

**INFLUENCE OF EDAPHIC FACTORS IN
THE ESTABLISHMENT OF COWPEA
RHIZOBIA AT BANGALORE**

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INFLUENCE OF EDAPHIC FACTORS IN THE ESTABLISHMENT OF COWPEA RHIZOBIA AT BANGALORE

by

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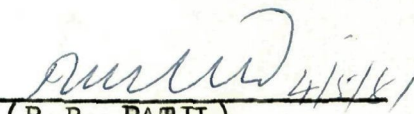
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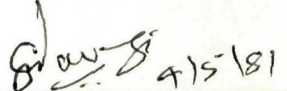
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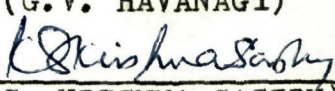

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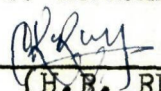
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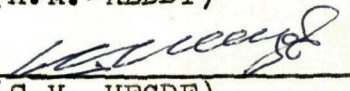
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INTRODUCTION

I. INTRODUCTION

Inoculation of grain and forage legumes with rhizobia has come to stay, and this practice is fairly widespread in most of the developed countries. In recent times the limited availability of chemical nitrogen fertilizers and their escalating prices have brought into sharp focus, the practical value of the legume-Rhizobium symbiosis as an economical and environmentally clean alternative. It provides a means of making available to the host plant, plentifully available atmospheric nitrogen, at negligible cost.

However, its usage and potential have made little impact in India. As the total area sown to grain legumes annually in India is nearly thirty million hectares, the potential for saving in fertilizer nitrogen costs, as well as in bridging the gap in their availability, is enormous. Estimates of the current usage reveal that less than one per cent of the area sown to legumes annually is inoculated with selected strains of Rhizobium (Sahney, 1976). This is due to various reasons, not the least of which, is the lack of studies to identify the causes for unsatisfactory performance of inoculants in many situations.

Hence, the need to determine the type of inoculation and cultivational practices suited to each of the different agro-climatic zones in the country is imperative.

In the last three decades, considerable amount of research effort has gone into identifying possible causes for the poor symbiotic performance of tropical legumes, especially in countries like Australia. This has added largely to our knowledge of the difficulties involved in establishing selected, highly efficient strains of Rhizobium in soils already colonized by large number of native, inefficient rhizobia. However, this work has mainly been confined to tropical forage legumes, while study of the ecology of rhizobia associated with grain legumes has received little attention.

Among the principal legume crops grown in the country, namely groundnut, pigeonpea, chickpea, green and black grams, cowpea,^{and} lentil, cowpea is, of late, becoming increasingly popular with the introduction of several high yielding and short duration varieties. Presently, it is sown in 1,19,400 hectares, annually in the state and 2.763 million hectares in the country (Anon. 1978). Traditionally it has been sown over vast tracts as an intercrop in finger millet, sorghum and maize, to provide vegetable when tender,

and grain at maturity, for human consumption and nutritious fodder for animals.

Its relationship with Rhizobium is promiscuous, as it tends to be infected by a majority of the strains of Rhizobium commonly present in soil. Hitherto, its symbiosis has been considered quite satisfactory. The apparent lack of response to inoculation with selected rhizobia being ascribed to the occurrence of suitable native rhizobia in soil, without identifying it with the strain used for inoculation. Adequate follow up studies to evaluate the performance and fate of initial seed coat inoculum, both during the growth of the crop and in soil, after the crop is harvested are needed. In the investigation envisaged herein, some attempts are made to obtain such information.

The benefit accruing from the association in soil between legumes and nodule bacteria introduced on their seed has been measured in terms of several parameters, such as nodulation, nitrogen fixation (as host nitrogen content), host dry matter production, grain and fodder yield. These criteria have proved adequate, provided appropriate controls are maintained. However, in each case the outcome of the association has hitherto been judged without identifying whether the strain infecting the host, is a native one or that introduced on the seeds. It is, therefore, essential to establish the

identity of strains that infect the host, with a view to assess the capability of selected strains of Rhizobium to infiltrate soil and establish there.

This is justified by the increase in symbiotic nitrogen fixation expected, when such strains are successful in competition with their native counterparts. The lack of such studies, especially in case of cowpea prompted the present investigation.

In the experiments envisaged herein, efforts to identify the strains nodulating the host legume—cowpea, over three seasons (over $1\frac{1}{2}$ year) were made. Valid comparison between these and the ones used for seed treatment, to assess the efficacy of seed inoculation as a means of transfusing the soil rhizobial complement as it were, to the best advantage of sown legumes, was contemplated.

The ubiquity of native rhizobia of the promiscuous cowpea group in the majority of Indian soils give prompt nodulation of newly sown legume hosts of the cowpea miscellany. This indigenous population has been considered adequate all along, not knowing its ability to fix nitrogen. Despite the broad nodulation response obtained, failure to fix sufficient quantities of nitrogen may arise due to low numbers of the desirable rhizobia entering the soil on seed. It may also be due to poor multiplication and establish-

ment of these rhizobia, as a result of adverse soil conditions such as low soil moisture content, unfavourable temperature, nutritionally unfavourable conditions (for the host), especially with regard to phosphorus (Franco, 1976), and keen competition from resident rhizobia. The absence of suitable host plants in the common rotations followed may also limit the multiplication and the perpetuation of the introduced strains of rhizobia in soil.

Although it has been known that these factors are often responsible for the diminished effectiveness of inoculants, their effects are not clearly defined.

Possible remedies and simple modifications in agronomic practices such as application of phosphatic fertilizer and seed pelleting need to be tried and tested. Also, sowing of the best adapted host variety and the use of matching strains of rhizobia under the conditions obtaining in a given region, are assuming greater importance with the release of a large number of varieties. These are prerequisites to improvement in yields, to meet the growing demand for pulses and protein rich fodder. In the present work, attempts were made to study these prerequisites for obtaining maximum nitrogen fixation under the conditions prevailing at Bangalore.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Survival on the seed

Rhizobia must survive the process of introduction on the seed coat in adequate numbers, to provide an initial population for further colonization. That they often do not, is apparent from the work of Cloonan and Vincent (1967). The difficulty of obtaining nodulation in Dolichos lablab and Vigna sinensis, where only one in ten trials was nodulated, was traced to poor survival of inoculants on the seeds. In further trials, they found that increased inoculum levels enhanced nodulation.

Large number of rhizobia on seed favours survival before planting, which in turn favours rhizobial multiplication in the rhizosphere, and hence, early nodulation. White (1967) obtained larger proportions of nodulated plants, increased number of nodules per plant and yield, when the number of rhizobia on the seed were increased from 4×10^3 to 4×10^6 . Burton (1964) reported that recovery of rhizobia from alfalfa seed was related directly to the amount of inoculum supplied. The number of rhizobia needed for effective nodulation varies with the species of legume, as well as conditions. Thus, according to Burton and Curley (1965), soybeans required 2×10^5 cells per seed, while alfalfa ⁿseeded only 5×10^2 . As per Ireland and Vincent (1968), Trifolium subterraneum required 10^5 - 10^6

rhizobia per seed. Cloonan (1966) stated that Dolichos lablab needed 10^6 rhizobia per seed, which was much more than that needed by Vigna sinensis, for obtaining 50 per cent or better nodulation in the crown region.

Seeds of most grain legumes must be sown close to the soil surface to obtain good stands. Thus, the rhizobia on them are exposed to moist heat. In pot experiments Philpotts (1967) noted that higher the soil temperature at sowing depth, the lower was the percentage of plants with nodules, as also the number of nodules per plant with cowpeas sown at depths ranging from one to four inches. Inferring from their studies under field conditions, Brockwell and Phillips (1970) recommended sowing at greater depths to reduce mortality due to high temperatures near the soil surface. Rhizobia belonging to the tropical cowpea miscellany vary in their tolerance to moderately elevated temperatures ranging from 35° to 42.5°C . However, they tend to be inordinately susceptible to moist heat. Experiments by Bowen and Kennedy (1959) indicated a decimal reduction time of 2.9 to 8.3 hours depending on strains at 40°C in moist soil. Sandy soils low in organic matter often reach temperatures of 40°C and upwards in the tropics.

When seeds inoculated with peat cultures were exposed to 40°C for eight hours, mortality of rhizobia was very high.

Hence, only 50 per cent of plants from such seeds were nodulated. Improved nodulation was achieved by using a higher level of inoculation and also by pelleting treated seed with materials other than lime, in the work of Diatloff (1969). Burton (1975) also opined that rhizobia on seeds could not survive for long at 40°C or above. Wilkins (1967) observed that in the case of medic rhizobia native strains were better adapted for survival at high soil temperatures than introduced strains.

Several factors such as high temperature, relative humidity etc., which militate against the survival of inoculant rhizobia during the critical stage of introduction and in the period immediately following the sowing of legume seeds have been studied by Philpotts (1977), Date (1968) and Iswaran (1971) and they have suggested that pelleting can mitigate the deleterious effects of these factors.

In order to improve the survival of applied rhizobia on seed, Dadarwal and Sen (1971) suggested either soaking of soybean seeds overnight, or the use of peat inoculum in preference to other forms of inoculum. Following the initial exposure to moist heat, the surviving rhizobia on seeds are often subjected to a spell of desiccation as the top-soil loses moisture, thus further decimating them. Moreover, the surviving rhizobia must then maintain viability till the host radicle becomes receptive to them after cotyledonary

combined nitrogen reserves have been depleted, and then they must attain infective numbers. Purchase and Nutman (1957) have demonstrated that a density of 10^4 cells/locus was essential for initiating infection, in clover.

Survival in soil

Rhizobia have been known to survive in soil in the absence of any host plant for as much as thirty to forty years (Albrecht and McCalla, 1937). Jensen (1961) demonstrated Rhizobium trifolii and R. leguminosarum in soil after 14 years and Bergersen (1970) after 11 years. However, such reported extensive longevity of rhizobia is based on the demonstration of one or a few surviving cells. Apparent contradiction of such reports by frequent quantitative determinations, which indicate a rapid decline of most rhizobia under most conditions (Iswaran et al. 1970) is adequately explained by the fact that, cells once dried have the capacity to subsist in a metabolically dormant state (Vincent et al. 1962; Marshall, 1968).

In a study of survival of Rhizobium in soils from seven locations Pant and Iswaran (1970) observed that counts and time were significantly and negatively correlated. Maximum longevity observed in Coimbatore black soil was 55 weeks, and the minimum of 16 weeks was seen in Coimbatore red soil. They did not obtain any correlation between survival and any soil constituent.

Effect of organic amendments:

Choudhury et al (1968) revealed that R. trifolii and R. lupini had a higher growth rate in soils where their hosts were present, and also in soil whose fertility had been enhanced by means of organic amendments. Parker et al (1975) have determined that 0.25 mg of available organic matter in soil can support the growth of upto 3×10^4 cells of Rhizobium.

In contradiction to this, Danso and Alexander (1974) have reported that organic amendments failed to arrest the decline of R. trifolii in soil. Their findings also indicated that R. meliloti did not readily lose viability on moist sand, desiccated soil, or solutions free of organic nutrients. In fact, both species of Rhizobium survived well in dry, non-sterile soil, although numbers sharply declined on inoculation on to desiccated sand.

Moisture stress:

Repeated drying and wetting of soil has been shown to have an adverse influence on the survival of seed borne rhizobia by BChatell (1975).

Shimishi et al (1967) proposed that frequent irrigation cycles did not affect the symbiotic process in groundnut. Precise studies made by Sprent (1973) revealed that any prolonged spell of dryness may altogether stop the

process of nitrogen fixation, even though the host plant tides over the situation, and continues normal physiological activity on the resumption of favourable conditions. Subsequent nodulation which occurs may come too late to be of any use to the host. In field trials with Vicia faba, Sprent (1972) observed that soil water content and nitrogen fixation were highly correlated. Slow drying over a six week period resulted in progressive reduction of nitrogen fixation activity, which was restored by irrigation.

Foulds (1971) observed that in air dried soil at 22°C the numbers of Rhizobium meliloti and lotus rhizobia declined to less than 10 cells per gram in 14 to 71 days depending on the soil sample. R. trifolii survived better under the same conditions and revived on re-wetting to 40 per cent moisture.

Desiccation and high temperature of the soil in the long interval between monsoons may decimate the rhizobial population built up during the monsoon, and in some cases, may even eliminate it entirely (Vyas and Prasad, 1960). Diatloff (1967) reported that plants from five species of tropical legumes, namely, Vigna sinensis, Glycine tomentosa, Cassia mimosoides, Psoralea tenax and Vigna lanceolata, lacked nodules during excessively wet or dry conditions in black earth soil. Habish and Mahdi (1976) contended that under field conditions, the number and weight of nodules were

affected by the interval between irrigations, while plant growth was affected by the amount of water applied. Twenty-five to 35 per cent soil moisture was ideal for cowpeas and 75 mm of water applied at seven day intervals gave the best growth.

Short periods of water-logging (as short as one day) adversely affected vegetative growth and seed yield, reducing it by as much as 50 per cent at flowering in the case of cowpea, according to Minchin et al (1978).

Pelleting

As cited earlier, the competitive position of introduced rhizobia can be improved by increasing the rate of inoculation, to afford a safety in numbers. It can also be improved by reducing the mortality rate of inoculum on the seed. As a first step, seeds should be sown as soon as possible after treatment to minimise exposure to adverse conditions such as desiccation and sunlight (Date, 1976). According to Iswaran (1971) soybean rhizobia survived only for a short time on seeds, and hence he emphasised that treated seeds should be sown immediately.

Seed pelleting with lime, though originally developed as a means to establish temperate legumes in acid soils (Longergan et al (1955), has now become a widely adopted procedure for inoculum establishment (Hastings and Drake, 1960; Murguia and Date, 1965; Jones et al. 1967;

Radcliffe et al 1967; and Iswaran and Chhonkar, 1971).

Date (1969) noted that lime pelleting and adhesive afforded a high degree of protection to peat inoculant on seed in the field, although under laboratory conditions it produced conflicting results. Experimenting with several seed coating materials (13 types) and adhesives (6 types), Radcliffe et al (1967) postulated that seed pelleting resulted in a ten fold better survival of peat inoculant. However, they also found that only one of the seven pelleting combinations with peat suspension in 40 per cent gum arabic had adequate number of survivors for nodulation after eight days of storage. Norris (1971) has described the advantage of seed pelleting of inoculated Dolichos lablab, indicating that rock phosphate was superior to lime in improving survival of inoculants and that the strain CB 756 survived better on seed than strain CB 159. In further experiments, Norris (1972) has observed that both rock phosphate and bauxite were superior pelleting materials for seeds of tropical legumes. Finely divided bauxite as an excellent pelleting material has been advocated by Norris (1973) for obtaining the increased recovery of inoculant strains from nodules.

By contrast, Herridge and Roughley (1974) working with five tropical legumes, viz., Vigna sinensis, Glycine wightii, Lupinus albus, Desmodium uncinatum and Glycine max, concluded that rock phosphate pelleting compared with lime

for seeds of these species, inoculated with alkali producing rhizobia, does not improve their longevity on seed.

The particulate matter in a pellet absorbs moisture, thus keeping the seed relatively dry and hence permitting the use of a large inoculum of Rhizobium permitting their introduction in greater numbers (Burton, 1975). Besides, protecting the emerging radicle from excess soil acidity (Iswaran and Chhonkar, 1971), or soil salinity, as per Chhonkar et al (1971), the pellet provides an effective physical barrier against high temperature and dessication, which militate against survival of the inoculum (Cloonan and Vincent, 1967; Date and Cornish, 1968; and Brockwell and Whalley, 1970). It also provides an excellent vehicle for the application of vital trace elements such as molybdenum to the crop with minimum wastage and appropriate placement, as has been shown by Date and Hillier (1968).

However, it has been shown that the protection so afforded enhances longevity of different species of Rhizobium to varying extents with R. meliloti, out living both R. trifolii and R. japonicum by Carter (1963) and Brockwell et al (1972).

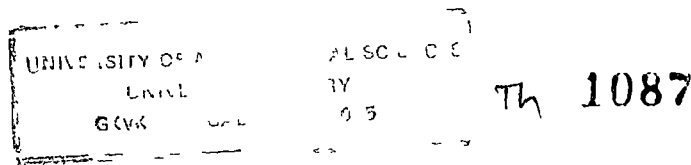
In contrast to the above, Adams and Lowther (1970) have stated that lime pelleting reduced the survival of Rhizobium on the seed.

Persistence

Effect of rotation

Only if elite strains of rhizobia are able to translate their initial advantage of placement near the seed into a large carry-over population of viable cells, distributed randomly in soil will they be available for nodulation of subsequent crops. A large population to start with would ensure better subsistence over the hot dry summer season. This is greatly emphasised by the findings of Nash and Moerman (1967). They observed that surviving soybean nodule bacteria (10^4 cells per gram) in red or sand veld soils appeared to be inadequate to obtain optimum nodulation of a subsequent crop (after five years) without additional seed inoculation. The ability to nodulate legumes other than their normal host plants may be an ecological factor in maintaining their survival during the summer fallow period (Bergerson, 1970). Diatloff (1969) proposed introduction of rhizobia along with the seeds of cereals and succeeded with R. japonicum,

Nutman (1969) and Nutman and Ross (1970) found that rhizobia were few, sometimes absent, in fields continuously cropped to non-legumes, or rotations without legumes. They also found that these low numbers were unaffected by mineral or organic manuring. De Escuder (1972) noted that R. leguminosarum and R. trifolii were more abundant in soil than R. lupini and R. meliloti, especially where



their respective hosts had been grown recently. Quite contrary to this, Kumar Rao and Dart (1979) found no effect of pigeon pea in the previous season on the population of cowpea type rhizobia in soil.

Vincent (1958) observed that after 10 to 15 years of cereal cultivation Rhizobium meliloti had not survived in soil. He attributed this lack of survival to the extreme conditions prevailing in the periods during which the ground was fallow.

In contrast to this, Bergersen (1970) has shown that nodule bacteria associated with naturalised hosts that are economically useless and often of low symbiotic efficiency with sown pasture clovers, may attain populations in excess of 10^5 per gram of soil.

Saprophytic competence:

That saprophytic competence varies with species (Chatel and Greenwood, 1973b) and strain was shown by Brockwell et al (1972), Chatel and Parker (1973) and Chatel and Greenwood (1973a & c). Dudman and Brockwell (1968) working with R. trifolii, noted a significant trend for the proportion of recoverable inoculum strains to diminish with lapse of time between sowing and isolation. Strain CB 756 established poorly and tended to be variable in effectiveness on Lablab purpureus, while CB 1024 established

well and persisted under field conditions as per Harridge and Roughley (1974).

Cutting

When fodder legumes are cut or grazed, the effect on their root systems and their accompanying nodular complement is quite drastic. According to Whiteman (1970) cutting was followed by a sharp reduction in nodule number and weight, whereas partial defoliation resulted in the number of nodules increasing in some cases, while the weight per nodule decreased. This indicated that while some nodules were lost, new ones were initiated. In another experiment, Whiteman and Lulham (1970) opined that the reduction in nodule weight per plant was due to a reduction in different parameters in different species. Accordingly, in Phaseolus atropurpureus, it decreased due to a decline in mean weight per nodule whereas in Desmodium uncinatum it was due to reduction in the number of nodules per plant. Besides this, they also observed that grazing caused a greater reduction in nodule number and weight than mowing to a height of three inches from the ground.

In a study on white clover, Chu and Robertson (1974) inferred that the changes in nodule dry weight per plant consequent upon defoliation were due to nodule decay and sloughing off of nodules. Severe defoliation, tantamount to cutting, resulted in degeneration of nodular leghaemoglobin within three days, and it took such plants at least

10 days to resume normal nitrogen fixation activity, according to them. Shading lowered the photosynthetic efficiency of the host and has been shown to have an effect akin to partial defoliation on the nodules, by Summerfield et al (1976).

Phosphate fertilization

The marked response of legumes to applied phosphorus is well known (Subba Rao, 1973; and Sundara Rao, 1972). The response has been related to soil nitrogen content (Khare et al. 1968); to the efficiency of Rhizobium spp. (Gupta and Sen, 1966); to the nodulation of the host (Murlidharan and George, 1971; Sharma and Garg, 1973). However, in contrast to this, Tewari (1965) stated that cowpeas showed no significant response to fertilizer phosphate and potash.

