobial counts. Populations of rhizobia also varied significantly ($P \leq 0.05$ and 0.01) along crop rows (AR) and between crop rows (BR) (Tables 10a, 11a and 12a). There were substantially larger numbers of rhizobia in the regions of crop growth, along the rows, as compared to the inter-row space.

Cultivation of inoculated soybean in the first season resulted in overall increase in rhizobial population in the field, although population of rhizobia found between crop row (BR) were lower than the original population of indigenous rhizobia (Table 10a). The density of rhizobia found along crop rows (AR) averaged 9.3×10^3/g soil, while that obtained between crop rows (BR) was 3.8×10^2/g soil. These populations were approximately 1600% greater than, and 32% less than the original population of indigenous soybean rhizobia (Appendix 1), respectively.

In the second season, when the first season soybean crop was followed by either soybean or maize, significantly ($P \leq 0.01$) higher population of soybean rhizobia was obtained under continuous soybean cropping (SS) than for soybean-maize (SM) rotation at boar sampling positions (Table 11b). Under continuous soybean cropping (SS), rhizobia counts per gram of soil along the crop rows and in the inter-row spaces were 1.6×10^6 and 6.3×10^4 respectively. These were approximately 1000 and 63 times greater than those obtained

Table 10: Analysis of variance (a) and means (b) of soybean rhizobial population per gram of field soil sampled from along (AR) and between (BR) crop rows after the first soybean crop

(a) Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Replication	2	0.344	0.172	1.30n.s
Sampling position	1	2.885	2.886	22.50*
Error	2	0.256	0.128	
Total	5	3.484		

n.s = not significant; *significant ($P \leq 0.05$)

C.V. = 19.80%

(b) Mean soybean rhizobial counts

Replication	Sampling position			
	AR	BR	Total	Mean
1	4.59	2.63	7.22	4.07×10^{2} (3.61)*
2	3.76	2.56	6.32	1.45×10^{3} (3.61)
3	3.56	2.56	6.12	1.15×10^{3} (3.06)
	9.30×10^{3}(3.97)			3.80×10^{2}(2.58)

LSD (5%) between position means = $\text{Log}_{10}0.25$.

( )* Values in parentheses are transformed mean counts of soybean rhizobia per gram of soil.

Table 11: Analysis of variance (a) and means (b) of soybean rhizobial population per gram soil from two soybean/maize cropping sequences sampled along (AR) and between (BR) crop rows

(a) Analysis of variance

Source of variation	Degree of freedom	Sum of square	Mean square	F-value
Replication	2	0.028	0.014	1.00n.s
Type of cropping sequence (A)	1	2.176	2.176	157.60**
Sampling position (B)	1	17.017	17.017	1232.40**
A X B	1	1.147	1.147	83.10**
Error	6	0.083	0.014	
Total	11	20.450		

n.s. = not significant; **significant ($P \leq 0.01$);

C.V = 2.7%

(b) Means of soybean rhizobial counts

Type of cropping	Sampling position AR	BR
Soybean/soybean (SS)	$1.6x10^6$(6.20)	$6.3x10^4$(4.80)*
Soybean/maize (SM)	$1.6x10^3$(3.20)	$1.0x10^3$(3.00)

LSD (1%) between (sequence and position) means = $Log_{10}0.40$

( )* = Values in parentheses are transformed mean counts of soybean rhizobia per gram of soil.

Table 12: Analysis of variance with single degree of freedom comparisons (a) and means (b) of soybean rhizobial population under three orthogonal sets of soybean/maize cropping sequences and their interactions with two sampling positions assessed after third season

(a) Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Replication	2	0.130	0.06	1.48n.s
Type of cropping sequence (A)	3	6.630	2.213	50.20**
SSS vs SSM (B)	1	0.330	0.330	0.75n.s
SMS vs SMM (C)	1	0.190	0.190	4.30n.s
SSS and SSM vs SMS and SMM (D)	1	6.407	6.407	145.60**
Sampling position (E)	1	33.370	33.370	758.40**
AxE	3	4.446	1.482	33.62**
BxE	1	0.108	0.108	2.45n.s
CxE	1	0.188	0.188	4.27n.s
DxE	1	4.150	4.150	94.30**
Error	14	0.617	0.044	
Total	23	45.193		

n.s = not significant; **significant ($P \leq 0.01$);
C.V. = 4.90%

(b) Means of soybean rhizobial counts/g soil.

Cropping sequence	Sampling position	
	Along crop rows	Between crop rows
SSS	3.20×10^{5} (6.50)	1.60×10^{3} (3.20)*
SSM	1.60×10^{6} (6.20)	1.30×10^{3} (3.10)
SMS	3.20×10^{4} (4.50)	1.60×10^{3} (3.20)
SMM	2.80×10^{4} (4.45)	5.00×10^{2} (2.70)

LSD (1%) between (sequence and position) means = Log_{10} 0.33.
( )* = Values in parentheses are transformed mean of soybean rhizobia per gram of soil..

after the maize crop (SM). From the data, it was also evident that maize depressed soybean rhizobial population. For instance, the density of rhizobia obtained along the crop rows (AR) after maize crop (SM) was 1.6 x 10^3 rhizobia/g soil which is 5.8 times lower than the level (9.3 x 10^3 rhizobia/g soil) obtained after the first season soybean crop.

Data on soybean rhizobial count in the third season obtained after the SSS, SSM, SMS and SMM cropping sequences are presented in Table 12. Population of rhizobia varied significantly ($P \leq 0.01$) with cropping sequence and sampling position, and was also significantly influenced by the type of crop planted in the second season (Table 12a).

Soybean rhizobial counts along crop rows (AR) of the third season crops (Table 12b and Figure 3) showed that significantly ($P \leq 0.01$) higher populations of rhizobia occurred in soils when both soybean and maize followed two consecutive soybean crops (SS) than when maize was grown as the second crop. There was no significant difference in soybean rhizobial counts between rows (BR) under SSS, SSM and SMS cropping sequences, but significantly ($P \leq 0.05$) soybean rhizobial populations were obtained under SMM (Figure 3).

5.3.3 Population of soybean rhizobia in fallow soil, soybean and maize cropping in the glasshouse

The establishment and survival of soybean rhizobia in potted IITA soil left under fallow or cropped to soybean or maize are summarized in Table 13 and Figure 4. Rhizobial populations varied significantly ($P \leq 0.01$) amongst the different treatment pots (Table 13a). There was also substantial variation in the patterns of rhizobial populations with time as indicated by the significant ($P \leq$

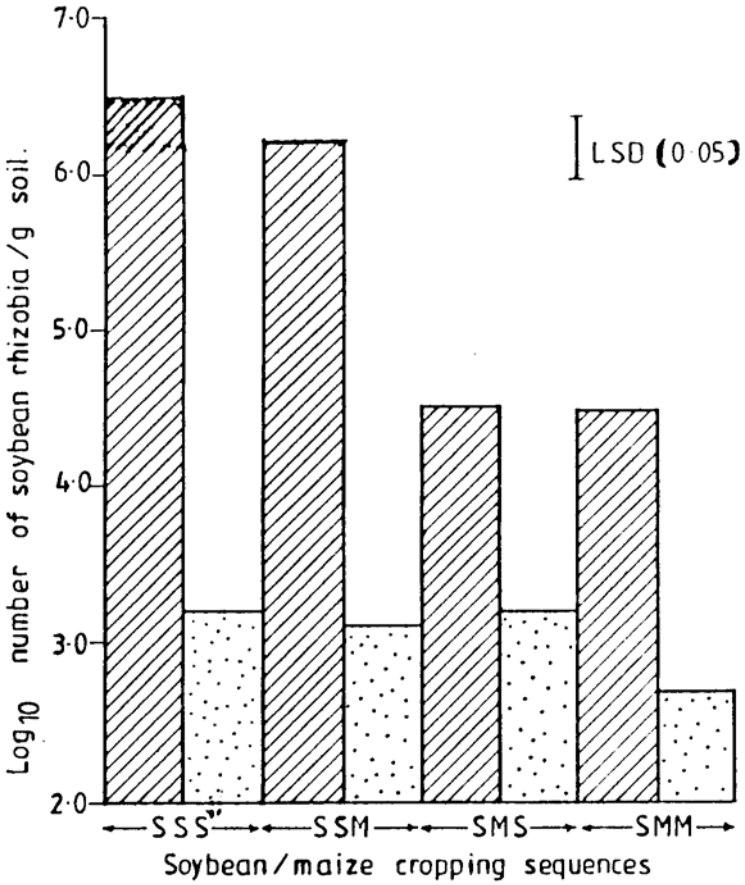


Fig. 3 Effects of four soybean/maize cropping sequences on $\log_{10}$ numbers of soybean rhizobia per gram of soil sampled from along (▨) and between (⁘) Crop rows

0.05) interaction between cropping treatment and time of sampling (Table 13a).