Competition for nodulation

When new strains of Rhizobium are introduced into soils already having a large indigenous population of rhizobia, able to nodulate a given host, they are at a disadvantage to begin with. In order to nodulate the host they must compete successfully with numerous native rhizobia (Ham et al. 1971; Abel and Erdman, 1964; Holland, 1970).

The relationship between numbers of introduced as

against those of resident rhizobia has been elegantly quantified by Ireland and Vincent (1968) under laboratory conditions with Trifolium spp. Similar experiments to gauge the intrinsic competitive ability of inocula have also been conducted by Labandera and Vincent (1975), and Marques Pinto et al (1974). Where multi-strain inocula are used, these rhizobia must compete among themselves for nodulation sites, besides competing with the native population in soil (Johnson and Means, 1964; Caldwell, 1969); and for nutrients with other soil micro-organisms (Caldwell, 1969; Rovira, 1961).

In opposition to Caldwell, in 1969, Dube et al working with Rhizobium japonicum contended that, inoculant strains fared better in their struggle for dominance over native rhizobia, if they were spared the ordeal of first struggling among themselves by use of single strain inocula. They also observed that re-inoculation of treated plots in subsequent planting brought about an increase in the leghaemoglobin content of the nodules formed and that, higher levels of inoculum led to better competitiveness and improved nodule size. These findings oppose those of Read (1953) who stated that competition between strains was not related to their ability to establish themselves in the field.

From this, it is clear that, in order that the introduced rhizobia become established in soil, they must be

conferred some special advantage. Despite these unfavourable odds, certain competitive strains do establish themselves in soil as has been demonstrated by the work of Brockwell and Dudman (1968). By means of a gel immune diffusion technique, they showed that strain WA 67 of Rhizobium trifolii produced nodules on the host roots not only in competition with a large native population, but also with two other strains, TA 1 and UNZ 29 in a mixed inoculum.

Strain differences in ability to colonise host root and soil in the presence of the host were demonstrated by Chatel and Greenwood (1973a). One strain TA 1 was consistently inferior to the other three which comprised another introduced strain (UNZ 29), and two locally isolated strains. Similar studies with these strains in the absence of the host and also with different species in Rhizobium were made by Chatel and Greenwood (1973b).

Rhizobia being essentially rhizosphere organisms are apparently encouraged electively in the rhizosphere of legumes in general (Rovira, 1961) and in some instances, specifically by a given host cultivar (Robinson, 1967). Their strategic placement initially by way of inoculation would be a distinct advantage in colonizing the rhizosphere according to Bergersen (1970).

Vincent and Waters (1953) have traced the progress of different strains of clover rhizobia in the host rhizosphere prior to infection and found that the host exercised

specific selection with regard to the strain that could nodulate it. Similar conclusions have been drawn by Abel and Erdman (1964) in their studies with soybean. Robinson (1969a & b; 1967) has demonstrated the existence of selective pressures on the part of the host in the field with clover on strains of R. trifolii. Besides this, another way of helping inoculants to establish, is to ensure their introduction itself in a big way, at least numerically. Initial numerical superiority attained by higher inoculum levels, decidedly confers a competitive advantage and has been demonstrated by Kapusta and Rouenhorst (1973) and Weaver and Frederick (1972), in case of soybean and in Trifolium spp. by Holland (1970). Ireland and Vincent (1968) reported that 10^5 - 10^6 rhizobial cells per seed were required for successful introduction when seeds were sown in soils heavily infested with native homologous strains of Rhizobium.

Weaver and Frederick (1974) recommended an inoculum containing one thousand times as many rhizobia as are present in one gram of the soil where beans are planted, in order to obtain 70 per cent or more nodules from the applied inoculum.

In soils possessing a large complement of highly infective but poorly effective native rhizobia, competition for nodulation sites on the host is intense. The negative effect of indigenous rhizobia due to their ineffectiveness

on introduced clovers has been reported from California by Holland (1970). Competitive effective strains were required in large numbers to swamp the established inhabitants. A similar problem arises where rhizobia established as effective symbionts for a given host are poorly adapted to fix nitrogen with other hosts in the cowpea miscellany, or on other species of the same host, as was documented by Vincent (1954).

Spread

Seed inoculation concentrates the inoculum close to the host radicle, whereas competing soil rhizobia are distributed randomly in soil. As a direct consequence, when roots of the growing crop traverse, the soil in the vicinity of the native populace, the added strains are at a distinct disadvantage, unless they are able to move down and sideways from the point of sowing. Hence, nodules on the lateral and secondary roots are most likely the regimen of the native populace. It is noteworthy, that rhizobia do not move away to any appreciable distance from the seed, unless they are washed down into soil by rain water in the field (Hamdi, 1970; Worral and Roughley, 1975).

An apparent consequence is that the seed inoculum is not likely to bring about nodulation below the crown, especially under dryland conditions. Thus, crown nodules are probably the main stay of the host with its elite inoculum

as its association lower down in soil with poorly effective native bacteria is not likely to be of much service. Norris (1971) on finding that the total number of nodules was inversely related to crown nodulation, has inferred that the soil moisture at planting significantly affects nodulation.

Working with Vigna sinensis and Vicia atropurpurea, Dart and Wildon (1970) observed that added nitrogen suppressed nodulation on the tap root while not affecting the process on secondary roots.

Weaver and Frederick (1972) have shown that increasing the level of inoculum caused increased tap root nodulation with a dosage of 2×10^3 rhizobia per seed being ideal for soybeans. Soil moisture content at germination has been shown to influence the strain of Rhizobium which will infect the host by Masterson and Sherwood (1974). However, spread of inoculant rhizobia along with the growing tip of secondary and lateral roots is quite possible, but their movement along with water in soil is more likely, as has been suggested by Hamdi (1970), Brockwell et al (1972) and Martin (1971).

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Rhizobium strains

Five strains of cowpea rhizobia obtained from the culture collection of the Department of Agricultural Microbiology, University of Agricultural Sciences, G.K.V.K. Campus, were used in the present studies. Among them, three strains, namely, UASB 94, 101 and 125 were local isolates proven for their efficiency on cowpea, groundnut, black gram and green gram in field trials conducted over a period of three years. The remaining two proven strains, namely, CB 756-M₂, and CB 1024 were obtained from the Division of Tropical Pasture Crops of the Commonwealth Scientific and Industrial Organization, Australia. Strain CB 756-M₂ was a streptomycin resistant mutant derived from strain CB 756. This strain was used in studies on survival in soil. The cultures were maintained on yeast extract mannitol agar (YMA) slants, containing small amounts of calcium carbonate.

Legume host

Cowpea variety IGFRI-895 was obtained from the Indian Grassland and Fodder Research Institute, Jhansi, for the present study. This cowpea variety, being a long annual, was amenable to one or two cuttings and capable of rapid regrowth after each cutting.

Seed treatment with Rhizobium

Cowpea seeds were treated with lignite based cultures of Rhizobium. For this, five ml of 10 per cent gum arabic solution was added to half a kg of seeds which were then rolled in a glass jar. Later 10 g of lignite inoculant of Rhizobium was sprinkled over the seeds and rolling continued till the seeds were completely coated with the culture.

Pelleting

For pelleting, seeds inoculated as above were moistened with a further 5 ml of gum arabic sticker. Finely powdered pelleting material was added in excess, to the jar, and the moist seeds rolled further by means of a rotating as well as tumbling action of the jar, till a uniform and apparently dry pellet was obtained. Pelleted seeds were allowed to dry in shade for a few minutes before sowing.

Survival on the seed

A laboratory experiment was conducted to determine the longevity of inoculated rhizobia on the seed, under various conditions normally obtained in the field. Seeds in 50 g lots were treated with 1 g of lignite based culture containing 10^9 viable cells per gram of any given strain of Rhizobium. Two strains, namely, UASB 125 and CB 1024 were taken for the study. Seeds treated with lignite inoculant

were pelleted as described earlier. Such treated seeds were exposed to the following conditions in storage:

A. Unpelleted: 1. Kept in shade.

2. Exposed to direct sunlight.
3. Placed in a closed plastic box and kept in shade.
4. Covered with a wet muslin cloth and kept in shade.

- B. Pelleted: 1. Pelleted with lime and kept in shade.
2. Pelleted with lime and exposed to sunlight.
 3. Pelleted with bauxite and kept in shade.
 4. Pelleted with bauxite and exposed to sunlight.
 5. Pelleted with rock phosphate and kept in shade.
 6. Pelleted with rock phosphate and exposed to sunlight.

Samples of 10 seeds were drawn in triplicate randomly from each treatment at the following intervals after inoculation: 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 24.0 hours. Each sample was shaken with 100 ml of sterile tap water containing 2 g of washed sterile sand (coarse) for five minutes on a rotary shaker. The aliquot was then taken for enumeration of rhizobia by the dilution plate count method on yeast extract mannitol agar (YMA) with Congo red dye (Vincent, 1970).

Survival in soil

Plants of cowpea, finger millet, sorghum, Acanthospermum spp., Cyperus spp., Cynodon dactylon and Mimosa pudica were raised in pots in triplicate. Each plant was inoculated with a broth culture containing 10^9 cells per ml at the rate of 2 ml per plant seven days after sowing. Strain CB 756-M₂ of Rhizobium, a streptomycin resistant mutant, was used in this experiment. The population of this strain in the rhizosphere, as well as in soil away from the roots of each plant was enumerated at flowering of the respective species. Enumeration was done using YMA containing 250 µg of streptomycin sulphate per ml by the dilution plate count method. From the data obtained the capacity of the introduced strain of Rhizobium to survive in the rhizosphere of non-host plants . as compared to the host - cowpea, was assessed.

Survival in soil under water stress

To study the effect of post sowing water stress on survival and competition for nodulation by seed inoculum, an experiment with plants raised in two inch plots was set up in the green-house. This was studied with two types of soil conditions, namely, (i) sterile (non-competitive soil) and (ii) unsterilised (competitive soil). In the former the soil was sterilized in the pots by autoclaving at 20 psi for one hour on two successive days. Two

strains of Rhizobium, UASB 125 and CB 756-M₂, were taken for the experiment. Seeds were surface sterilised with 0.1 per cent acidified HgCl₂ solution and washed with 10 changes of sterile water before inoculating with lignite based culture of the test strain of Rhizobium. Seeds inoculated with each strain were pelleted with three different materials, namely, lime, bauxite and rock phosphate. Seed treatments were as follows:

1. Uninoculated control (common to both strains of rhizobia).
2. Seeds inoculated.
3. Seeds inoculated and pelleted with lime.
4. Seeds inoculated and pelleted with bauxite.
5. Seeds inoculated and pelleted with rock phosphate.

Treatments 2 to 5 were repeated with two different strains of Rhizobium, namely, UASB 125 and CB 756-M₂.

Watering schedule

All the seeds were sown into soil brought to field capacity by addition of measured quantities of sterile tap water. Then the pots were separated into 10 sets of 3 pots each. The first set of three pots were watered from the first day onwards. The second set from the second day, the third set from the third and likewise the tenth set was watered for the first time ten days after

sowing and daily thereafter. Regardless of the day of initial watering, all the pots were kept at field capacity by addition of sterile tap water daily after the initial watering until the experiment ended on the 20th day. Then the plants were uprooted and examined for nodulation.

Effect of organic amendments on survival of inoculant strain

To assess the saprophytic competence of Rhizobium strain CB 756-M₂ in the presence of the native population of rhizobia an experiment was set up. Soil sieved through 2 mm mesh was dispensed in 100 g lots into 350 ml saline bottles, plugged with cotton. The treatments were:

1. Sterile soil + 1 ml inoculum - control (sterile soil + 1 ml of sterile broth as a blank treatment was also maintained).
2. Unsterile soil + 1 ml inoculum.
3. Unsterile soil + farm yard manure + 1 ml inoculum.
4. Unsterile soil + cowpea residue + 1 ml inoculum.

Farm yard manure and cowpea residues were added to make up 0.1 g carbon per 100 g air-dry soil, and mixed thoroughly with the dry soil before dispensing into bottles. Each treatment was replicated thrice. In each case, the soil was brought to field capacity with sterile tap water before adding the inoculum consisting of 1 ml of well grown broth culture of strain CB 756-M₂ containing 10^9 cells per ml. Thereafter, sterile water was added each time the soil

reached wilting point as measured by loss in weight, to bring it back to field capacity. Enumeration was done at weekly intervals for four weeks from samples of 1 g soil drawn from each bottle. Dilution plate counting was done on YMA plates with and without streptomycin sulphate. Congo red indicator at 0.25 per cent and actidione at 0.02 mg per ml were added to both types of media. The difference in the colony counts between the latter and the former were taken as the number of native rhizobia.

Response to inoculation and persistence of introduced strains

Field experiments were conducted during Kharif 1976 in red sandy loam soil of the Agricultural College farm, University of Agricultural Sciences, Hebbal, having a pH of 5.8, available nitrogen content of 23 kg/ha, available phosphorus of 12 kg P_2O_5 /ha and potash 47 kg/ha. The population of resident rhizobia was estimated by the Most Probable Number Method (MPN) (Vincent, 1970), with *Siratro* as the test host (10^5 cells/g). Each treatment plot measured 5 m x 4 m. The crop was sown in 45 cm rows with 25 cm between plants in each row.

The following eight treatments, each replicated three times, were included:

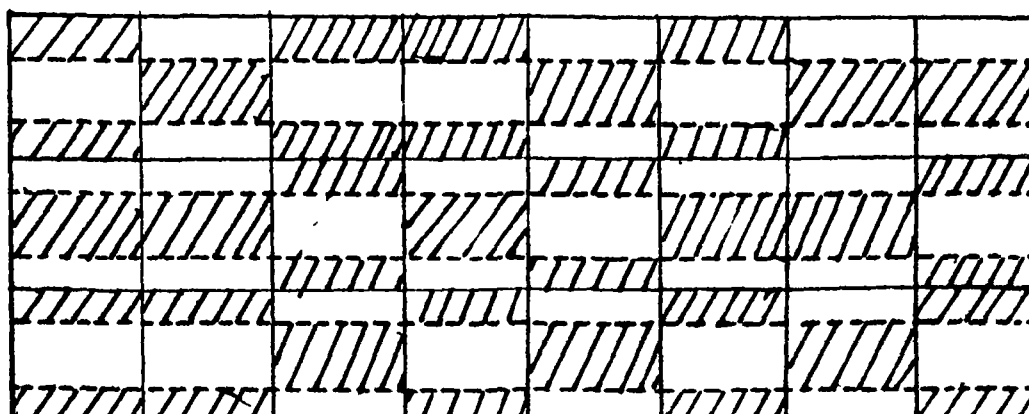
1. Uninoculated control.
2. Nitrogen control (40 kg N/ha).

Fig. 1: Lay-out of field experiment on response to inoculation
(Randomized complete block design)

I	III	IV	VII	VIII	V	VI	II
III	II	I		VIII	VII	VI	IV
II	VI	IV	III	V	VII	VIII	I

- I = Uninoculated control
 II = Nitrogen control
 III = Inoculated with UASB 94
 IV = Inoculated with UASB 101
 V. Inoculated with UASB 125
 VI. Inoculated with CB 756-M₂
 VII. Inoculated with CB 1024
 VIII. Inoculated with mixture of all strains

Fig. 2: Lay-out of split-RCBD on effect of cutting on
nodulation and persistence*



 Cut

 Uncut

*This design was superimposed on the RCBD shown in Fig. 1:

3. Inoculated with strain UASB 94.
4. Inoculated with strain UASB 101.
5. Inoculated with strain UASB 125.
6. Inoculated with strain CB 756-M₂.
7. Inoculated with strain CB 1024.
8. Inoculated with a mixed inoculum of the 5 strains mentioned above.

A basal dose of 50 kg P₂O₅ per ha was applied before sowing. The lay out plan of the field experiment is given in Fig. 1.

Effect of cutting on nodulation and competitiveness of inoculants

At 20 per cent flowering, a split plot design was imposed on the original RCBD with cutting and no cutting as the two sub-plot treatments with the eight main plot treatments retained as such. The lay out plan of the experiment after imposition of the new treatments is shown in Fig. 2. Nodule number and shoot dry weight at 20, 40, 60, 70 and 100 days from the date of sowing were recorded. For this, five plants were dug out carefully from individual plots taking care to select plants at random (hence three samples per treatment). Plant shoots were separated and dried at 75°C to constant weight and their dry weights were recorded.

Nodule number per plant was also recorded at 70 and 100 days after sowing to observe the nodulation pattern

after cutting. Grain and fodder yields were recorded at harvest.

Influence of crop rotation on the persistence of inoculant strains

After the first season crop, a second split plot experiment was imposed on the original split RCBD with minimum disturbance of the existing layout and soil conditions. The sub-plot treatments newly imposed were:

1. Continuous legume (cowpea IGFR1 895)
2. Cereal non-host - finger millet
3. Fallow (unweeded)
4. Bare fallow (weeded)

The layout is shown in Fig. 3. From the plots continuously under legume host, five plants were randomly dug out at initiation of flowering and nodule number per plant was recorded. One hundred and fifty nodules selected at random were serotyped. In the other treatments, the population of soil rhizobia was estimated by dilution-plate-count method before sowing and at the time of harvest, using YMA containing actidione and Congo red (Vincent, 1970).

At the end of the second season (Rabi 1976), the third and final crop of cowpea was taken to study the persistence and competitive ability of the introduced Rhizobium strains. For this, each of the original treat-

Fig. 3: Lay-out of split-split-RCHD on effect of crop rotation on persistence of inoculum*

a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d
a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d
a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d

a = Cowpea-cowpea-cowpea
b = Cowpea-ragi-cowpea
c = Cowpea-fallow-cowpea
d = Cowpea-bare fallow-cowpea

*This design was superimposed on the RCHD shown in Fig. 1 in the second season

Fig. 4: Layout of experiment on re-inoculation on persistence and competitiveness of inoculum*

 Re-inoculated with original strain
 Uninoculated in third season

*This design was superimposed on the RCHD shown in Fig. 1 in the third season

ment plots as included under experiment on response to inoculation was divided into two sub-plots. Seeds sown into one of the sub-plots were uninoculated while those sown in the other were re-inoculated with the same Rhizobium strains as in the original experiment.

The layout plan is given in Fig. 4. Nodules obtained from five plants drawn from each sub-treatment plot were serotyped following the procedure of Dudman and Brockwell (1968). Persistence of the introduced Rhizobium strains was estimated from the data on serotyping obtained from the uninoculated plots. Similar data obtained from the re-inoculated plots were used to assess the competitive ability of the experimental strain.

Antisera preparation and serotyping of nodules

Antisera against strains UASB 94, 101, 125 and CB 1024 were raised in rabbits as per guidelines of Vincent (1970). Pure cultures of each strain were grown on agar slopes. Growth so obtained was washed with two changes of sterile saline, aseptically, followed by centrifuging. The final antigen was made up with physiological saline (8.5 g NaCl per litre of distilled water), to contain 10^8 cells per ml. One ml of such antigen was injected after thoroughly mixing it with 1 ml of Difco complete adjuvant intramuscularly into the thigh muscle. A second booster dose of 1 ml of antigen alone was given after 30 days, intraveinously into the ear vein. A sample of blood was

drawn a week later and its antibody titre was tested by the simple tube agglutination method. Wherever necessary booster dose of antigen was repeated till a titre of 1:3200 was obtained. Separate rabbits were used for each experimental strain of Rhizobium.

For gel diffusion serotyping 1 per cent Difco agar (serological grade) in physiological saline (as before) was used. Gel plates containing seven arrays of wells of size 4 mm diameter x 4 mm depth were prepared using a template designed by the author.

Sera obtained as per details above were used in typing by the Ouchterlony gel immune diffusion method as modified by Date (1970).

Pelleting and phosphate fertilization

To study the effect of phosphorus fertilisation, pelleting and strains of Rhizobium on cowpea variety IGFRI-895, a split-split plot experiment was laid out in the field. Main-plot treatments included three levels of P_2O_5 application as single superphosphate, at 25 kg, 50 kg and 75 kg per ha. The sub-plot treatments were:

1. Control uninoculated.
2. Inoculation with strain UASB 125.
3. Inoculation with CB 756-M₂.
4. Inoculation with strain CB 1024.
5. Inoculation with a mixture of the above three strains (in equal parts by weight of lignite inocula).

Fig. 5: Lay-out of field experiment on phosphate fertilization and pelleting on nodulation and competitiveness

25 kg P ₂ O ₅ /ha					50 kg P ₂ O ₅ /ha				
I	2	4	1	3	III	3	1	4	2
III	1	2	3	4	II	2	1	3	4
II	4	2	1	3	I	4	3	2	1
V	2	4	3	1	V	4	3	1	2
IV	3	4	2	1	IV	1	3	2	4
50 kg P ₂ O ₅ /ha					75 kg P ₂ O ₅ /ha				
I	4	1	2	3	II	1	2	3	4
III	3	1	2	4	I	2	4	1	3
V	2	4	1	3	III	4	1	2	3
II	4	2	1	3	V	3	1	2	4
IV	2	1	3	4	IV	1	2	4	3
75 kg P ₂ O ₅ /ha					25 kg P ₂ O ₅ /ha				
III	2	3	4	1	I	4	2	1	3
IV	3	4	2	1	V	1	4	2	3
V	2	3	1	4	III	2	1	3	4
II	4	2	1	3	IV	2	4	1	3
I	3	2	4	1	II	3	2	1	4
25 kg P ₂ O ₅ /ha					75 kg P ₂ O ₅ /ha				
V	4	3	2	1	IV	4	3	2	1
II	1	2	3	4	III	3	1	2	4
I	3	2	4	1	II	1	3	4	2
III	1	3	2	4	V	4	1	3	2
IV	2	3	1	4	I	4	2	3	1
50 kg P ₂ O ₅ /ha									
II	4	3	2	1					
III	3	4	2	1					
IV	2	4	1	3					
I	1	4	3	2					
V	2	1	4	3					

1. Unpelleted
2. Bauxite pelleted
3. Lime pelleted
4. Rock phosphate pelleted

I = Uninoculated controls; II = Inoculated with UASB-125

III = Inoculated with CB 756-M₂ IV = Inoculated with CB-1024

V = Inoculated with mixture of all strains

The sub-sub-plot treatments were:

1. Control - No pelleting
2. Pelleting with bauxite
3. Pelleting with lime
4. Pelleting with rock phosphat

Individual plots measured 1.5 m x 1.5 m and their layout plan is described in Fig. 5. The experimental dose of P_2O_5 was applied as a single basal dose in bands 5 cm away from the seed. Seeds were sown in 30 cm rows with 15 cm between the plants.

The native population of rhizobia in the experimental plots was estimated by the most probable number method prior to sowing.

Random samples made up of five plants were drawn from each plot at 30, 60 and 90 days after sowing. The number of nodules per plant and the dry weight of each plant were recorded.

Host variety versus rhizobial strain

To identify the best combination of cowpea variety and Rhizobium strain under the conditions obtaining in Bangalore, a split-split-plot experiment using 12 cultivars of the host with five rhizobial strains was laid out in micro-plots. Appropriate uninoculated and nitrogen controls were maintained. The crop was sown in $1m^2$ (net

Fig. 6: Layout of experiment on host-versus bacterial genome on yield

Varieties	Replication 1												Replication 2												Replication 3											
	TREATMENTS												Replication 2												Replication 3											
	1	3	5	2	7	8	4	6	1	3	5	2	7	8	4	6	1	3	5	2	7	8	4	6	1	3	5	2	7	8	4	6				
C-152	1	3	5	2	7	8	4	6	1	3	5	2	7	8	4	6	1	3	5	2	7	8	4	6	1	3	5	2	7	8	4	6				
Co Pusa-2	3	2	1	5	8	7	6	4	3	2	1	5	8	7	6	4	3	2	1	5	8	7	6	4	3	2	1	5	8	7	6	4				
Co 2-1	1	3	6	7	4	2	5	8	1	3	6	7	4	2	5	8	1	3	6	7	4	2	5	8	1	3	6	7	4	2	5	8				
Co 288	2	5	6	4	7	3	8	1	2	5	6	4	7	3	8	1	2	5	6	4	7	3	8	1	2	5	6	4	7	3	8	1				
Co P-S-68	1	3	7	8	5	2	4	6	1	3	7	8	5	2	4	6	1	3	7	8	5	2	4	6	1	3	7	8	5	2	4	6				
Dixilee	4	7	9	3	5	2	6	1	4	7	8	3	5	2	6	1	4	7	8	3	5	2	6	1	4	7	8	3	5	2	6	1				
IGPRI-895	5	1	3	7	2	8	4	6	5	1	3	7	2	8	4	6	5	1	3	7	2	8	4	6	5	1	3	7	2	8	4	6				
Iran Grey	6	5	7	8	2	4	1	3	6	5	7	8	2	4	1	3	6	5	7	8	2	4	1	3	6	5	7	8	2	4	1	3				
Lolita	1	8	3	6	4	2	5	7	1	8	3	6	4	2	5	7	1	8	3	6	4	2	5	7	1	8	3	6	4	2	5	7				
Mississippi purple	8	6	3	1	2	4	5	7	8	6	3	1	2	4	5	7	8	6	3	1	2	4	5	7	8	6	3	1	2	4	5	7				
Selection-2	8	4	6	1	2	7	3	5	8	4	6	1	2	7	3	5	8	4	6	1	2	7	3	5	8	4	6	1	2	7	3	5				
Virginia	2	4	8	5	1	7	3	6	2	4	8	5	1	7	3	6	2	4	8	5	1	7	3	6	2	4	8	5	1	7	3	6				

1 = Uninoculated control; 2 = Nitrogen control; 3 = Inoculated with UASB 94;

4 = Inoculated with UASB 101; 5 = Inoculated with UASB 125; 6 = Inoculated with CB 756-M2

7 = Inoculated with CB 1024; 8 = Inoculated with mixture of all strains

size) plots in 30 cm rows with 15 cm spacing between the plants. The layout plan is shown in Fig. 6. The grain yield was recorded from each plot. The varieties used were:

- | | |
|---------------|------------------------|
| 1. C-152 | 7. IGFR1-895 |
| 2. Co Pusa-2 | 8. Iran Grey |
| 3. Cowpea 2-1 | 9. Lolita |
| 4. S-288 | 10. Mississippi Purple |
| 5. FS-68 | 11. Selection-2 |
| 6. Dixilee | 12. Virginia |

Field as well as laboratory observations recorded were tabulated and analysed statistically as per guidelines contained in the publication of Sunderraj et al. (1968).

Spread of introduced rhizobia: The spread of inoculant rhizobia in soil from the point of introduction on the seed was studied as follows. Intact root systems of plant samples drawn in the previous experiments were spread on a graph sheet so as to simulate their probable distribution in soil. Fifty nodules were selected at random from each treatment and their locations along the X and Y axes of the graph noted. These nodules were serotyped to identify the strain infecting them and from this an approximate idea of the vertical and horizontal spread of inoculants was obtained. These results are presented in Figures 7, 8, 9 and 10.

RESULTS

IV. RESULTS

By and large, grain and forage legumes do respond to artificial inoculation with selected strains of rhizobia, on their seeds. However, inoculation as a means of introducing new strains of rhizobia may be ineffective, because of low numbers of rhizobia on the seed, and their failure to establish in soil in competition with native strains. It may also be due to certain soil factors such as low pH, nutrient deficiency particularly of phosphorus and molybdenum, and so on. Long absence of the host legume, or presence of non-host crops which do not favour the growth of rhizobia, may be other reasons for such failures. Further, complications may arise on account of the introduced strain of Rhizobium not matching with the large number of host varieties now being grown. Some of the above factors limiting nodulation and nitrogen fixation were studied, using cowpea as a test crop, under laboratory, green house and field conditions.

Survival of inoculant rhizobia on seed and in soil

On the seed:

It is essential that sufficient numbers of rhizobia survive on the inoculated seed, so that they can provide an adequate nucleus population for the colonization of soil, and subsequently, nodulation of the host. The viability of Rhizobium strains UASB 125 and CB 1024 on seeds of cowpea treated with lignite based inoculant, under

various conditions of storage, simulating those obtaining in the field, were studied by dilution plate counting, over a period of 24 hours. Counts obtained at different intervals are presented in tables 1 and 2. Of the two strains used, the former grew fast and produced extracellular gum copiously, while the latter grew slowly and produced less gum. Survival was better in terms of number of viable cells, and more prolonged when treated seeds were kept in shade, than when they were exposed to sunlight on a clear morning. However, time versus survival data indicate that more than 50 per cent of the rhizobia on seeds were killed during the very first hour of storage. Thereafter, a significant decline in numbers, at one per cent level, occurred in almost all the treatments at every half hour, upto two hours from treatment. At the end of six hours, only a negligible number of viable cells remained on the seeds under most storage conditions studied.

The highest mortality rate of 75 per cent in the first half hour was noted when inoculated seeds were exposed to sunlight. They lost practically all their viable cells within four hours - retaining less than hundred cells after two hours and none after four hours.

Among the pelleting materials tried, lime offered the best protection and was closely followed by bauxite. Rock phosphate, however, proved to be a rather poor protectant

Table 1: Viability of Rhizobium UASB 125 (cells x 10³/seed) on cowpea seed

Treatment	Time in hours									
	0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	24.0	
<u>Unpelleted</u>										
Control: Shade I	49.10 (100)	35.96 (73.24)	25.33 (51.58)	6.20 (12.62)	5.38 (10.95)	0.77 (1.57)	0.45 (0.92)	0.04 (0.08)	0.01 (0.01)	
Sun II	49.16 (100)	12.36 (25.13)	1.85 (3.76)	0.93 (1.89)	0.59 (1.2)	0.07 (0.15)	0.01 (0.02)	0 0	0 0	
Wet cloth III	49.95 (100)	40.27 (80.62)	38.37 (76.82)	23.29 (46.83)	18.64 (37.32)	9.92 (19.80)	12.82 (25.67)	1.49 (2.97)	0.79 (1.57)	
Plastic box IV	48.82 (100)	37.86 (77.55)	31.50 (64.52)	22.50 (46.09)	19.62 (40.19)	13.43 (27.51)	8.69 (17.8)	2.77 (5.67)	1.33 (2.92)	
<u>Pelleted</u>										
Lime Shade V	44.60 (100)	23.64 (53.00)	20.56 (46.10)	18.60 (41.7)	12.42 (27.85)	11.47 (25.92)	9.03 (20.25)	5.99 (13.43)	2.63 (5.90)	
Sun VI	46.17 (100)	20.12 (43.58)	14.65 (31.73)	11.62 (25.07)	7.03 (15.23)	7.41 (16.05)	5.46 (11.83)	4.27 (9.24)	1.62 (3.51)	
Rock Shade VII phosphate	43.67 (100)	16.93 (38.79)	13.65 (31.25)	9.63 (22.05)	4.47 (10.24)	2.35 (5.38)	2.43 (5.86)	1.60 (3.65)	0.50 (1.15)	
Sun VIII	44.87 (100)	12.33 (27.48)	9.81 (21.86)	5.49 (12.24)	2.52 (5.62)	1.96 (4.37)	0.39 (0.86)	0.07 (0.15)	0 0	
Bauxite Shade IX	45.95 (100)	22.18 (48.27)	18.64 (40.57)	18.36 (39.96)	12.55 (27.31)	7.00 (15.93)	3.36 (7.32)	1.38 (3.00)	1.23 (2.67)	
Sun X	46.47 (100)	22.35 (48.09)	18.18 (39.12)	11.31 (24.34)	6.90 (14.85)	3.60 (7.75)	2.15 (4.63)	0.38 (0.82)	0.29 (0.62)	

Values in parentheses indicate survival as a percentage of the original number on the seed.

against sunlight, and was comparable to storage of unpelleted seeds covered with a wet cloth in shade, in this respect.

After 24 hours of storage, the highest number of viable cells/seed (2.63×10^3) of strain UASB 125 were retained on seeds pelleted with lime and stored in shade. The survival at 24 hours was also comparable to that on seeds pelleted with bauxite and stored in shade as well as to that on seeds stored in a closed plastic box, without pelleting; this treatment having the highest retention of 1.33×10^3 viable cells/seed among the unpelleted treatments.

Taking 1×10^4 viable cells/seed as standard, the treatments tested can be graded as follows in descending order of total cell viability with strain UASB 125 after two hours of storage. Unpelleted seeds covered with a wet cloth were best, followed by unpelleted but stored in a closed plastic box, than by lime pelleted and stored in shade and lastly by bauxite pelleted and stored in shade. The normal practice of dry seeds after treatment, in shade, kept the required viable number for one hour only in the case of both the experimental strains.

Although the longevity of strain CB 1024 on cowpea seeds showed the same trend in the different treatments as in the case of UASB 125, it proved to be far more vulnerable

Table 2: Viability of Rhizobium CB 1024 (cells x 10³/seed) on cowpea seed

Treatment	Time in hours									
	0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	24.0	
<u>Unpelleted</u>										
Control: Shade	I	44.84 (100)	21.97 (48.99)	15.68 (34.96)	5.83 (13.00)	1.02 (2.27)	0.26 (0.57)	0.01 (0.03)	0.002 (0.004)	0 0
Sun	II	46.00 (100)	13.68 (29.73)	7.67 (16.67)	3.35 (7.28)	0.65 (1.40)	0.004 0	0 0	0 0	0 0
Wet cloth	III	45.15 (100)	30.99 (68.62)	26.71 (59.15)	17.33 (38.37)	10.47 (23.18)	4.50 (9.96)	2.35 (5.19)	0.80 (1.26)	0.33 (0.73)
Plastic box	IV	45.55 (100)	31.31 (68.73)	26.50 (58.17)	18.21 (39.97)	13.34 (29.28)	7.44 (16.33)	5.73 (12.57)	2.12 (0.04)	0.67 (0.01)
<u>Pelleted</u>										
Lime	V	43.20 (100)	33.37 (77.23)	26.30 (60.87)	20.10 (46.52)	15.48 (35.83)	9.78 (22.63)	9.14 (11.15)	5.37 (12.43)	1.74 (0.04)
	VI	43.53 (100)	31.16 (71.57)	21.36 (49.06)	16.64 (38.22)	10.33 (23.72)	6.08 (13.95)	4.93 (11.31)	0.87 (1.99)	0.05 (0.001)
Rock phosphate	VII	44.86 (100)	22.46 (50.06)	17.77 (39.60)	10.76 (23.93)	5.57 (12.42)	3.51 (7.81)	0.23 (0.50)	0.004 0	0 0
	VIII	46.58 (100)	20.88 (44.81)	10.68 (22.93)	4.00 (8.59)	1.30 (2.78)	0.47 (1.00)	0.001 (0.02)	0 0	0 0
Bauxite	IX	45.56 (100)	35.40 (77.69)	25.17 (55.25)	22.27 (48.87)	16.50 (36.21)	12.09 (26.52)	5.62 (12.33)	1.50 (3.28)	0.25 (0.54)
Sun	X	44.21 (100)	31.72 (71.76)	23.32 (52.75)	13.75 (31.10)	9.54 (21.57)	5.35 (12.10)	2.96 (6.69)	1.21 (2.74)	0.05 (0.11)

Values in the parentheses indicate survival as a percentage of the original number on the seed.

to direct sunlight - practically disappearing from the seed surface within two hours, unlike the latter, which remained viable to a larger extent over a period of four hours. However, the two strains showed only marginal differences in the death rate in the case of seeds stored in an air-tight plastic box. Albeit, the decline of strain CB 1024 was sharper, and relatively more rapid under all the conditions of storage tried. The various treatments in the decreasing order of survival of strain CB 1024, taking 1×10^4 cells/seed as standard, were : bauxite pelleted and kept in shade was the best, followed closely by lime pelleted and kept in shade, then by unpelleted and stored in a plastic box, and lastly by unpelleted and covered with a wet cloth.

At 24 hours, the highest survival of strain CB 1024 (1.74×10^3 cells/seed) was obtained when the seeds were pelleted with lime and stored in shade, followed by storage in a plastic box without pelleting. Covering treated seeds with a wet cloth, maintained the viability of rhizobia on them for six hours, which was comparable to pelleting with bauxite.

One problem, commonly met with in the present studies was the rapid growth of contaminant fungi, at 24 hours, in the treatment where a wet cloth was used to cover the seeds. However, such fungal growth occurred only to a very limited

extent in the seeds stored in a plastic box, and was practically absent in the pelleted treatments.

In soil:

The ability to survive, or even possibly thrive in the rhizosphere of non-host species, especially weeds may be a desirable character that could ensure the survival of inoculants over the hot, dry summer season. In the present case, this was studied by using a streptomycin resistant mutant - M₂ of strain CB 756. A common leguminous weed Mimosa pudica supported a population of 10³ cells per gram soil in its rhizosphere. This reservoir of viable cells contributed to maintaining a population of 10⁻¹⁰³ cells in the soil away from its roots. Among the common non-leguminous weeds, Cynodon dactylon supported the growth of CB 756-M₂ to some extent. Cyperus spp., did not favour the growth of this strain, or possibly had an unfavourable effect on it. Despite this, some cells were able to subsist in soil away from its roots. Similarly, Acanthospermum spp., did not encourage the growth of rhizobia in its rhizosphere, but a scant population subsisted in soil away from its roots. The roots of sorghum plants did not harbour rhizobia to any appreciable extent while the rhizosphere of finger millet plants supported the growth of cells in their tens and hundreds only. However, introduced rhizobia survived better in the non-rhizosphere soil in pots where these crops were grown. Cowpea, the host legume, supported a maximum population

Table 3: Survival of Rhizobium CB 756-M₂ in
the rhizosphere of non-host plants

Crop/weed	Rhizosphere	Non- rhizosphere
Cowpea	17.1×10^5	23.3×10^3
Ragi	100	110
Jowar	17	390
<u>Cynodon dactylon</u>	483	630
<u>Cyperus</u> spp.	0	17
<u>Acanthospermum</u> spp.	1	7
<u>Mimosa pudica</u>	3.82×10^3	2.40×10^3

An initial population of 1×10^9 cells was added as
inoculum to each pot

of 10^5 - 10^6 cells per gram soil of the inoculant strain in its rhizosphere. It also helped to contribute to the soil pool of rhizobia, by virtue of its high rhizosphere effect.

Effect of organic amendments:

Rhizobia being preeminently soil organisms, form an important part of the soil microbial population. Addition of farm yard manure (FYM) is known to favour better survival and multiplication of bacteria in soil. An experiment was designed to study the effect of organic amendments on the survival of the test strain CB 756-M₂ in sterile and non-sterile soils. From the data in table 4 it is clear that introduced rhizobia maintained a relatively large profile in soil, in the absence of competition, even when no nutrient additions were made. In the face of competition from their native counterparts, and possibly also from other microbes in soil, their numbers were subdued as is seen in treatment two. The addition of FYM triggered their multiplication, but the initial increase in population was not sustained, and ultimately numbers declined to a basal level within four weeks. Incorporation of cowpea residues in place of FYM actually diminished numbers during the first and second week after inoculation. Despite this initial setback, numbers rose in the third week and finally reached a lower basal level at the end of four weeks.

Native rhizobia were better adapted to saprophytic

Table 4: Effect of organic amendments on survival in soil of Rhizobium CB 756-M₂ as compared to native rhizobia (cells x 10³)*

Treatment	Time in weeks											
	0			I			II			III		
	N	I	N	N	I	N	N	I	N	N	I	I
Sterile soil (control)	0.00	17.00	0.00	4.06	0.00	1.24	0.0	1.24	0.0	980.00	0.00	54.00
Unsterile soil	21.40	19.00	4.30	0.34	7.6	39.0	41.0	6.91	0.52	0.18		
Unsterile soil + FYM	21.40	47.00	0.54	52.20	2.07	434.0	32.0	76.20	2.79	0.67		
Unsterile soil + cowpea residues	21.40	34.00	0.172	0.092	5.20	0.041	4.6	5.41	0.741	0.59		

*Each value is the mean of three replicates.

N = Native rhizobia

I = Introduced rhizobia

survival than the introduced strain CB 756-M₂, in the long term, as they attained comparatively higher numbers at the end of four weeks, than the inoculant strain, despite their much lower initial population in soil. However, the addition of FYM tended to stimulate the growth and multiplication of the introduced strain far more than the native rhizobia for a short time, but it was unable to maintain the high numbers achieved for a long time, - four weeks in this case.

Effect of moisture stress before germination:

Enumeration of rhizobia on the seed after treatment, by plate counting gives an idea of the number of viable cells that are introduced by inoculation. However, it does not indicate whether this surviving number is sufficient to bring about nodulation of the host especially, under adverse conditions. Having got an idea of the number of cells that survive the process of sowing in the earlier experiment, it was decided to set up an experiment to find out to what extent such surviving cells could nodulate the host. Hence, survival of rhizobia as affected by moisture regimes in soil was studied. Varying degrees of water stress in soil were created by withholding watering for varying lengths of time after sowing.