During the first 70 days of planting, the rhizobial numbers per gram of soil declined from 1.00×10^8, an initial level obtained after inoculation to 1.58×10^2, 6.31×10^2 and 2.51×10^2 for fallow soil, soybean and maize cropping respectively. These values were not significantly different (Table 13b). In the subsequent 70 day period of planting, population of soybean rhizobia in soil cropped with maize declined further while those in fallow soil and in soil cropped with soybean increased (Figure 4). At 140 days after soil inoculation, rhizobial counts per gram of soil in pots planted with maize (50) was significantly ($P \leq 0.05$) lower than those obtained for soybean (2512) or fallow (630) pots (Table 13b).

The results showed that maize suppressed rhizobial multiplication, and this agrees with observations obtained in the fiel.

5.4 DISCUSSION

Observations made in this study showed that nodulation of soybean and population of soybean rhizobia in soil were greatly influenced by soybean-maize cropping sequences. There was a low nodule recovery for the introduced soybean rhizobium at the end of the first season. In the two subsequent seasons, continuous soybean (SSS) encouraged high infectivity of mutant IRj 2114 rhizobium, and the total rhizobia population in the soil. Under rotation with maize (SMS), however, total soybean nodulation, percentage recovery of nodules due to the inoculant rhizobium, and rhizobial population

Table 13: Analysis of variance (a), and means (b) of soybean rhizobia counts in potted soil, under different croppings and sampled at 2 different times after soil inoculation.

(a) Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Type of cropping (A)	2	4.750	2.380	10.62**
Time of Sampling (B)	1	0.010	0.010	0.03n.s
AxB	2	1.716	0.858	3.90*
Error	12	2.690	0.220	
Total	17	9.166		

**Significant ($P \leq 0.01$); *Significant ($P \leq 0.05$);

n.s = not significant; C.V = 18.20%

(b) Mean soybean rhizobial counts/g soil.

Type of cropping	Sampling time (days)	
	70	140
Fallow soil	158 (2.20)	630 (2.80)
Soybean	631 (2.80)	2512 (3.40)
Maize	251 (2.40)	50 (1.70)

LSD (5%) between (cropping and time) means = $Log_{10}0.84$.

( )* Values in parentheses are transformed mean counts of soybean rhizobia per gram of soil.

in the soil were all adversely affected.

Increased total nodulation of soybean plants, and nodulation due to the introduced IRj 2114 mutant rhizobium obtained and continuous soybean cropping (Table 8) reflected the important role that host plants play in the establishment and multiplication of rhizobia in the field. Through nodule disintegration (Bushby 1981; Brockwell et al., 1988) and multiplication in the rhizosphere (Rovira, 1961), population of the introduced IRj 2114 mutant increased as indicated by higher nodule percent recoveries obtained in the second (38%) and third (60%) seasons. Results obtained in the present study are in conformity with observations made by Kolling et al., (cited by Freire, 1976) and Dunigan et al. (1984).

The results also showed adversely affected nodulation of soybean plants. Total nodulation of the plants and recovery of nodules formed by the mutant rhizobium were substantially lowered (by 42% and 18% respectively) for the third season soybean crop that followed maize (Table 9). This adverse effect of maize crop was also reflected by the significantly ($P \leq 0.01$) lower soybean rhizobial populations obtained when maize was grown as a second crop (Table 12). These field observations on the populations were supported by data obtained from pot. experiment (Table 13 and Figure 4).

It is known that in the absence of appropriate host crops, soybean rhizobial populations in the soil decline. Hiltbold et al. (1985), for instance observed a decline of soybean rhizobial population when cotton followed soybean in rotation. Nutman and Hearne (1979) also observed declining populations of rhizobia under continuous cereal crops. Poor survival and multiplication of rhizobia under the influence of non-host rhizosphere was probably responsible for this decline in rhizobial populations.