From the data presented in table 5 it can be made out that with-holding watering upto the third day did not adversely affect rhizobial survival, as all the plants tested were nodulated. Thereafter, a decline in the number

Table 5: Effect of initial water stress on survival of inocula and nodulation of cowpea

Treatment	Time in days to first watering									
	1	2	3	4	5	6	7	8	9	10
Uninoculated (i)	-	-	-	-	-	-	-	-	-	-
(ii)	+	+	+	-	-	-	-	-	-	-
UASB 125 (i)	+	+	+	***	***	-	-	-	-	-
(ii)	+	+	+	+	***	-	-	-	-	-
UASB 125 + Lime pellet (i)	+	+	+	+	+	+	***	-	-	-
(ii)	+	+	+	+	+	+	+	-	-	-
UASB 125 + Bauxite pellet (i)	+	+	+	+	+	+	+	-	-	-
(ii)	+	+	+	+	+	+	+	-	-	-
UASB 125 + Rock-phosphate pellet (i)	+	+	+	-	-	-	-	-	-	-
(ii)	+	+	+	+	-	-	-	-	-	-
CB 756-M ₂ (i)	+	+	+	-	-	-	-	-	-	-
(ii)	+	+	+	-	-	-	-	-	-	-
CB 756-M ₂ + Lime pellet (i)	+	+	+	+	+	+	-	-	-	-
(ii)	+	+	+	+	+	+	***	-	-	-
CB 756-M ₂ + Bauxite pellet (i)	+	+	+	+	+	+	***	-	-	-
(ii)	+	+	+	+	+	+	-	-	-	-
CB 756-M ₂ + Rock phosphate pellet (i)	+	+	+	+	-	-	-	-	-	-
(ii)	+	+	+	+	-	-	-	-	-	-

(i) - Sterile soil

(ii) - Unsterile soil

+ - 3 out of 3 replications nodulated

*** - Two out of three replications nodulated

+* - One out of 3 replications nodulated

of nodulated plants set in, which finally ended on the seventh day, with no further nodulation occurring thenceforth. Nodulation ceased to occur after the fifth day in the unpelleted treatments, with both strains of Rhizobium tried. Similarly it also ended on the fourth day, when rock phosphate was used for pelleting treated seeds, showing that rock phosphate did not protect the rhizobia from desiccation to any appreciable extent. Inoculated seeds that were pelleted with lime or bauxite, retained sufficient rhizobia for nodulation upto the seventh day. There were no differences in nodulation between the competitive and non-competitive situations in any of the treatments tried. Nodulation did not occur after the third day, in the control with unsterile soil. Strain UASB 125 nodulated the host more often on the seventh day than strain CB 756-M₂, under similar conditions. In general, both the strains were able to nodulate the host for a longer period under stress, when they were protected by means of pelleting.

A composite field experiment was laid out to study the response of cowpea variety IGFRI-895 to five different strains of Rhizobium, and to study the competitive ability of these inoculants in forming nodules when compared to native rhizobia. Further, treatments were overlaid to know the effect of cutting on nodule senescence, release of bacteroids, and their subsequent multiplication and ability to reinfect the host and form nodules. Finally, the effect

of rotations and following on the persistence of the introduced rhizobia with and without re-inoculation as well as the vertical and horizontal spread of inoculants were traced.

Response to inoculation:

Nodule number increased from the twentieth to sixtieth day in all cases, but the differences in mean nodule number/plant between treatments and control were significant only at sixty days. The uninoculated control without nitrogen had the largest mean number of nodules/plant (143), followed by inoculation with strain UASB 125 at 60 days. Although the number of nodules/plant in the inoculated treatments were less than those in the uninoculated plants, they were considerably larger in size, more fleshy, with deep red colouration inside, generally confined to the tap root and crown regions and borne invariably in clusters. By contrast, the large number of nodules borne by the uninoculated plants were small, often dirty white or green inside and scattered over the roots and on laterals.

This fact is further corroborated by the significantly higher dry matter obtained in all the inoculated treatments as compared to the uninoculated control. The dry weights of inoculated plants were on par with those of plants in the nitrogen control. There was no correlation between nodule number and dry weight. Differences noted in nodule number

Table 6: Response of cowpea variety IGFRI-895 to different strains of Rhizobium (Nodule number and shoot dry weight in g/plant)

Treatment	Time in days from sowing					
	20		40		60	
	N. No.	D.W.	N. No.	D.W.	N. No.	D.W.
Uninoculated control	27	1.73	103	3.86	143	8.65
Nitrogen control	26	2.88	74	5.57	94	10.90
Strain UASB 94	24	3.37	66	3.45	91	12.08
Strain UASB 101	23	3.10	66	4.93	82	11.73
Strain UASB 125	23	2.77	59	5.66	120	12.00
Strain CB 756-M ₂	25	2.78	65	4.73	87	10.92
Strain CB 1024	24	2.48	56	4.93	67	11.57
F value	0.41	2.83	1.10	3.46	4.185	7.13
Significant at 1 per cent	NS	*	NS	*	**	**
C.D. at 5 per cent level					35.49	1.29

* Significant at five per cent level

N. No. - Nodule number/plant

D.W. - Dry weight in g/plant

and shoot dry weight were more pronounced at 60 days as compared to those at 20 or 40 days.

Effect of cutting:

Cutting is known to bring about shedding and senescence of nodules, and thereby contribute to the soil rhizobial pool, which may serve as inoculum for subsequent nodulation. To study the effect of cutting on nodule senescence and re-generation with growth, one set of plants ~~was~~ cut close to the ground, while the other was left as such. Nodule number and dry weight of shoots observed five days and thirty-five days after cutting are recorded in table 7. The collected nodules were serotyped to know the ability of released rhizobia to infect and nodulate the cut plants when they had regenerated.

Almost 100 per cent nodule senescence occurred after cutting of the shoots. The rate of re-nodulation was higher than that of uncut plants upto 100 days. A comparison between 40-day old plants grown from seed (table 6) and 35 day old plants, re-generated after cutting, revealed that the former had a far larger number of nodules. Un-inoculated plants had a higher mean number of nodules than the inoculated plants after cutting, but their nodules were smaller in size and restricted to the periphery of lateral roots, whereas those on inoculated plants were larger, and near the tap root. Eventhough no secondary inoculum was applied, the infectivity of inoculants was enhanced after

Table 7: Response to inoculation and cutting in cowpea variety IGFR1-895.
(Dry weight of shoots, nodule number/plant and fodder weight
at 5 and 35 days after cutting)

Treatment	Time in days after cutting									
	5					35				
	Cut		Uncut		N. No.	Cut		Uncut		N. No.
	N. No.	D.W.	N. No.	D.W.		N. No.	F.W.	N. No.	F.W.	
Uninoculated control	1	3.40	79	35.17	27	17.17	91	25.33		
Nitrogen control	2	5.10	35	42.30	14	17.67	55	25.33		
UASB 94	0	4.50	43	43.67	15	24.67	45	27.50		
UASB 101	2	4.57	47	46.00	14	21.00	41	28.00		
UASB 125	0	4.33	42	41.00	16	19.00	35	26.00		
CB 756-M ₂	1	4.60	47	42.67	16	20.00	56	25.33		
CB 1024	2	4.27	44	38.87	16	19.33	32	25.67		
Mixed inoculum	2	4.40	45	45.9	17	17.67	54	26.33		

Each value is the mean of three replications, which are, in turn, the mean of five individual observations

N. No. - Nodule number/plant
D.W. - Dry weight in g/plant
F.W. - Fodder weight in kg/plot

Analysis of variance for dry weight per plant
for data in Table 7

Source	F-test	C.D.
<u>Rhizobium</u> strain	NS	-
Cutting	**	7.27
R x C	NS	2.099

Analysis of variance for fodder weight per plot

Source	F-test	C.D.
<u>Rhizobium</u> strain	**	12.78
Cutting	**	7.13
R x C	*	4.79

cutting. The response of plants to native rhizobia in terms of nodule number, was massive, and the retention of nodules formed by them on 100-day old uncut plants was significantly higher, compared to those formed by the inoculants. Although dry weights of individual plants were significantly higher in the inoculated treatments, as compared to control, their aggregate fresh weights in terms of fodder yield per plot, did not differ from that of the control plots.

Effect of crop rotation

Continuous cultivation of the host - cowpea:

Given the favourable stimulus of a host root system in soil, it is expected that rhizobia will thrive such as when cowpea follows cowpea over 3 seasons. From the results presented in table 8, it is apparent that, not merely rhizobia in general, but even a specific strain will build up its strength in soil under such circumstances. There is a marked increase in percentage infectivity of the inoculant strains in general and of strains UASB 94, UASB 125 and CB 1024 in particular. That the strains have maintained their individuality with respect to their competitiveness is defined by the highly significant Chi-square test.

A comparison of the percentage infectivity of inoculants in the second season crop with their overall

Table 8: Percentage infectivity of inoculants on
cowpea in the second season

Strains	Total No. of nodules	No. identical with inoculum	Percentage infectivity	
			Season II	Season I
UASB 94	282	126	84.00	66.30
UASB 101	248	93	62.00	59.50
UASB 125	286	131	87.30	81.25
CB-756-M	268	114	76.00	61.87
CB 1024	245	128	85.30	76.87
χ^2 value			39.02	

mean infectivity in the first season shows that each strain has improved in its share in the nodule complement of the host.

Effect of re-inoculation:

Native rhizobia in the uninoculated treatments continued to form a significantly larger number of nodules (mean = 31) than the inoculants (mean = 19) in the third season. Re-inoculation of the seeds with the experimental strains did not in itself change the number of nodules formed. Re-inoculation in combination with the effects of Rhizobium strain and also with the residual effect of cutting, significantly reduced the number of nodules formed, when compared to the native rhizobia, despite the addition of fresh inoculum in the third season. The residual effect of cutting in the uninoculated sub-treatments resulted in a marginal increase in the number of nodules per plant. Similarly, the interaction between cutting and crop rotation had no definitive residual effect on nodule number per plant. All the first order interactions except cutting x crop rotation, as well as the second order interactions were highly significant. Thus, treatment effects in various combinations brought about a significant change in the number of nodules per plant, although individually they did not affect it materially. This is further evident from the distinct significance of the effects of Rhizobium strain on inoculation which did not bring forth any clear cut

Table 9: Residual effect of crop rotation, cutting and inoculation on cowpea-Rhizobium symbiosis.
Number of nodules/plant in the third season
(30 days after sowing)

Treatment	Strains							
	C (I)	N.C. (II)	94 (III)	101 (IV)	125 (V)	756-M ₂ (VI)	1024 (VII)	Mixed (VIII)
<u>Number of nodules per plant</u>								
<u>Uninoculated</u>								
<u>Cut</u>								
a	35	24	28	16	22	17	12	17
b	31	24	16	21	18	17	18	16
c	29	26	20	19	18	13	13	22
d	27	25	16	20	24	17	17	17
<u>Uncut</u>								
a	40	24	21	21	21	19	14	20
b	34	21	18	20	20	19	17	18
c	35	21	13	21	20	16	15	17
d	25	20	22	22	18	16	12	14
<u>Reinoculated</u>								
<u>Cut</u>								
a	40	23	23	20	23	15	19	15
b	35	27	23	19	21	18	16	18
c	29	24	23	14	20	17	18	16
d	23	21	21	18	21	19	11	15
<u>Uncut</u>								
a	36	25	19	22	20	17	14	17
b	32	20	18	20	14	18	16	16
c	24	22	17	14	17	17	14	17
d	25	26	21	18	17	18	18	17

Rotations: a - Cowpea-cowpea-cowpea C = Uninoculated control
 b - Cowpea-ragi-cowpea N.C. = Nitrogen control
 c - Cowpea-fallow-cowpea
 d - Cowpea-bare fallow-cowpea

Analysis of variance for data in Table 9

Source	F-test	C.D. at 5%
Rhizobium (A)	**	1.64
Inoculation (B)	NS	-
Cutting (C)	*	0.82
Rotation (D)	**	1.19
A x B	**	2.38
A x C	**	2.38
A x D	**	3.37
B x C	**	1.19
B x D	NS	-
C x D	*	1.68
A x B x C	**	3.37
A x B x D	**	4.76
A x C x D	**	4.76
B x C x D	**	2.38
A x B x C x D	**	3.89

Table 9-a: Additional statistical information. Mean nodule number per plant per subtreatment for cutting

	Treatments							
	I	II	III	IV	V	VI	VII	VIII
<u>Uninoculated</u>								
Cut	30.50	24.75	20.00	19.00	20.50	16.00	15.00	18.00
Uncut	33.50	21.50	18.50	21.00	19.75	17.50	14.50	17.00
<u>Inoculated</u>								
Cut	31.75	23.75	22.50	17.75	21.25	27.25	16.00	16.00
Uncut	29.25	23.25	18.75	18.50	17.00	17.50	15.50	16.00
Mean	31.25	24.31	19.94	19.94	19.06	17.06	15.25	17.00

Table 9-b: Additional statistical information. Mean
nodule number per plant per sub-treatment
for rotations

	Rotation			
	a	b	c	d
<u>Uninoculated</u>				
Cut	21.370	29.125	20.000	20.37
Uncut	22.500	14.630	19.750	18.63
<u>Inoculated</u>				
Cut	22.125	22.125	20.125	18.63
Uncut	21.250	19.250	17.750	20.00
Mean	21.840	19.030	19.410	19.410

numerical trend.

Am mono-crop rotation with cowpea, grown continuously, supported the highest number of both inoculants as well as native rhizobia in soil. This is reflected in the mean number of nodules per plant, ^{it} being the highest in this sub-treatment, among the four different types of rotations tried. Survival of native rhizobia under the cereal crop, ragi, was better than that under weeds or bare fallow, as the nodule number in this sub-treatment is the second highest and larger than that in the latter two, among the uninoculated treatments. However, the same cannot be said of the inoculant strains. No distinct differences could be made out between the weeded and unweeded fallows.

The data in table 10 show that nodulation at 60 days was higher in all cases, irrespective of the treatments, than at 30 days. Rhizobium strain and crop rotation have maintained their distinct influence on nodule number per plant at the 60th day as in the observation taken at 30 days. The superior infectivity of native rhizobia has been retained in that the number of nodules per plant in the uninoculated control treatments with and without combined nitrogen are far more than in the inoculated treatments. Nodule number per plant is highest with persistent rhizobia and similarly in the treatments with the residual effect of cutting than in the re-inoculated treatments and those with no residual effect of cutting.

Table 10: Residual effect of crop rotation, cutting and inoculation on the cowpea-Rhizobium.
Number of nodules per plant in the third season (60 days after sowing)

Treatment	Strains							
	C (I)	N.C. (II)	94 (III)	101 (IV)	125 (V)	756-M ₂ (VI) ²	1024 (VII)	Mixed (VIII)
<u>Nodule number per plant</u>								
<u>Uninoculated</u>								
<u>Cut</u>								
a	103	94	73	81	86	87	74	85
b	86	82	64	82	77	79	86	87
c	88	88	87	65	84	57	72	85
d	85	94	68	73	73	67	63	88
<u>Uncut</u>								
a	92	94	76	78	86	71	81	86
b	91	89	87	81	74	67	67	69
c	98	87	68	69	78	64	89	61
d	78	85	62	75	80	68	70	63
<u>Reinoculated</u>								
<u>Cut</u>								
a	96	82	86	87	77	67	87	75
b	97	81	75	68	78	68	72	74
c	85	86	65	63	67	58	64	77
d	79	92	66	60	64	71	56	72
<u>Uncut</u>								
a	95	92	85	83	80	73	72	73
b	93	78	79	75	78	65	70	78
c	92	93	64	74	65	64	65	84
d	78	74	62	68	67	67	57	57
<u>Rotations:</u>								
a - Cowpea-cowpea-cowpea					C = Uninoculated control			
b - Cowpea-ragi-cowpea					N.C = Nitrogen control			
c - Cowpea-fallow-cowpea								
d - Cowpea-barefallow-cowpea								

Analysis of variance for data in Table 10

Source	F-test	C.D. at 5%
<u>Rhizobium</u> : (A)	**	8.83
Inoculation (B)	**	4.42
Cutting (C)	NS	-
Rotation (D)	**	6.25
A x B	NS	-
A x C	NS	-
A x D	NS	-
B x C	NS	-
B x D	NS	-
C x D	NS	-
A x B x C	*	17.67
A x B x D	NS	-
A x C x D	NS	-
B x C x D	NS	-

Table 10-a: Additional statistical information. Mean nodule number per plant per subtreatment; for cutting

	Treatments							
	I	II	III	IV	V	VI	VII	VIII
<u>Uninoculated</u>								
Cut	90.50	89.50	73.00	77.75	80.00	72.50	73.50	86.25
Uncut	89.75	88.75	73.25	75.75	79.50	67.50	76.75	69.75
<u>Inoculated</u>								
Cut	89.25	85.25	73.00	69.50	71.50	66.00	69.75	74.00
Uncut	89.50	84.25	72.50	75.00	72.50	67.25	66.00	73.00
Mean	89.75	86.91	74.50	75.87	68.31	71.56	75.87	75.75

Table 10-b: Additional statistical information, mean
nodule number per plant per subtreatment:
for rotation

	Rotation			
	a	b	c	d
<u>Uninoculated</u>				
Cut	85.37	80.38	78.25	77.63
Uncut	83.00	78.13	76.75	72.63
<u>Inoculated</u>				
Cut	82.13	76.63	70.63	70.00
Uncut	81.63	77.00	75.13	86.25
Mean	83.03	78.03	75.19	71.63

However, when their numbers were augmented by re-inoculation the inoculants diminished this difference in the reinoculated/^{into}cutting treatments. Mean number of nodules was highest in the sub-plots cropped to legume continuously, followed by cereal in rotation with legume, then by unweeded fallow rotated with legume and lastly by bare fallow in the intermission between successive legume crops. This ^{is} enlarged population was essentially comprised by the inoculant strains as confirmed by the markedly increased percentage infectivity in these plots shown in table 16.

Re-inoculation had not affected nodule number per plant in any of the strains tested at 30 days, but at 60 days under its influence the inoculant strains formed significantly fewer nodules when compared to those formed by native rhizobia. However, the introduced rhizobial strains continued to retain their identity with regard to the number of nodules they formed on the host in the third season.

The second and third order interactions of the imposed treatments were significant in their effect on nodule number at 30 days, but not at 60 days, except for the interaction among rhizobial strain, re-inoculation and cutting.