Data collected also showed that populations of soybean rhizobia were consistently higher along crop rows (AR) than in the spaces between the rows (BR) (Tables 10, 11, 12). Johnstone (1964) and Moerman (1965) also found, after soybean harvest, that while there were virtually no soybean rhizobia between the crop rows, soil near the crowns of the old plants (about 15 cm radius) was still heavily infected.

It is reported that rhizospheres of both host and non-host plants significantly influence the growth of soil rhizobial populations (Rovira, 1961; Robinson, 1967; Pena-Cabriales and Alexander, 1983; Jensen and Sorensen, 1987). Diatloff (1969), for example, found a high stimulation of soybean rhizobia by both oats and soybean. Chowdhurry et al. (1968) also demonstrated the stimulating effects of rhizosphere in the sterile soil and showed that serradella rhizosphere markedly stimulated Rhizobium lupini.

It is generally recognized that rhizobia grow and multiply in rhizospheres in response to available nutrient (carbohydrates, amino acids and vitamins) contained in root exudates (Date and Brockwell, 1978). The high density of roots found along crop rows (AR) and rhizobia release into soil by decaying nodules were therefore responsible for the high populations of rhizobia found in this region as compared to the inter-row spaces.

It was evident from the study that successful establishment and build-up of populations of rhizobia was dependent on the presence of host plant. It was also evident that in soybean-maize rotation, successful establishment and rapid multiplication of an introduced soybean rhizobium depends significantly on whether soybean or maize follows the first inoculated soybean crop.