Effect of crop rotation, cutting and re-inoculation on dry weight of plant tops:

In the preceding tables an account of the

Table 11: Residual effect of crop rotation, cutting and inoculation on the cowpea-Rhizobium symbiosis (30 days after sowing). Dry weight of shoots (g/plant) in the third season

Treatment	Strains							
	C (I)	N.C. (II)	94 (III)	101 (IV)	125 (V)	756-M ₂ (VI)	1024 (VII)	Mixed (VIII)
<u>Uninoculated</u>								
<u>Cut</u>								
a	1.44	1.84	2.56	2.06	2.57	2.44	2.29	2.38
b	1.26	1.83	2.14	2.96	2.52	2.43	2.46	2.19
c	1.35	2.00	1.93	2.18	2.35	2.65	2.21	2.61
d	1.24	1.93	1.97	2.36	2.57	2.70	2.29	2.14
<u>Uncut</u>								
a	1.40	2.05	2.10	2.06	2.75	2.47	2.23	2.38
b	1.50	1.95	2.20	2.11	2.43	2.47	2.61	2.25
c	1.65	1.98	2.19	2.49	2.50	2.08	2.31	2.36
d	1.53	2.18	2.30	2.47	2.53	2.21	2.39	2.77
<u>Reinoculated</u>								
<u>Cut</u>								
a	1.71	2.03	2.36	2.19	2.35	2.45	2.05	2.31
b	1.42	2.15	2.28	2.12	2.70	2.50	2.29	2.78
c	1.51	2.21	1.99	2.53	2.43	2.30	2.29	2.45
d	1.46	2.03	1.84	2.40	2.48	2.09	2.47	2.39
<u>Uncut</u>								
a	1.45	2.51	2.17	2.41	2.55	2.25	2.46	2.14
b	1.52	1.95	2.13	2.41	2.48	2.43	2.16	2.40
c	1.50	2.21	2.02	2.05	2.44	2.59	2.76	2.59
d	1.23	2.14	2.60	2.17	2.76	2.38	2.36	2.13

Rotations: a - Cowpea-Cowpea-Cowpea C = Uninoculated control
 b - Cowpea-Ragi-Cowpea N.C. = Nitrogen control
 c - Cowpea-Fallow-Cowpea
 d - Cowpea-Bare fallow-Cowpea

Analysis of variance for data in Table 11

Source		F-test	C.D. at 5%
<u>Rhizobium</u>	(A)	**	0.17
Inoculation	(B)	NS	-
Cutting	(C)	NS	-
Rotation	(D)	NS	-
A x B		**	0.24
A x C		NS	-
A x D		NS	-
B x C		NS	-
B x D		NS	-
C x D		NS	-
A x B x C		**	0.34
A x B x D		**	0.47
A x C x D		**	0.47
B x C x D		**	0.24
A x B x C x D		**	0.39

Table 11-a: Additional statistical information. Mean nodule number per plant per sub treatment: for cutting

	Treatments							
	I	II	III	IV	V	VI	VII	VIII
<u>Uninoculated</u>								
Cut	1.520	2.043	2.198	2.282	2.552	2.308	2.390	2.438
Uncut	1.323	1.923	2.150	2.212	2.503	2.555	2.313	2.330
<u>Inoculated</u>								
Cut	1.430	2.203	2.230	2.260	2.558	2.413	2.433	2.320
Uncut	1.530	2.110	2.118	2.310	2.490	2.340	2.280	2.483
Mean	1.451	2.070	2.174	2.266	2.526	2.404	2.354	2.393

Table 11-b: Additional statistical information. Mean
nodule number per plant per subtreatment:
rotations

	Rotation			
	a	b	c	d
<u>Uninoculated</u>				
Cut	2.200	2.149	2.160	2.150
Uncut	2.180	2.190	2.195	2.298
<u>Inoculated</u>				
Cut	2.181	2.280	2.214	2.145
Uncut	2.241	2.185	2.270	2.227
Mean	2.200	2.200	2.201	2.203

Table 12: Residual effect of crop rotation, cutting and inoculation on cowpea-Rhizobium symbiosis
60 (60 day old). Dry weight of shoots in g/plant in the third season

Treatment	Strains							
	C (I)	N.C. (II)	94 (III)	101 (IV)	125 (V)	756-M ₂ (VI)	1024 (VII)	Mixed (VIII)
<u>Uninoculated</u>								
<u>Cut</u>								
a	22.02	24.30	26.20	25.87	25.90	24.53	23.27	26.63
b	21.00	25.63	25.53	31.37	27.30	25.20	23.50	25.93
c	21.47	26.60	27.53	27.57	27.93	26.47	25.83	28.70
d	20.13	23.30	28.17	26.97	24.17	26.90	24.73	25.73
<u>Uncut</u>								
a	19.30	27.57	26.30	27.00	28.47	26.30	25.77	26.27
b	20.70	20.67	30.77	30.07	24.60	27.57	24.10	23.93
c	21.40	26.77	30.20	24.80	24.47	26.93	24.53	27.00
d	22.57	24.53	29.07	26.53	25.63	27.80	26.50	29.07
<u>Reinoculated</u>								
<u>Cut</u>								
a	19.47	26.23	27.60	26.63	28.60	27.77	26.93	27.57
b	22.20	27.57	27.10	27.23	28.77	26.47	26.00	28.70
c	21.40	23.53	26.60	26.83	28.87	28.57	27.93	27.47
d	22.30	26.30	25.97	27.83	29.20	27.73	25.50	25.53
<u>Uncut</u>								
a	22.73	28.40	27.77	29.97	28.57	28.50	25.90	26.50
b	21.03	23.57	27.77	25.06	30.20	30.30	26.63	29.33
c	21.73	26.10	24.83	26.97	29.87	27.43	26.60	28.50
d	18.63	23.57	26.30	27.70	28.53	29.53	28.03	27.00
<u>Rotations:</u>								
a	- Cowpea-cowpea-cowpea				C	= Uninoculated control		
b	- Cowpea-ragi-cowpea				N.C.	= Nitrogen control		
c	- Cowpea-fallow-cowpea							
d	- Cowpea-barefallow-cowpea							

Analysis of variance for data in Table 12

Source		F-test	C.D. at 5%
Rhizobium	(A)	**	1.43
Inoculation	(B)	**	0.71
Cutting	(C)	NS	-
Rotation	(D)	NS	-
A x B		**	2.02
A x C		NS	-
A x D		NS	-
B x C		NS	-
B x D		NS	-
C x D		NS	-
A x B x C		**	2.85
A x B x D		**	4.03
A x C x D		NS	-
B x C x D		**	4.03
A x B x C x D		**	3.29

Table 12-a: Additional statistical information. Mean nodule number per
plant per subtreatment; for cutting

	I	II	III	IV	V	VI	VII	VIII
<u>Uninoculated</u>								
Cut	20.99	24.89	29.08	27.10	25.78	26.65	25.23	26.58
Uncut	21.16	24.93	26.86	27.85	26.30	25.78	24.33	26.77
<u>Inoculated</u>								
Cut	21.03	25.41	26.67	27.43	29.29	28.94	26.29	27.83
Uncut	21.34	25.90	26.57	27.13	28.86	27.65	26.59	27.32
Mean	21.13	25.28	27.28	27.40	27.56	27.26	25.61	27.13

Table 12-b: Additional statistical information.
 Mean nodule number per plant per
 subtreatment: for rotations

	Rotations			
	a	b	c	d
<u>Uninoculated</u>				
Cut	24.84	25.68	26.51	25.01
Uncut	25.87	25.05	25.76	26.46
<u>Inoculated</u>				
Cut	26.23	26.76	26.40	26.29
Uncut	27.29	26.74	26.50	25.91
Mean	26.06	26.06	26.29	25.92

residual effects of cutting, and crop rotation as well as the effect of re-inoculation on the number of nodules formed on the host crop in the third season has been rendered. The effects of these experimental variables on the weight of dry matter produced by the host shoots is presented in tables 11 and 12, at 30 and 60 days, respectively.

At 30 days, mean dry weight per plant was the lowest in the uninoculated control treatment without combined nitrogen. This was also evident from the significant improvement in dry weight due to inoculant strains even at one per cent level of probability, as well as by their effect in combination with re-inoculation. However, re-inoculation as such did not alter the dry weight of plants materially.

Although continuous raising of cowpea conferred a distinct advantage to the population of rhizobia in soil, as seen from the data on nodule number, this advantage did not apparently reflect in the dry weight of shoots. There were no differences among the rotation treatments with regard to shoot dry weight.

The residual effect of cutting did not affect shoot dry weight in the third season.

Competition:

In order to nodulate the host, rhizobia introduced along with the seed must be able to compete

Table 13: Percentage infectivity of inoculant rhizobia in 40 and 60 day old cowpea plants

Strains	Total number of nodules		Percentage infectivity		Mean
	at 40 days	at 60 days	at 40 days	at 60 days	
Native uninoculated	309	428	0	0	0.00
UASB 94	198	273	54	64	59.00
UASB 101	198	247	49	59	54.00
UASB 125	176	359	67	82	74.50
CB 756-M ₂	180	265	42	71	56.50
CB 1024	169	200	73	68	70.50
X ² values			26.68**	13.91**	

In each case 100 nodules were taken at random for typing, regardless of the total number present in the treatment

**Significant at 1%

Table 14: Percentage infectivity of inoculant rhizobia in 70 and 100 day old cowpea plants, with and without cutting

Strain	Total number of nodules				Percentage infectivity			
	Cut		Uncut		Cut		Uncut	
	100 days	70 days	100 days	70 days	100 days	70 days	100 days	70 days
Native Uninoculated	274	237	82	0	0	0	0	0
UASB 94	134	128	45	71	69	69	69	69
UASB 101	124	140	42	62	58	58	64	64
UASB 125	106	127	43	85	86	86	79	79
CB 756-M ₂	167	140	49	63	77	77	51	51
CB 1024	97	131	49	81	83	83	73	73
χ^2 value				**	**	**	NS	NS

Where 100 or more nodules were present, only 100 were taken for serotyping at random, otherwise all the nodules were typed

**Significant at 1%

Table 15: Effect of phosphate fertilization and pelleting on infectivity of Rhizobium strains UAS B 125, CB 756-M2 and CB 1024 in field grown cowpeas

Strains	25 kg P ₂ O ₅ /ha				50 kg P ₂ O ₅ /ha				75 kg P ₂ O ₅ /ha				Mean
	C ₀	C ₁	C ₂	C ₃	C ₀	C ₁	C ₂	C ₃	C ₀	C ₁	C ₂	C ₃	
<u>Native uninoculated</u>	1.	100	83	101	103	143	143	120	156	175	153	145	
	2.	0	0	0	0	0	0	0	3	0	0	0	
	3.	0	0	0	0	0	0	0	6	0	0	0	
<u>UASB 125:</u>	1.	84	97	78	85	112	92	102	117	125	123	114	
	2.	29	38	38	31	34	39	42	31	43	38	35	
	3.	58	76	76	62	68	78	84	62	86	76	70	72.30
<u>CB 756-M₂:</u>	1.	82	80	64	88	106	93	84	118	109	145	152	
	2.	26	32	31	24	23	28	35	24	28	22	23	
	3.	52	64	62	48	46	56	70	48	56	44	46	54.83
<u>CB 1024:</u>	1.	83	72	90	81	120	117	110	110	154	143	156	
	2.	31	31	29	27	32	23	32	25	21	37	29	
	3.	62	62	58	54	64	46	64	50	42	64	58	57.83
<u>Mixed:</u>	1.	82	91	82	84	81	122	121	146	140	133	149	
	2.	35	41	36	31	33	44	42	34	43	40	38	
	3.	70	82	72	62	66	88	84	68	86	80	76	76.16
X ² value		51	87	76	60	61	98	101	48	108	86	73	

1. Total number of nodules in that observation.
2. Number identical with inoculant among those typed.
3. Percentage infectivity.

successfully with numerous native rhizobia that are already present in soil. In order to know the capability of the five strains used in the present experiments, to compete with native rhizobia when outnumbered by a ratio of nearly $1:10^4$, nodules obtained from plant samples taken from the field were serotyped by the Ouchterlony gel-immune diffusion technique, to identify the strain of Rhizobium in them. These data expressed as a percentage of the number of nodules taken for typing gave an idea of the competitive ability of each strain, and are presented in tables 13, 14 and 15.

The mean infectivity of all the five strains over a period of 60 days was more than 50 per cent, indicating that they were successful competitors against native rhizobia. Among them, strain UASB 125 stood out as being clearly superior to the rest, tallying an average of 74.5 per cent of the nodules on the host. CB 1024 was a close second with an average of 70.5 per cent. All the strains except CB 1024 improved their infectivity from the 40th to the 60th day, and even in the case of CB 1024, the drop in infectivity was only marginal. Strains UASB 125 and CB 1024 were the most competitive, while UASB 101 was a poor competitor, the other two being intermediate, in this respect. The Chi-square test for proportions of nodules formed by the experimental strains was highly significant. Progressive increase in infectivity of the experimental strains with time was seen from 40 to 60 days

and further from then on to the seventieth day after sowing. The increase in percentage infectivity recorded thereafter upto the hundredth day, was especially prominent in case of strain UASB 101, whereas in the case of strain CB 756-M₂ it actually declined during this phase of plant growth.

At sixty days, regardless of the treatment, the mean dry weight per plant had increased as compared to that at 30 days, but it was significantly higher in the inoculated treatments (mean = 26 g) when compared to the uninoculated control, without combined nitrogen (mean = 21 g). Re-inoculation of seed in the third season brought about a significant increase in the dry weight of plant tops as compared to plants that were nodulated by inoculant strains of rhizobia carried over from the initial inoculation three seasons (12 months) ago. Cutting and crop rotation continued to have no residual effect, as such, or in combination, on the dry weight of shoots. A combination of Rhizobium strain and re-inoculation enhanced shoot dry weight significantly. These two factors in turn interacted with cutting and crop rotation to bring about a significant increase in the dry matter production.

Persistence of introduced rhizobia

Persistence of the introduced rhizobia was measured by their percentage infectivity in the third season crop. Strains CB 1024, UASB 94 and 125 persisted fairly well in soil, over the three seasons, strain CB 756-M₂, though

Table 16: Persistence of inoculant rhizobia in the third season as affected by cutting, crop rotation and reinoculation

Strains	Cut					Uncut				
	Conti- nuous cowpea	Ragi	Fallow	Weeded fallow	Conti- nuous cowpea	Ragi	Fallow	Weeded fallow		
<u>Uninoculated</u>										
Control	(a) (b)	310 12	250 0	265 0	255 4	275 0	272 0	294 0	235 0	
UASB 94	(a) (b)	219 69	193 42	262 23	204 21	228 68	261 35	204 36	186 11	
UASB 101	(a) (b)	243 54	245 22	194 13	248 14	233 47	244 28	206 18	226 11	
UASB 125	(a) (b)	267 63	230 52	252 31	219 39	258 64	223 42	234 27	241 30	
CB 756-M ₂	(a) (b)	262 49	236 26	172 16	200 11	212 43	201 21	193 15	203 18	
CB 1024	(a) (b)	223 59	258 38	216 22	188 23	244 61	200 31	266 30	210 25	

continued..

Table 16 (contd.)

Strains	Cut				Uncut			
	Conti- nuous cowpea	Ragi	Fallow	Weeded fallow	Conti- nuous cowpea	Ragi	Fallow	Weeded fallow
<u>Reinoculated</u>								
Control	{a} {b}	287 0	290 10	256 0	236 0	255 16	279 0	275 0
UASB 94	{a} {b}	257 93	226 82	196 84	197 73	254 91	236 79	292 72
UASB 101	{a} {b}	260 86	204 78	188 54	180 49	250 68	224 53	221 44
UASB 125	{a} {b}	231 94	233 100	202 78	191 76	280 89	233 81	194 73
CB 756-M ₂	{a} {b}	201 78	205 65	174 69	214 63	220 84	195 73	193 68
CB 1024	{a} {b}	261 87	215 76	191 72	168 65	215 88	210 79	194 71

(a) Total number of nodules in the treatment

(b) Percentage infectivity of the strain from those typed.

Table 16-a: Mean infectivity of inoculants as
influenced by cutting and reinoculation

Mean infectivity of individual strains of Rhizobium

Strains	Cut	Uncut	Grand mean
<u>Uninoculated</u>			
UASB 94	38.75	37.50	38.125
UASB 101	25.75	26.00	25.875
UASB 125	46.25	40.75	43.500
CB 756-M ₂	25.50	24.25	24.875
CB 1024	35.50	36.75	36.125
<u>Reinoculated</u>			
UASB 94	83.00	77.50	80.250
UASB 101	66.75	54.25	60.500
UASB 125	87.00	79.75	83.375
CB 756-M	68.75	69.00	68.875
CB 1024	75.00	77.50	76.250

Table 16-b: Mean infectivity as affected by cutting

Crop rotation	Cut			Uncut		
	Reinoculated	Uninoculated	Mean	Reinoculated	Uninoculated	Mean
Cowpea-cowpea-cowpea	87.60	58.80	73.20	84.00	56.50	70.25
Cowpea-ragi-cowpea	80.20	36.00	76.10	73.00	31.40	52.20
Cowpea-fallow-cowpea	71.40	21.00	46.20	65.60	25.20	45.40
Cowpea-bare fallow-cowpea	65.20	21.60	43.40	63.80	19.00	41.40
Mean	55.225			52.3		

persistent, did not maintain numbers as well as the former three strains. Strain UASB 101 persisted relatively poorly under the same conditions. Differences in persistence between plots which received additional rhizobia from sloughed and shed nodules after cutting as compared to plots where no such effect was carried over were only marginal. Re-inoculation of plots with the same strain as that first introduced and which persisted after the original introduction, strongly favoured the competitive-position of the strain, enhancing its percentage infectivity dramatically.

Persistence was highest in plots where the host roots were continually present as in a mono-crop sequence of cowpea. It was considerably lower in a rotation of finger millet with cowpea, further diminished in fallow land where weeds grew uninhibited. In soil freed of all vegetation, regularly, persistence was the lowest. In plots that were inoculated a second time in the third season after fifteen months, these differences were virtually wiped out.

Unlike the other parameters, namely nodule number per plant and dry weight of shoots, the percentage infectivity remained fairly consistent for a given strain, both, with the same crop at different stages of growth as well as with different crops sown in sequence with an intermission of nearly a year.

Response of cowpea to phosphorus fertilization and seed pelleting:

To study the effect of simple modifications in agronomic practices such as application of phosphate fertilizer and use of pelleting on the response of cowpea to different strains of Rhizobium in terms of nodulation and dry matter production, an experiment was undertaken under field conditions. The results obtained are presented in tables 17 and 18.

Effect on nodule number per plant:

Application of 25, 50 and 75 kg P_2O_5 per ha had no effect on nodule number, at 30 and 60 days, but at 90 days the effect was significant.

Introduced rhizobial strains differed significantly with respect to nodule number, in all the three stages of plant growth studied, as compared to resident rhizobia in soil. Plants inoculated with the experimental strains had significantly fewer nodules than those in the uninoculated control treatment, at all levels of phosphate application. Pelleting with lime, bauxite, or rock phosphate had no effect on nodule number per plant at any of the stages of growth, at any of the levels of phosphate tested.

Phosphate application in combination with strain of Rhizobium was highly significant at 30 and 60 days, but not at 90 days. Application of 75 kg phosphorus brought about maximum nodulation with native rhizobia at 30, 60 and

Table 17: Effect of phosphate fertilization, pelleting and Rhizobium strain on nodule number per plant in 30, 60 and 90 day old cowpea

Strain	25 kg/ha				50 kg/ha				75 kg/ha			
	C ₀	C ₁	C ₂	C ₃	C ₀	C ₁	C ₂	C ₃	C ₀	C ₁	C ₂	C ₃
<u>30 days</u>												
Native uninoculated	21	22	30	26	19	22	26	18	27	26	37	30
UASB 125	17	12	21	23	32	25	32	20	24	29	30	21
CB 756-M ₂	26	22	19	24	32	40	39	26	15	18	31	16
CB 1024	24	21	19	20	22	24	17	19	25	18	18	34
Mixed	28	18	30	19	24	29	27	14	24	38	22	27
<u>60 days</u>												
Native uninoculated	21	17	22	27	26	23	28	27	31	32	27	38
UASB 125	17	21	24	22	25	23	30	19	40	31	20	29
CB 756-M ₂	21	17	9	22	18	19	27	16	23	29	22	20
CB 1024	23	19	16	25	19	16	30	20	28	16	30	29
Mixed	22	23	8	23	10	8	30	23	33	21	16	28
<u>90 days</u>												
Native uninoculated	33	27	34	34	48	48	46	40	52	58	51	48
UASB 125	28	32	26	28	37	31	31	34	33	39	42	51
CB 756-M ₂	27	27	21	29	35	31	28	33	36	36	48	51
CB 1024	28	24	30	27	40	39	37	34	37	51	48	52
Mixed	27	30	27	28	27	41	40	34	49	47	44	50

Each value represents the mean of 3 replications, which are in turn the mean of 5 individual observations

C₀ = Unpelleted; C₁ = Lime pelleted; C₂ = Bauxite pelleted
C₃ = Rock phosphate pelleted

Analysis of variance for data in Table 17

Source	F-test	C.D.
<u>30 days</u>		
Phosphate (P)	NS	-
<u>Rhizobium</u> strains (R)	**	2.93
P x R	**	5.07
Pelleting	NS	-
P x C	NS	-
R x C	NS	-
P x R x C	NS	-
<u>60 days</u>		
Phosphate	NS	-
<u>Rhizobium</u> strains	**	1.49
P x R	**	2.58
Pelleting	NS	-
P x C	NS	-
R x C	NS	-
P x R x C	NS	-
<u>90 days</u>		
Phosphate	**	7.08
<u>Rhizobium</u> strains	**	3.42
P x R	NS	-
Pelleting	NS	-
P x C	NS	-
R x C	NS	-
P x R x C	**	10.52

Table 17-a: Additional statistical information
mean nodule number per plant per
treatment (pelleting)

<u>Rhizobium strains</u>	<u>P levels</u>			
	<u>P_I</u>	<u>P_{II}</u>	<u>P_{III}</u>	<u>Mean</u>
<u>30 days</u>				
R ₀	24.75	21.25	30.00	25.34
R ₁	18.25	27.25	26.0	23.84
R ₂	22.75	34.25	20.00	25.67
R ₃	21.75	26.50	23.75	22.00
R ₄	23.75	26.00	27.75	25.84
Mean	22.25	25.85	25.50	
<u>60 days</u>				
R ₀	21.75	26.25	32.00	26.57
R ₁	21.00	24.25	30.00	25.09
R ₂	17.25	20.00	23.50	20.25
R ₃	20.75	21.25	25.75	22.59
R ₄	21.50	22.75	24.50	22.92
Mean	20.45	22.90	27.15	
<u>90 days</u>				
R ₀	32.00	45.50	52.25	43.25
R ₁	28.50	33.75	40.00	34.09
R ₂	26.00	31.75	42.75	33.50
R ₃	27.25	37.50	47.00	37.25
R ₄	28.00	35.50	47.50	37.00
Mean	28.35	36.80	45.90	

Table 17-b: Mean nodule number per plant per
treatment for Rhizobium strains

<u>Rhizobium</u> strain	Mean
R ₀	31.75
R ₁	27.67
R ₂	26.47
R ₃	27.28
R ₄	28.58

Table 17-c: Mean nodule number per plant per
treatment for phosphate levels

Phosphate levels	Mean
P _I	23.68
P _{II}	28.51
P _{III}	32.85

90 days. Nodule number correlated directly with phosphate level.