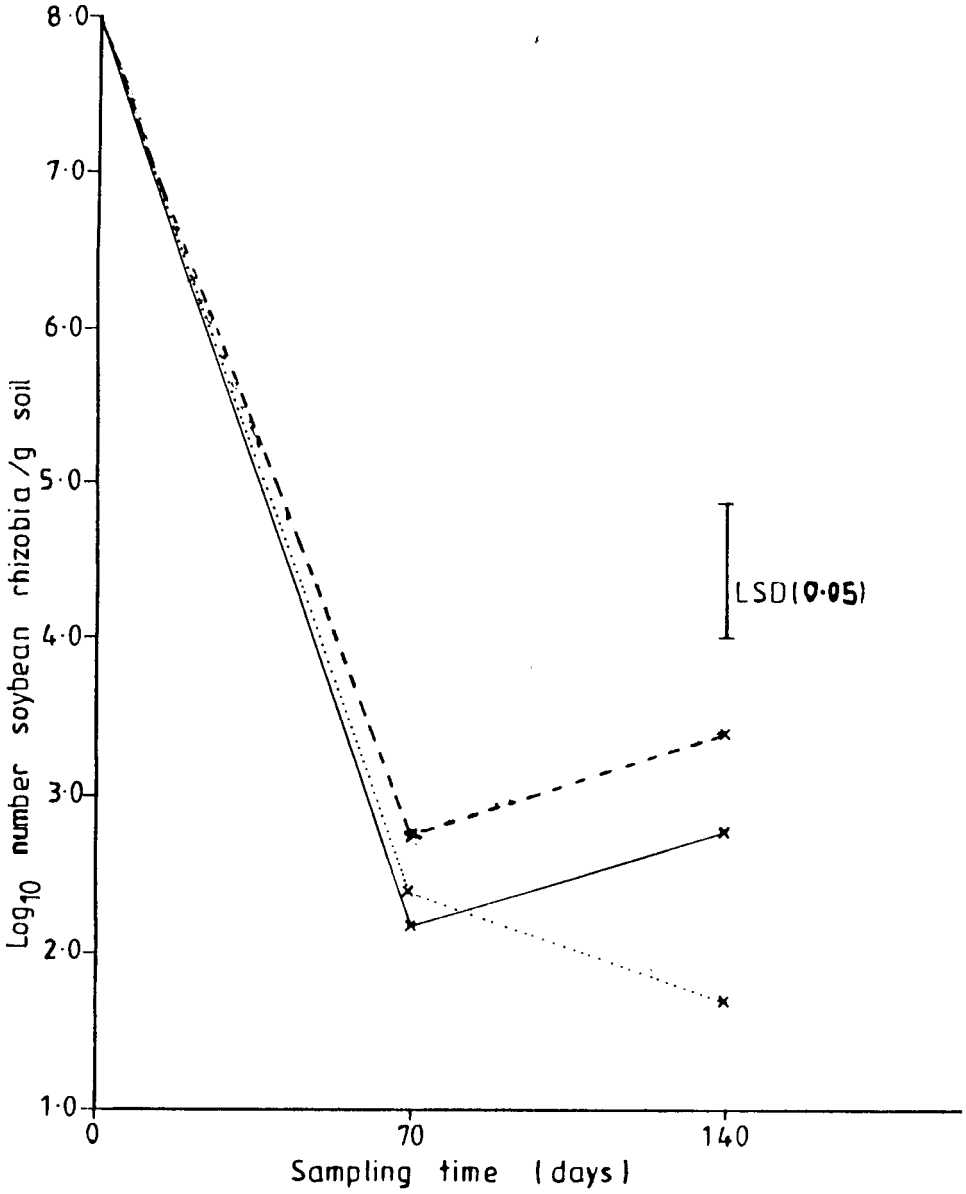


Fig. 4 Effect of soybean (– – – –) , maize (·····) or fallow (——) Cropping on soybean rhizobia population in potted IITA soil sampled at 0, 70 and 140 days after soil inoculation.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The work reported in this thesis investigated the possibility of obtaining long term benefits from introduced highly effective strains of soybean rhizobia (Bradyrhizobium japonicum) in the existing cropping systems, particularly rotations.

Soybean and other legumes are normally grown in rotation with cereals and other crops to reduce the build-up of diseases, pests and weeds, and also to exploit the contributions of the legumes, through nitrogen fixation, to the soil nitrogen economy. In such a cropping system, an introduced B. japonicum strain has to compete with the existing microflora for available substrate for growth. It has to survive in the absence of specific host and later compete for nodule sites whenever the specific host is planted (Freire, 1976; Elkan, 1987). There is a need therefore to establish a better understanding of the persistence of both introduced and indigenous soybean rhizobial populations in soils under different cropping sequences.

Marked positive responses of soybean to inoculation with IRj 2114 mutant rhizobium obtained in this study can be attributed to the high competitiveness of the strain and advantages provided by the inoculation process. By seed inoculation, high numbers of selected rhizobia are concentrated in the proximity of emerging plant roots thus creating high chances of infection for the strain. For an introduced strain, successful establishment, multiplication and effectiveness will depend however, on its ability to adopt to its new environment (Parker et al., 1977; Vincent, 1977; Dunigan et al., 1984).

During the present study, soybean nodulation in the first season (after inoculation) due to the mutant rhizobium was very low (15%) and only buildup after repeated soybean cropping to an average of 51% in third season (60% for SSS and 42% for SMS). Low initial percentage recovery of nodules formed by introduced rhizobial strains have also been reported by earlier workers (Johnson et al., 1965; Caldwell and Vest, 1970; Cardwell and Johnson, 1971). In Kawanda, Uganda, for example, response to inoculation of soybean was obtained from subsequent soybean crops rather than from the inoculated crop (Anon., 1953). It seems, therefore, that an improved soybean rhizobial strain introduced through inoculation undergoes intense selection for survival and competitiveness during the first cropping season. Successful adaptation is followed by the build-up of populations of the introduced rhizobium in the soil.

Because of this apparent period of adaptation, following introduction, the type of crop that follows the inoculated legume crop is important for the successful establishment of introduced rhizobium. In the present study, for instance, there was low total nodulation of soybean plants and percentage of nodules formed by the introduced mutant rhizobium, in the third season, when maize followed the inoculated soybean crop. The maize crop grown in the second season did not stimulate multiplication of the introduced rhizobium.

Although non-legume rhizospheres are known to stimulate rhizobial growth, their effects are generally smaller than stimulation caused by the legume rhizospheres (Date and Brockwell, 1978, Pena-Cabriales and Alexander 1983). This low stimulation effects of non-legume crops means therefore that careful consideration should be given to the type of cropping sequences to

he followed when introducing new highly effective strains of soybean and other annual legume rhizobia. The present study showed for instance that soybean inoculated with introduced rhizobium should be followed by a second soybean crop to ensure adequate establishment and build-up of introduced rhizobium.