The interactions of phosphate level with Rhizobium strain and pelleting did not affect nodule number per plant at any age of the crop studied. The interaction of phosphate level with Rhizobium strain and pelleting affected nodule number only at 90 days, and not earlier. Seventy-five kg P_2O_5 along with native rhizobia and lime pelleting gave the maximum mean number of nodules per plant (58).

Effect on shoot dry matter:

Phosphorus application did not affect plant dry weight at 30 days, but increased it at the higher levels of P_2O_5 at 60 and 90 days. Application of 75 kg P_2O_5 per ha, gave the maximum dry weight per plant both at 60 and 90 days, but at 60 days the inoculants increased dry matter content, over the uninoculated control. Inoculant strains were superior to native rhizobia in respect of plant dry weight produced.

The interaction of phosphorus and Rhizobium strain was significant only where the effect of Rhizobium was significant, while the effect of pelleting was significant only where the effect of Rhizobium was not significant.

Phosphorus level and pelleting had no effect on dry weight in combination, while Rhizobium strain and

Table 18: Effect of phosphate fertilization, pelleting and *Rhizobium* strains on mean dry weight per plant at 30, 60 and 90 days after sowing in cowpea variety IGFRI-895

Strain	P _I				P _{II}				P _{III}			
	C ₀	C ₁	C ₂	C ₃	C ₀	C ₁	C ₂	C ₃	C ₀	C ₁	C ₂	C ₃
<u>30 days</u>												
Native uninoculated	1.04	1.33	1.14	1.12	1.27	1.19	1.27	1.25	1.04	1.07	1.20	1.08
UASB 125	0.97	1.19	1.45	1.16	1.22	1.17	1.54	1.41	0.92	1.09	1.06	0.95
CB 756-M ₂	1.74	1.27	1.32	1.07	1.36	1.24	1.03	1.41	0.94	1.37	1.31	1.22
CB 1024	0.90	1.21	0.98	1.13	1.09	1.13	1.20	1.39	0.98	0.78	1.10	1.13
Mixed	1.18	1.20	1.08	1.23	1.27	1.54	1.24	1.36	1.00	1.04	1.10	1.06
<u>60 days</u>												
Uninoculated	7.68	12.23	12.67	12.41	11.62	12.81	15.44	13.17	11.34	10.91	15.97	14.61
UASB 125	10.80	9.18	8.12	16.30	12.55	13.52	15.75	13.14	12.13	14.57	16.25	16.83
CB 756-M ₂	11.12	14.25	13.56	10.27	11.67	13.18	13.98	12.66	12.69	16.51	16.59	17.49
CB 1024	9.81	14.39	15.73	13.73	9.08	14.57	14.06	15.84	10.32	14.06	17.93	17.84
Mixed	15.55	10.21	16.12	14.92	10.00	14.21	15.03	15.36	10.26	13.67	14.78	14.98
<u>90 days</u>												
Uninoculated	20.75	20.64	20.48	22.29	24.36	24.08	24.06	23.75	31.58	32.40	35.65	35.72
UASB 125	25.14	24.74	26.67	26.27	35.20	33.10	37.95	37.76	44.90	56.39	59.11	49.14
CB-756-M ₂	26.65	26.71	27.10	27.15	32.85	38.51	37.87	37.04	44.07	55.26	56.00	56.00
CB 1024	27.16	28.29	26.50	25.63	32.02	42.19	40.23	41.83	48.90	61.72	58.26	60.85
Mixed	29.83	27.34	27.13	27.36	38.14	43.86	38.36	42.26	51.45	59.00	60.00	60.00

Each value represents the mean of three replications which are in turn, the mean of five individual observations.

C₀ = Unpelleted; C₁ = Lime pelleted; C₂ = Bauxite pelleted; C₃ = Rock phosphate pelleted

Analysis of variance for data in Table 18

Source		F-test	C.D.
<u>30 days</u>			
Phosphate	(P)	NS	-
<u>Rhizobium</u> strain	(R)	NS	-
P x R		NS	-
Pelleting	(C)	**	0.08
P x C		NS	-
R x C		NS	
P x R x C		*	0.35
<u>60 days</u>			
Phosphate		*	1.44
<u>Rhizobium</u> strain		NS	-
P x R		NS	-
Pelleting		**	1.11
P x C		NS	-
R x C		*	2.48
P x R x C		**	4.29
<u>90 days</u>			
Phosphate		**	2.98
<u>Rhizobium</u> strain		**	1.83
P x R		**	3.18
Pelleting		NS	-
P x C		NS	-
R x C		NS	-
P x R x C		NS	-

Table 18-a: Mean Additional statistical information,
mean dry weight per plant as influenced
by phosphate level, strain or Rhizobium
and pelleting

<u>Rhizobium</u> <u>strains</u>	P levels			
	P _I	P _{II}	P _{III}	Mean
<u>30 days</u>				
R ₀	1.16	1.25	1.20	1.17
R ₁	1.40	1.34	1.01	1.25
R ₂	1.35	1.26	1.21	1.28
R ₃	1.08	1.21	1.00	1.10
R ₄	1.18	1.35	1.05	1.20
Mean	1.24	1.29	1.07	1.20
<u>60 days</u>				
R ₀	11.25	13.26	13.21	12.58
R ₁	11.04	13.74	14.95	13.25
R ₂	12.30	12.88	15.82	13.67
R ₃	13.43	13.39	15.04	13.96
R ₄	14.20	13.65	13.43	13.76
Mean	12.45	13.39	14.49	13.45
<u>90 days</u>				
R ₀	21.04	24.07	33.84	26.32
R ₁	25.71	36.02	52.39	38.04
R ₂	26.66	36.57	52.96	39.73
R ₃	26.90	39.07	57.44	41.14
R ₄	27.92	40.73	57.62	42.09
Mean	25.65	35.30	50.85	37.31

Table 18-b: Mean dry weight per plant per treatment
for Rhizobium strains

<u>Rhizobium</u> strain	Mean
R ₀	13.35
R ₁	17.09
R ₂	17.96
R ₃	18.73
R ₄	19.02

Table 18-c: Mean dry weight per plant per treatment
for phosphate levels

Phosphate levels	Mean
P _I	13.10
P _{II}	16.66
P _{III}	22.14

pelleting together were significant at 5 per cent level of probability, only at 90 days, and not at 30 and 60 days. Phosphate application combined with inoculation and pelleting was significant at 5 per cent level at 30 days and at 1 per cent level of probability at 90 days. However, it was not significant at 60 days.

Sixty day old plants were more responsive to phosphate level and inoculation with Rhizobium as compared to 30 or 90 day old plants.

Effect on grain and fodder yield:

The effects of phosphate fertilization, inoculation and pelleting on grain yield of cowpea variety IGFRI-895 are presented in table 19. The highest grain yield of 391 g per plot was obtained with mixed inoculant at 75 kg P_2O_5 , followed by inoculation with CB 1024 and CB 756-M₂. Thus, the same strain did not give similar effect on grain and fodder yield. Pelleting either singly or in combination with other treatments did not affect grain and fodder yield of cowpea.

Maximum fodder yield was obtained with strain CB 756-M₂ supplemented with 75 kg P_2O_5 per ha, followed by strains CB 1024 and mixed inoculant, at the same level of phosphorus.

All the strains used viz., UASB 125, CB 756-M₂ and CB 1024 as well as their combination gave significantly higher

Table 19: Effect of phosphate fertilization, pelleting and Rhizobium strains on grain yield (in grams/plot) of cowpea

Fertilizer in kgs.	Uninoculated	UASB 125	CB 756-M ₂	CB 1024	Mixed
<u>10 kgs</u>					
C ₀	220.30	271.00	230.33	246.33	228.33
C ₁	260.00	243.30	256.67	248.00	281.67
C ₂	233.30	234.30	252.00	267.67	280.00
C ₃	266.00	272.67	232.33	268.33	251.67
<u>20 kgs</u>					
C ₀	268.70	278.67	265.33	294.00	255.67
C ₁	315.80	288.67	267.00	208.00	280.00
C ₂	272.67	392.67	295.33	299.67	290.00
C ₃	288.3	297.00	298.33	325.00	310.67
<u>30 kgs</u>					
C ₀	308.67	311.67	309.33	302.67	306.67
C ₁	339.67	339.33	392.33	378.33	361.33
C ₂	294.3	367.00	377.67	362.67	394.00
C ₃	278.3	359.67	339.00	357.00	391.00

Each value represents the mean of three replications, which are in turn the mean of 5 individual observations

C₀ = Unpelleted

C₁ = Lime pelleted

C₂ = Bauxite pelleted

C₃ = Rock phosphate pelleted

Analysis of variance for data in Table 19

Source		F-test	C.D.
Phosphate	(A)	**	0.03
<u>Rhizobium</u>	(B)	**	0.01
A x B		**	0.02
Pelleting	(C)	NS	
A x C		NS	
B x C		NS	
A x B x C		NS	

Table 20: Effect of phosphate fertilization, pelleting and Rhizobium strain on fodder weight (in kg/plot) in cowpea

Fodder in kgs	Uninocu- lated	UASBE125	CB 756-M ₂	CB 1024	Mixed
<u>10 kgs</u>					
C ₀	12.87	15.13	13.93	15.00	12.73
C ₁	13.73	16.30	16.37	16.73	15.40
C ₂	12.77	15.03	15.97	16.90	14.43
C ₃	14.00	15.73	15.43	15.67	13.00
<u>20 kgs</u>					
C ₀	14.63	16.47	17.00	16.27	16.60
C ₁	17.23	18.20	19.80	22.40	22.33
C ₂	17.57	19.70	22.30	19.13	20.20
C ₃	16.70	19.23	19.90	20.87	19.97
<u>30 kgs</u>					
C ₀	16.57	19.60	20.97	21.67	22.43
C ₁	20.80	24.10	25.90	25.30	25.33
C ₂	18.20	25.17	26.37	24.27	24.80
C ₃	17.97	24.20	25.43	24.27	23.30

Each value represents the mean of three replications which are in turn the mean of individual observations

C₀ = Unpelleted

C₁ = Lime pelleted

C₂ = Bauxite pelleted

C₃ = Rock phosphate pelleted

Analysis of variance for data in Table 20

Source		F-test	C.D.
Phosphate	(A)	**	2.33
<u>Rhizobium</u>	(B)	**	0.97
A x B		**	1.67
Pelleting	(C)	NS	
A x C		NS	
B x C		NS	
A x B x C		NS	

yield of grain and fodder at 1 per cent level of probability, when compared with the uninoculated control. Pelleting with lime, bauxite or rock phosphate, did not materially change the yield of cowpea, although it may have played a role in improving the competitive position of inocula, by aiding their survival on the seed.

Host variety versus Rhizobium strain interaction

The results presented so far, evidently give an idea of the utility of minor modifications in routine agronomic practices, such as soil fertility enhancement, by application of super phosphate, or the practice of pelleting inoculated seeds, or both, in achieving successful establishment of desirable strains of rhizobia in the field, and ultimately of realising higher crop yields. However, it may not be possible to achieve this objective entirely, by protected infiltration with suitable rhizobia. It is known that genetic factors in the host determine the preferential selection of a compatible rhizobial genotype for nodulation from the myriad strains in arable soils. To arrive at the best host legume (cultivar) and bacterial genome combination, under the agro-climatic conditions obtaining in Bangalore, a split-split plot experiment was laid out, using combinations of 12 cultivars of cowpea and five rhizobial strains. The results obtained in terms of grain yield are presented in table 21. Statistical analysis of the data gave an idea of

Table 21: Interaction of Rhizobium strains (5) with host (cowpea) varieties (12) under field conditions (grain yield in g/plot)

Cow pea varieties	Control	NO 3 control	UASB 94	UASB 101	UASB 125	CB 756-M ₂	CB 1024	Mixed	Mean
C-152	456.67	616.67	596.67	550.00	520.00	495.00	550.00	486.67	533.98
Co Pusa-2	346.67	496.67	425.00	430.00	440.00	450.00	418.33	536.67	441.67
Cowpea 2-1	495.00	630.00	633.33	506.67	620.00	501.67	531.67	498.33	552.83
S-288	218.33	388.33	416.67	361.67	343.33	301.67	346.67	398.33	346.87
FS-68	263.33	561.67	381.67	311.67	333.33	410.00	498.33	489.33	411.41
Dixiles	253.33	290.00	295.00	273.33	333.33	310.00	318.33	301.67	296.87
IGFRI-895	293.33	460.00	426.67	426.67	466.66	421.67	440.00	406.67	417.78
Iran grey	440.00	670.00	548.33	523.33	535.00	490.00	571.67	576.67	544.37
Lolita	570.00	633.33	666.67	626.67	593.33	576.67	503.33	643.33	601.67
Mississippi Purple	203.33	563.33	386.67	348.23	443.33	396.67	431.67	473.33	405.33
Selection-2	311.67	538.33	505.00	386.67	386.67	421.67	386.67	386.67	415.46
Virginia	196.67	321.67	246.67	241.67	300.00	231.67	220.00	278.33	254.58
Mean	336.86	516.60	460.69	414.72	442.91	417.22	434.72	457.08	

Each of the above observations is the mean of three replications, rounded off to the second decimal place.

Analysis of variance for data in Table 21

Source		F value			Signi- ficance	C.D.
		Calculated	Table			
			5%	1%		
Varieties	(1)	8.07	2.37	3.36	**	
<u>Rhizobium</u> strains	(2)	6.55	1.97	2.66	**	
(1) X (2)					N.S.	

the significant differences among the various host cultivars as well as the different strains of rhizobia used in the experiment. However, it also revealed that the variations did not interact significantly. Maximum yield of 670 g per plot was given by variety Iran Grey with the nitrogen from an application of 100 kg N per ha, in the nitrogen control. The mean yields of the varieties were the highest with the nitrogen control, followed by inoculation with strain UASB 94, then by mixed inoculum, which was followed by UASB 125 and CB 756-M₂ and finally by UASB 101. Among the varieties tried, Lolita gave the highest yield and in combination with UASB 94 it gave the second highest mean seed yield plot.

Variability among host genotypes was far greater than among rhizobial strains.

Spread of seed coat inoculum

Figure 7 shows the relative location of nodules formed by inoculant rhizobia on 60 day old plants from the point of sowing. At 30 days the vast majority of nodules are confined to the crown region and tap root and with in a distance of 8 cm laterally and 14 cm downwards indicating that migration of inoculum from the seed coat has been very limited, if at all any.

Figure 8: In plants whose roots were cut the inoculant strains are largely infective at greater depths

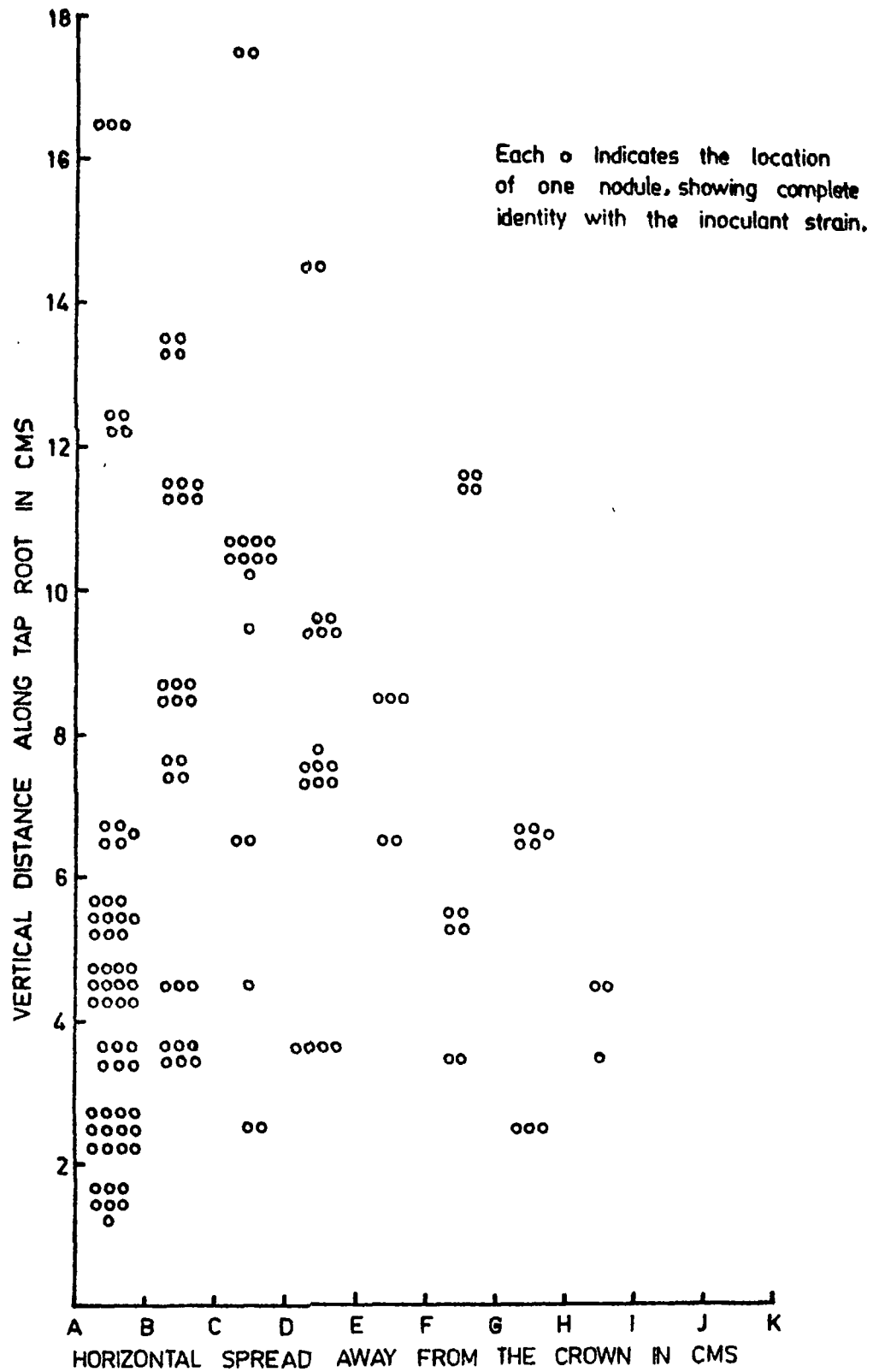
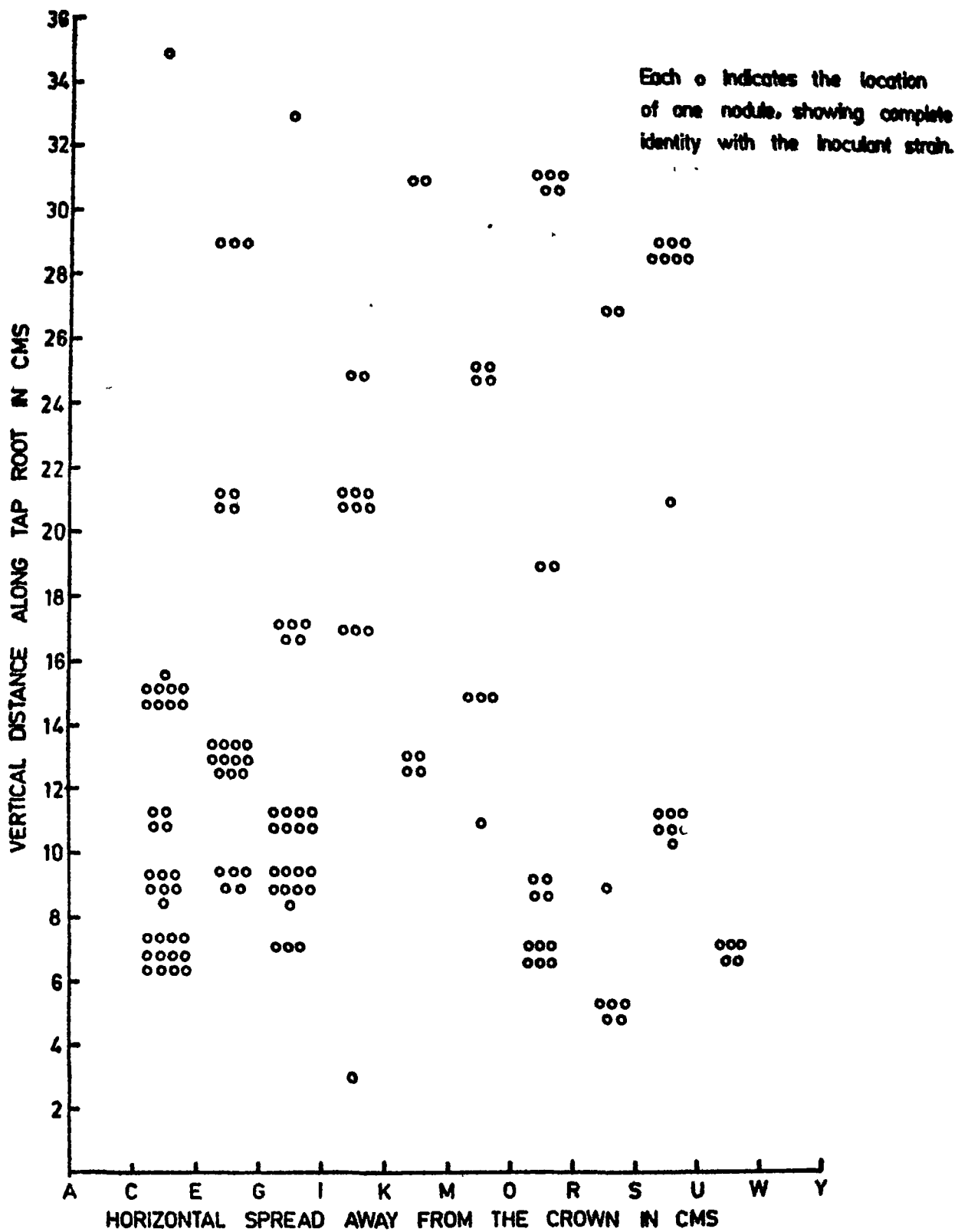