Finally, it is suggested that studies he carried out to establish the effects of soybean rotation with crops other than maize like some legumes on infectivity and effectiveness of introduced rhizobial strains. To gain a better understanding of the relationships between nodulation ability, competitiveness and saprophytic competence of introduced strains, the immunoflourescence technique could be used to study directly the autoecology of the rhizobia in soils and in natural rhizospheres (Bohlool and Schmidt, 1973; Reyes and Schmidt, 1979, 1981). This would be a significant improvement over the MPN and antibiotic techniques used in this study.

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Appendix 1: Rhizobial Populations, Physical and Chemical Characteristics of Experimental Soils before planning

Place of Collection	Sample Depth (cm)	RHIZOBIAL POPULATIONS		PHYSICAL CHARACTERISTICS				CHEMICAL CHARACTERISTICS											
		Soybean B. jap. (Cells /g Soil)	Cowpea	%Sand	%Silt	%Clay	Soil type	pH in H_2O (1:1)	% O.C	% T.N.	Ext.P (PPM)	Ca	Mg	Mn	K	Na	Al	Total Acidity	C.E.C
												M.e./100g Soil							
FASHOLA	0-15	0	8,500	94	4	2	Sand	5·40	0·610	0·060	2·700	1·42	0·009	0·006	0·007	0·150	0·220	0·300	2·090
IITA	0-15	560	12,900	83	10	7	Loamy Sand	5·00	1·230	0·140	12·100	2·840	0·810	0·001	0·19	0·008	0·07	0·100	4·030

B. jap Bradyrhizobium japonicum

Appendix 2: **Formulation of supplemental nutrients required for rhizobial and plant growth and amount of solution used per pot (Vincent, 1970).**

Nutrient supplied	Chemical used (and Concentration)		Amount of chemical
			Solution used per pot (ml)
Magnesium	$MgSO_4.7H_2O$	(10%)	6
Sodium	Nacl	(15%)	3
Manganese	$MnSO_4.6H_2O$	(0.1%)	3
Boron	H_3BO_3	(0.025%)	3
Calcium	$CaSO_4.5H_2O$	(0.005%)	3
Zinc	$ZnSO_4.5H_2O$	(0.005%)	3
Iron	$FeCL_3.6H_2O$	(10%)	3

Appendix 3: **Composition of Nitrogen-free nutrient solution (Anon. 1982).**

Element	Applied form	g/l
Ca	$CaCl_2.2H_2O$	294.100
P	KH_2PO_4	136.100
Fe	Fe-Citrate	6.700
Mg	$MgSO_4.7H_2O$	123.300
K	K_2SO_4	87.000
Mn	$MnSO_4.H_2O$	0.338
B	H_3BO_3	0.247
Zn	$ZnSO_4.5H_2O$	0.288
Cu	$CuSO_4.5H_2O$	0.100
Co	$CoSO_4.7H_2O$	0.056
Mo	$Na_2MoO_2.2H_2O$	0.048

Appendix 4: **Number (M) of rhizobia estimated by the plant infection (extracted from Vincent 1970).**

Ten-fold dilutions: (A = 10) with replicates per dilution.

Positive pouches	Dilution step (s)
n = 4	s = 10
40	7×10^{8}
39	386.9
37	3.4
36	1.8
35	1.0
34	5.9×10^{7}
33	3.1
32	1.7
31	1.0
30	5.8×10^{6}
29	3.1
28	1.7
27	1.0
26	5.8×10^{5}
25	3.1
24	1.7
23	1.0
22	5.8×10^{4}
21	3.1
20	1.7
19	1.0
18	5.8×10^{3}
17	3.1
16	1.7
15	1.0
14	4.8×10^{2}
13	3.1

Appendix 4: (Continued)

Positive pounches	Dilution step(s)
12	1.7
11	1.0
10	5.8×10^{1}
9	3.1
8	1.7
7	1.0
6	5.8 x 1
5	3.1
4	1.7
3	1.0
2	0.6
1	<0.6
0	
Approximate range	10^{9}

Factor, 95% fiducial limits (Conchran 1950) x, + by 3.8