Fig. 7. Spread of seed coat inoculum on roots of 60 day old cowpea plants.



on the lateral fringe of their respective spread while they occur at relatively shallower depths in case of uncut plants. One possible inference is that these bacteria have moved down after being released from nodules that were shed in the same lateral plane soon after cutting, along with fresh secondary and tertiary root growth. A comparison of Figures 1 and 2 tends to indicate such a trend.

Figure 9 illustrates the distribution of persistent rhizobia in soil, while Figure 10 shows the location of inoculant rhizobia as well as that of persistent cells that have remained in soil after introduction along with seeds in the initial crop of cowpea.

A striking common feature in all the former graphs is the slightly greater mean depth at which the inoculants are found in it, when compared to the distribution in Figure 10. The spread of the experimental strains laterally is notable and common to all of them. Possibly this is a consequence of the spread and mixing due to the cultivation, rather than active movement of cells.

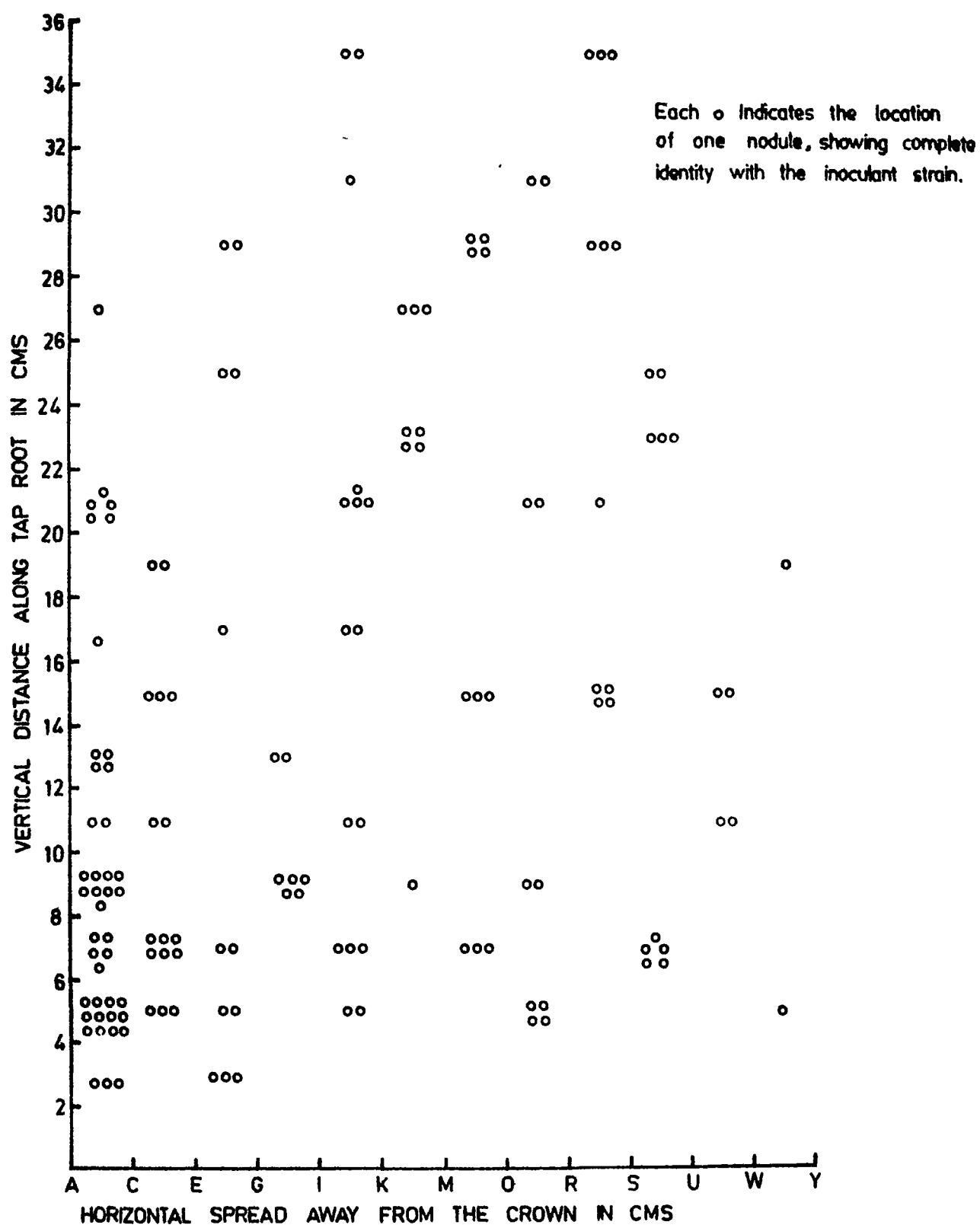


Fig.9. Spread of inoculants as affected by crop rotation.

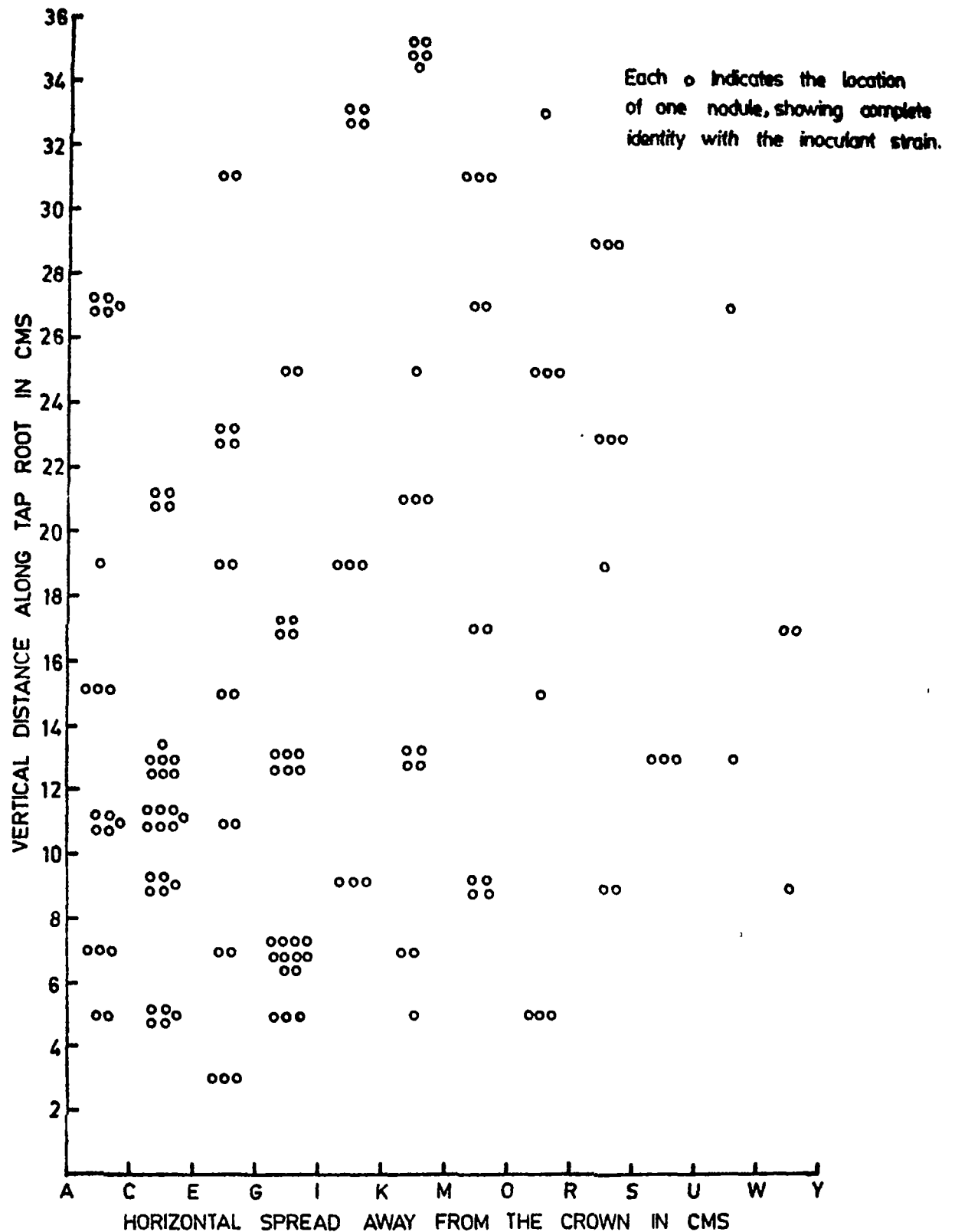


Fig.10. Location of persistent rhizobia on roots of cowpea in the (the) third season.

DISCUSSION

V. DISCUSSION

Cowpea type rhizobia are known to occur naturally and in substantial numbers in soil at many locations in India (Subba Rao, 1972). Nodulations of legumes of the cowpea miscellany is, therefore, obtained without any difficulty. However, adequate nitrogen fixation in the association with these indigenous rhizobia is not always obtained judging from the large response to inoculation with other rhizobia obtained (Sundara Rao, 1970). Introduction of strains of Rhizobium selected for their high nitrogen fixing efficiency is, therefore, essential. This is generally achieved by inoculating seeds with lignite based cultures of desirable rhizobia.

At times, rhizobia inoculated on seeds fail to reach the soil in sufficient numbers. This was one of the aspects studied herein. Apparently data in Tables 1 and 2 point out that poor survival of lignite based inoculum on the seed is one of the major factors responsible for this. Moreover, bright sunshine and windy conditions at sowing which dessicate the inoculated seeds coupled with routine agrarian practices, which expose treated seeds to these conditions, further aggravate the problem. Although, no specific minimal count of rhizobia per seed at sowing has been published under Indian conditions, guidelines worked out elsewhere for cowpea seeds could be followed with profit.

Cloonan (1966) suggested 10^3 - 10^4 viable cells of Rhizobium per seed of cowpea for obtaining effective nodulation, while Burton (1975) placed the requirement at 10^5 cells per seed. Again, Cloonan and Vincent (1967) noted that in hot, dry soils increased inoculum levels were a must for obtaining nodulation in cowpeas. However, 10^4 viable cells of the inoculant per seed would be a fair number for introduction into soil, already colonised by cowpea-type rhizobia. From the results on survival of rhizobia on seed, it is apparent that within an hour, seeds in the normal course of sowing ceased to carry the minimum requirement of 10^4 viable cells of Rhizobium on them, even if they are kept in shade. However, these findings are not uncommon, as a similar rapid decline of viable cells from broth inocula on seeds was noted by Vincent et al. (1962). Exposure to sunlight, per chance, even for a short duration of 30 minutes was found to be lethal to coated rhizobia
25 | and their numbers diminished to nearly 25 per cent of their original count. Pelleting with lime or bauxite helped retain a larger viable number of cells on the seed for at least 4-6 hours after treatment, provided they were stored in shade.

Storage of treated seeds in a plastic container capable of retaining moisture, or failing the availability of this, the use of a wet cloth to cover them, even for the short period between treatment and sowing, extended the

longevity of coated rhizobia by 3-4 hours. Hence, this practice could be advocated to farmers. The advantage of such storage is quite apparent in terms of ten to hundred fold increased survival and especially the extended period for which the required minimum of 10^4 cells per seed is maintained. The extra two to four hours thus made available for sowing operations after seed treatment by taking these simple precautions are of considerable practical importance to the farmer. Under Australian conditions, Brockwell and Whalley (1970) were able to extend the longevity of rhizobia on treated medic seeds by storing them in a plastic jar.

Another simple way of extending the longevity of rhizobia on treated seeds was by covering them with a wet cloth. However, a drawback in the usage of a wet cloth to retain moisture and humidity on the treated seeds was the undesirable growth of fungi, particularly after eight hours of storage. The possibility of dipping the cloth in a solution of fungicide, non-toxic to rhizobia, to suppress such fungal growth is worth considering for further studies.

Going in for higher level of inoculum within reasonable limits of cost benefit ratio, such as two to three times the present recommendation of 375 g/ha (of lignite based culture) would ensure higher rates of introduction of desirable rhizobia into soil.

The observed differences in numbers of survivors at any given time interval, between the two strains used in the present experiments were significant. This suggested that the fast growing strain which produced gum copiously and which was adapted to the local conditions (UASB 125) was able to survive better on seeds than the slow grower which produced gum sparingly and which was exotic (CB 1024). The presence of an average of 1.6×10^3 viable cells per seed of strain UASB 125 as compared to 53 cells of CB 1024 on lime pelleted seeds exposed to sunlight indicated that strain UASB 125 was much better adapted to overcome the rigours of strong sunlight -- mainly its drying and heating effects. Wilkins (1967) recorded that local isolates from tropical soils were better adapted to survival at high temperatures than exotic strains.

It should then be possible to identify such hardy strains for use in inoculants to ensure introduction of desirable rhizobia in adequate numbers into soil.

Rhizobia that have survived the process of introduction must be able to compete with their native counterparts for nodulation sites on the emerging radicle. Normally, an inoculation rate of 375 g of lignite based culture is recommended for treating cowpea seeds to be sown in one hectare. Thus 375×10^9 cells are introduced assuming initial count of the culture to be 10^9 cells/g.

In other words, each gram of the plough furrow layer of soil receives 185 cells (approximately), theoretically. In practice, however, $2-2.3 \times 10^8$ cells are concentrated on each seed of cowpea (assuming negligible mortality) depending upon whether 160 or 175×10^4 seeds are sown/ha (seed rate of 25-30 kg/ha). In a natural population of 10^8 bacterial cells/g of soil this would amount to a competitive ratio of $1:10^6-10^7$ of introduced rhizobial cells to total soil bacteria. In Indian soils, where counts of cowpea-type rhizobia range between 10^2-10^6 cells/g (Kumar Rao and Dart, 1979), the introduced strain would be out-numbered by a ratio of 10^2-10^4 . It can thus be plainly seen that the inoculant rhizobia must be exceptionally competitive as well as assured of a favourable environment, if they are to establish in soil. The above relation explicitly emphasises the importance of survival in numbers, on the seed in the period immediately following sowing.

Often the establishment of introduced rhizobia is short lived, despite their introduction in large numbers and lasts only till the roots of the sown host remain in soil. Accordingly, the results presented in Table 3 imply that without the stimulus of a host root system in soil, the numbers of rhizobia introduced by inoculation of an initial host crop tend to decline at rates dependent on their intrinsic ability to survive under

the conditions obtaining in soil.

In order to compete successfully and colonise soil, inoculant rhizobia must be able to make best use of the available, and especially added carbon substrates like farm yard manure to multiply and increase their numbers, saprophytically. Koleshko (1978) followed the development of nodule bacteria in soil and found cell generation to be a function of the content and availability of nutritive substances. Further, it was characterised by periods of adaptive, active growth and stabilization of numbers in the population. The duration of each period being determined by the sum of ecological factors. Avrov and Zhurbina (1968) were able to obtain improved nodulation by the application of straw. They also observed that straw permitted the multiplication of nodule bacteria when applied to soil. In the present case, application of farm yard manure at the rate of 0.1 g carbon per 100 g of air dried soil stimulated vigorous multiplication of strain CB 756-M₂ as well as non-descript rhizobia indigenous to the soil. Although the latter rhizobia did not attain large numbers for three weeks, their ultimate number surviving at the end of the fourth week was substantially larger than that of CB 756-M₂. Mere multiplication rate would not, therefore, indicate the ability of rhizobia to persist.

The number of cells of Rhizobium on the seed at sowing does not indicate whether this surviving minority

will be able to attain infective numbers in the rhizosphere of the germinating host, especially under adverse conditions. For instance, though sowing is always taken up after rains, there is no guarantee that rains will ensue after sowing is over. In fact, a feature of the monsoonal climate is that, precipitation occurs at irregular intervals than as a smooth flowing continuity. It is thus quite likely that the next shower of rain will occur from say one to anything upto 12 to 15 days after sowing, ⁴under such circumstances, the surface soil having been cultivated (harrowed) will soon dry up and the probability that seeds will germinate at moisture levels far below those required for the survival and multiplication of the rhizobia on them is very high. Under such circumstances desired strains of rhizobia fail to establish if rains do not occur for more than three days after sowing. On the resumption of favourable moisture regimen after rains, the seedling roots would necessarily be nodulated by native rhizobia. Experiments to simulate such breaks between two showers of rains from 1 to 10 days were conducted. In the trials recorded here, pelleting with either lime or bauxite extended the longevity of inocula so as to obtain nodulation, even if rains failed for seven days.

In studies on the survival of Rhizobium meliloti applied to medic seed pelleted with lime and sown into dry soil, Brockwell and Whalley (1970) obtained better survival

when inoculated seeds were pelleted, and when such seeds were stored in soil (by sowing) than in air tight jars they were able to recover about 10 per cent of the added peat inoculum after 200 days of storage. The wide disparity between longevity on seeds expressed above and that seen in the present studies can be explained by the different types of carrier used, the strains of rhizobia and environmental conditions.

If rains fail after sowing, the pellet on seeds would possibly act as a buffer, initially preventing sufficient moisture from reaching the seed, and thereby arresting seed germination and subsequently disintegrating when rain water washes it down into the soil. Thus, it would ensure that the seed germinates when there is also sufficient moisture for multiplication and movement of the inoculated rhizobia on it (Diatloff, 1967; Date, 1968; Brockwell and Whalley, 1970).

Better survival of inoculants on the seed confers a competitive advantage as seen in Table 15 where the pelleted treatments have a higher percentage infectivity than the unpelleted treatments. Possibly, the pellet, though short lived, remained long enough on the seed to protect the coated rhizobia against dessication, high temperature or acidic soil pH and ensured their survival in greater numbers. This conclusion is corroborated by the evidence in Tables 1 and 2, and concurs with the findings

N / of Date (1969), Cleonan and Vincent (1967) and Norris (1972).

Ireland and Vincent (1968) contended that pelleting seeds improved the relative performance of inoculants for a given number of rhizobia per seed. This made it possible for them to establish rhizobia in soil containing large numbers of homologous rhizobia, which otherwise would have required extremely heavy inoculum, according to them.

i / In the present study, it is plain that, although the experimental strains could make their mark in the absence of pelleting, the presence of a lime or bauxite pellet, did confer a distinct competitive advantage, as the soil had a very high population of native homologous rhizobia ($10^5/g$).

The capacity of efficient nitrogen fixing strains of Rhizobium to nodulate their host adequately under field conditions is crucial to the improvement of the crop. This is distinguished by significant improvement in the agronomic parameters ^{viz.,} dry weight, grain and fodder yield of the crop, as seen in the present investigation. However, their actual success or failure is revealed by nodule serotyping by the Ouchterlony gel immune diffusion technique.

In essence, the Ouchterlony gel immune diffusion method as modified by Date (1970) envisages the availability of sufficient quantity of antigen of the required concentration to fill an antigen well. However, this is true only

of nodules that are somewhat large in size, say, more than 2 mm in diameter. This would preclude the typing of numerous small nodules found in lateral and secondary roots of inoculated plants, or those which are still developing. Thus, selection of large nodules for typing would necessarily weigh in favour of the inoculant strains, and hence is likely to give inflated figures of their infectivity. This fact must be borne in mind while considering the data in Tables 13, 14 and 15. In the present work, it is seen that provided some simple precautions are taken to minimise mortality of rhizobia on treated seeds, all the five experimental strains have proved to be competitive. Albeit, a distinct gradation in this ability is recognisable with strains UASB 125 and CB 1024 at the topmost level, while strain UASB 101 is at the tail end. Hence, possibly beyond a certain threshold, mere numbers on seeds at sowing, do not decide competitiveness in themselves. Thereafter, the intrinsic capacity of each strain to colonise and multiply in soil, comes into play.

For clovers, Purchase and Nutman (1957) have determined that homologous nodule bacteria must reach a population density of 10^4 cells at the point of infection, before they can successfully initiate nodulation.

Though the precise population density required for infection of cowpeas has not been worked out, it is reasonable to assume that a similar phenomenon as in clovers is operative

here~~too~~. Hence, rhizobia introduced on seeds which survive the initial period of germination, till seedlings become receptive to them, must necessarily multiply in soil and attain infective numbers.

In the present case, based on serotype data, with the progressive increase in number of nodules per plant from 20 to 60 days, the fraction formed by inocula out of those typed, also increased. This signifies the ability of these strains to multiply in soil and overcome pressure from resident rhizobia as and when new receptive points develop in the vicinity of the tap root as indicated by data on horizontal and vertical spread. This, however, was not comparable to the capacity of resident rhizobia in terms of number of foci of infection, especially, those far removed from the tap root.

With regard to the number of nodules per plant, the inverse relationship between nodule number and size has been lucidly explained by Nutman (1963). Therein he states that, a kind of compensatory mechanism operated in bringing about an increase in the total nitrogen fixing tissue per plant by the formation of increasing numbers of small nodules when the amount of such tissues/nodules is low. It has been further shown that this character is by and large a function of the strain infecting the host, by Dilworth and Parker (1969), for a given species of host legume. Evidently, this relationship is vindicated

for 1900r | consistently in the present case, as per statistical interpretation of the data in Table 6. The tendency of native rhizobia to form numerous small nodules and thus make up for poor efficiency and low volume of nitrogen fixing tissue per nodule is quite prominent at flowering, as evident from the data in Table 6. Date (1970) has observed that effective nodules are recognised by their fewer numbers, larger size, pink central zone and healthy appearance of the host plant. He further states that ineffective nodules are small, white, very numerous and scattered over the entire root system. This, however, does not explain the relationship between nodule numbers and effectiveness entirely.

The insipient response to inoculation pertaining to dry matter of shoots seen at 20 and 40 days of growth, later resolved into superiority among the inoculated treatments over the uninoculated control. Probably, the nodules formed earlier became fully functional after 40 days and hence, their true performance was reflected in terms of shoot dry weight at 60 days.

A rough idea of the functional life of individual nodules gained from periodical visual observations indicated the period to range from 35-45 days. Nodulation being a continuous process, it is also possible that nodules formed later became functional and added to the contribution already being made by the first formed nodules, during this

period. All other factors being congenial, increased dry matter production must necessarily be the outcome of higher nitrogen fixing efficiency, but in turn, nitrogen fixing efficiency need not be directly proportional to nodulation response. There was no correlation between nodule number and dry weight, and between nodule number and effectiveness of rhizobia.

The inoculants continued to exhibit this trend after cutting and re-nodulation. In view of the fact that new growth in the proximity of the tap root was receptive to infection, rather than the tap root itself, after cutting, there was a lateral shift in the domain of the inoculants. This is evident from Fig. 7 indicating the location of nodules identifiable with the inoculants 35 days after cutting.

Greshoff et al. (197f) have demonstrated the viability of Rhizobium bacteroids from soybean nodules. In the light of this, it is quite likely that sloughed off nodules and especially those shed by the roots after grazing or cutting off of the shoots, contribute to the soil pool of rhizobia. This is also evident from the rise in percentage infectivity of inocula in plants that had re-generated 35 days after cutting, as compared to their mean infectivity before cutting. This suggestion is further fortified by the enhanced infectivity observed in the second season as compared to the mean for the first season. Thus, subsequent

sowings of legumes will benefit from the association with elite strains of Rhizobium, only if inoculants are able to translate the initial advantage of placement near the seed into a random distribution of viable cells throughout the soil. Further, when rhizobia introduced on seeds are able to tide over prolonged hot, dry conditions in summer, in the absence of their host, they will be available for nodulation of new host roots which traverse the soil in subsequent sowings. The present studies show that all the inoculant strains were able to persist somewhat poorly, as can be discerned from data presented in Table 19.

In the present studies, the inoculants, though eminently successful in competing against their native counterparts initially, were unable to sustain their numbers in the absence of the host.

Results pertaining to saprophytic survival of rhizobia in soil point out to the beneficial influence of host roots in maintaining their numbers. Normally, most pulses are not sown as pure crops in vast tracts under rainfed conditions in India. They are generally interplanted as row crops in either broadcast or drilled cereals such as finger millet, sorghum, pearl millet etc. This practice wherein legumes such as cowpea, lab-lab, green gram or black gram constitute every 8 or 10th row with cereals in between is known as 'akkadi' planting. The practice of

akkadi planting of legumes can thus serve as an important reservoir of cowpea-type rhizobia, and also aid in their perpetuation over the annual dry summer by building up a reservoir of viable cells in their rhizospheres during the monsoon. In places where a double cropping sequence is possible due to the availability of protective irrigation, a second legume crop would derive benefit from such rhizobial population build up achieved by akkadi planting in the first crop. Preparatory cultivation after the first crop to prepare the land for the second one would ensure lateral movement of rhizobia and thus distribute them in soil from points of concentration along the akkadi row. This is relevant, in that, the principal crops in India, namely, groundnut, pigeon pea and chick pea are generally sown as pure crops. Of these, the latter two are sown as rabi or semi rabi (post monsoon) crops in vast areas. Hence, an earlier inoculated legume crop, either pure or akkadi planted would help in the establishment of inoculants.

The poor showing of inoculants under the bare fallow suggests the poor capacity of inoculants to tide over the hot, dry summer months. Even where a bare fallow is not strictly practised in India, cattle are allowed to graze on the scant vegetation in the form of weeds and stubbles that comes up after the main crop is harvested. This close grazing, virtually strips the ground of all cover and is tantamount to a bare fallow. In a mono crop sequence, with a long intervening fallow period, as that followed in @

very large stretch of land, it would seem that the annual rainfed groundnut crop would thus require re-inoculation every season. The somewhat similar cropping pattern in most of the dry land areas would also demand inoculation at every successive sowing.

Nutman (1969) counted the numbers of four species of rhizobia, and found few, or sometimes none in soils continuously cropped to non-legumes, or in rotations without legumes, or in long term fallows. From a heavily inoculated and pelleted sowing of clover, Hely (1963) could recover 80 per cent nodules containing the inoculant in the first season, but by the end of the next season (uninoculated) the inoculant strains were not recovered; all the nodules having been formed by native rhizobia.

Apparently weeds failed to stem the drastic decline in the viable numbers of inoculant rhizobia in soil brought about by a unfavourable environment in the long, hot dry summer. Even the sparse leguminous weed flora did not arrest decline to any appreciable extent. This is quite conspicuous from the diminished proportion of inoculants recovered from the fallow plots from the subsequent crop. The favourable rhizosphere effect of Mimosa pudica (exhibited in pots) or other leguminous weeds was thus not translated into any tangible increase in recovery of inoculants in the field. However, when supplemented with more of their numbers, by way of inoculum in the third season, the inoculant rhizobia

nodulated the host forming most of its nodules.

This large response to inocula in the third season, especially with a supplemental dose of inoculum could be used to advantage. Cowpea for fodder followed by a pure crop of groundnuts in a double cropping sequence under rainfed conditions is possible. Cowpea being promiscuous (Doku, 1969) could be inoculated with a strain of Rhizobium that is highly efficient with groundnut to introduce desirable rhizobia in large numbers into the soil. Thick sowing of the inoculated fodder cowpea would ensure veritable permeation of the entire topsoil by these cells, thus making up for the inability of seed inocula to move appreciably in soil (Hamdi, 1970). The subsequent groundnut crop would then be practically assured of adequate nodulation by elite nitrogen fixers.

The application of high dosage of phosphorus stimulated nodulation in general in the present experiments. The rise in mean nodule number per plant with increasing dosage of phosphorus in all the treatments thus reveals the pitfalls in interpreting data on nodule numbers entirely on the basis of effectiveness in symbiotic nitrogen fixation. Nodulation was equally stimulated in the uninoculated treatments as in the inoculated treatments but the yield obtained in the latter was higher.

Phosphorus is undoubtedly the prime limiting nutrient in the establishment of tropical legumes

(Franco, 1976). In studies by Shaw et al. (1966) on a low fertility sodic soil, great increases in nitrogen fixation were recorded in response to increasing phosphorus application. In a field experiment, yield of dry matter was raised by a factor of 2.4 and yield of nitrogen by a factor of 3.3 as super phosphate equivalent was raised from 0 to 502 kg/ha and under the most favourable conditions of controlled environment, absolute nitrogen in tops was increased seven fold with increase of super phosphate equivalent from 0 to 752 kg/ha.

In the experiments described here, the higher shoot dry matter production at 30 kg P_{205} /ha in the treated plants which were effectively nodulated (inoculated) suggested that next to nitrogen, the most important limiting nutrient was phosphorus. This was very prominent at initiation of flowering (60 days) but not either before or immediately after (i.e. at 30 or 90 days). Finally, it was confirmed by the significantly higher yield of grain and fodder in the highest level of P_{205} as compared to the other two levels.

Deficiency of phosphorus, severely and frequently, limits nitrogen fixation by limiting the growth of the host plant (Norris, 1972). Although there are no clear demonstrations that it limits nitrogen fixation or nodulation, there are data suggestive of such effects in the present experiments. Possibly phosphate application

stimulated root growth, which made more points available for infection and native rhizobia were best able to take advantage of this, although the inoculants did follow up at higher levels of phosphate application.

Claims that phosphate fertilization has improved nodulation appear in reports of green house and field trials with several species (DeMooy and Pesek, 1966; Kaushik and Singh, 1969; Gates, 1974). The commonly observed increased nodulation in plants when fertilised with phosphate (Shwa et al. 1966; Andrew and Robbins, 1969; and Gates, 1974) may also indicate that maximum nitrogen fixation rates require more phosphorus than maximum growth, or it may mean simply that healthy plants have a small proportion of nitrogen poor structural and storage tissue than phosphorus deficient plants. Cowpea variety IGFRI-895 responded very well to phosphate fertilization when applied as a single basal dressing in bands. Initially, the response in terms of nodulation and dry weight of the Aerial portion of plants was rather obscured possibly by a combination of various factors, such as response to inoculation, pelleting and soil fertility corresponding to nutrients other than phosphorus. However, the response resolved with time and growth into distinct superiority in terms of grain yield and weight of fodder per plot at increased levels of P_2O_5 . The increase in these two parameters recorded when 75 kg P_2O_5 was applied per hectare (highest dose) was almost 50 per cent when

compared to plots where 25 kg P_2O_5 /ha was applied. This suggests that the present recommended dose (50 kg P_2O_5 /ha) is still inadequate, and could be enhanced to 75 kg/ha profitably in terms of increased returns per acre.

Breeding of pulse crops for resistance to pests and diseases and also for rapid and quantitative response to applied fertilizers has received considerable attention in recent years. Improved varieties so evolved have been tested in agronomic trials at several locations for evaluation of their performance in the field. The quest for host versus bacterial genome combinations best adapted to any particular region with a view to obtaining maximum benefit from the association is of prime importance. Host selection for any given strain of Rhizobium has considerable bearing on the ultimate symbiotic effectiveness of the combination. A marked host and strain interaction in nodulating competitiveness related to symbiotic effectiveness was found in case of soybean cultivars with Rhizobium japonicum strains by Diatloff and Brockwell(1978). This is of added significance in Indian conditions where soil is already inhabited by a multitude of rhizobial genotypes, which are generally less effective than the superior inoculant strains. The choice of a strain of Rhizobium which will fix the greatest amount of nitrogen with a particularly well adapted host genotype under the conditions obtaining in the region is thus imperative. In the current investigation the cowpea cultivar Lolita has proved to be the best

among the 12 varieties tried in combination with strain UASB 94. However, field observations revealed a far greater range of heterogeneity among the host genotypes than among the five bacterial genotypes, as gauged from the yield of grain per plot. Also, it is conspicuous that all the experimental strains have proved to be superior to the native rhizobia though they were not as good as a dressing of 100 kg N/ha., in their supply of combined nitrogen to the host. This begs further systematic study with a wider range of bacterial genotypes to identify the ideal strain as per specifications laid above. Working with four cowpea genotypes and four strains of Rhizobium, Minchin et al. (1978) learnt that no single strain was clearly superior for all the cultivars, although isolates from tropical soils, generally gave greater seed yields than exotic strains. Findings in the current studies are quite parallel to this as strains UASB 94 and 125 performed better than CB 756-M₂ and CB 1024 in terms of mean yield obtained with the 12 host varieties.

In summary, it can be said that in order to obtain a positive response to inoculation, it is essential that introduction of desirable rhizobia in sufficient numbers on the seed is ensured. Possible ways of achieving this are by means of pelleting or storage of treated seeds in plastic containers or by covering them ^{with} a moist cloth. However, considerable efforts by extension agencies would be required to realise the widespread use of pelleting. Moreover availability of cheap pelleting materials would pose another difficulty,

hence the latter methods offer better prospects for quick and large scale adoption by farmers. Once they have been introduced, such rhizobia should be encouraged to multiply and establish in soil by periodical sowing of their appropriate host legume. When a long fallow period intervenes between successive sowings their numbers in soil need to be augmented by annual re-inoculation. Albeit, the need for repeated inoculation is determined by the ability of inoculant rhizobia to reside in soil and especially their ability to tide over adverse conditions. Besides this, cropping pattern and the type of rotations practised under a given set of agro-climatic conditions will have a bearing on the persistence of inocula.

The spread of seed inoculum is largely a result of cultivation such as ploughing, earthing up, weeding and hoeing and to a lesser extent due to the movement of water and least due to the ability of cells to migrate from the point of initial introduction. Hence, timely inter-cultivation may help in distributing inocula throughout the soil so as to be available to the young and growing roots of the crop.

Adequate phosphate fertilization in combination with the above mentioned measures to help in the establishment of inocula is most essential if maximum nitrogen fixation is to be realised. Also due to importance to the compatibility of

strains of Rhizobium with host cultivars needs to be given in varietal trials conducted for crops at a given location to derive the best host-Rhizobium combination.

SUMMARY

VI. SUMMARY

The benefit from elite nitrogen fixing strains of Rhizobium, introduced into soil on the seed, can be derived only if they are able to establish in soil, and in years following their initial application, if they are able to persist therein.

Chori | Seed inoculation being the principal mode of introduction of rhizobia into soil, their survival in adequate numbers during the process of sowing and immediately thereafter, is crucial to their colonization of the emergent host radicle, and soil. In the present studies, desiccation of lignite based inocula coated on seeds was very rapid and lethal, so much so that, inoculation was rendered virtually ineffectual within an hour, in the normal course of operations. Survival of the depleted inoculum was prolonged by 2 to 6 hours when treated seeds were covered with a wet cloth, or kept in a closed plastic box. Pelletting with lime or bauxite also extended longevity of inocula upto 8 hours. The practical value and significance of such simple treatments to extend the viability of rhizobia on treated seeds has been discussed.

Survival in soil and in the rhizosphere of several non-host plants was assessed in pot culture trials. Two commonly grown cereal crops, namely finger millet and sorghum, supported the multiplication of the experimental strains of

Rhizobium only to a limited extent. Among three species of common non-leguminous weeds tried, Cynodon dactylon harboured cells in their tens and hundreds in its rhizosphere, while Cyperus spp., and Acanthospermum spp., did not encourage multiplication of the introduced rhizobia at all. A commonly occurring leguminous weed, namely Mimosa pudica, maintained populations upto 10^3 - 10^4 cells/g in its rhizosphere.

Organic amendments such as farm yard manure, or cowpea residue brought about a spurt in the numbers of introduced rhizobia when added to soil so as to provide 0.1 g carbon/100 g air dry soil. However, the rise in numbers was ephemeral, lasting only for two weeks at best.

A simple green house experiment indicated that if soil moisture is not replenished after sowing for a period of three days, the process of infection by surviving rhizobia on the seed would not be affected. However, any further period without water significantly diminished the chances for infection and nodulation by the seed coat inoculum. In the absence of pelleting nodulation ceased to occur after the fifth day, while the presence of a lime or bauxite pellet extended this threshold to the seventh day. Thereafter nodulation did not occur if watering was withheld. The presence of native rhizobia in soil did not have any influence on these phenomena.

Even under the most ideal conditions seed inoculation can introduce only a limited number of rhizobia. These were outnumbered heavily in the experimental plots by a ratio of nearly $1:10^4$ in soil. Despite these odds the experimental strains were able to compete successfully with strain UASB 125 and CB 1024 accounting for nearly 70 per cent of the nodules formed on the host.

Cowpea variety IGFRI-895 responded positively to inoculation with five different strains of selected rhizobia, giving significantly higher dry matter/plant and yield under field conditions, as compared to uninoculated plants, nodulated by native rhizobia.

Cutting of plant tops at flowering resulted in shedding of practically all the root nodules. The inoculant strains accounted for a significantly higher proportion of the nodules formed subsequently when the plants re-generated. This indicated that cutting conferred a competitive advantage to the inoculant strains by virtue of their augmented numbers in soil emanating from the nodules shed following removal of plant tops.

Monoculture with legumes is seldom followed in India. Even in tracts where groundnut is grown year after year, there is a long summer fallow period between successive crops taken in the monsoon. Invariably legumes are either rotated with cereals or sown as intercrops in them. In field experiments conducted over three seasons it was

revealed that presence of the host roots continually in soil was most conducive to the establishment, multiplication and persistence of selected strains of rhizobia introduced along with the first sown crop. Rotations including a cereal - finger millet with cowpea, an unweeded fallow and a weeded or bare fallow with cowpea, supported the sustenance of decreasing numbers of the original inoculant strains of Rhizobium in that order. Survival of inoculants was poorest when land was rendered weed free in the long summer fallow period (bare fallow). The five strains tried in the experiment, although able to compete successfully with native rhizobia for nodulation in the presence of the host, were unable to maintain their competitive position in the third season in plots where the host was absent in the second season. However, when supplemented with a second dose of inoculum in the third season they accounted for more than 80 per cent of the nodules on the host. The importance of akkadi planting of legumes, as well as their relevance in a double cropping sequence in the light of these facts have been discussed in detail.

Cowpea variety IGFRI-895 showed a significant positive response to applied phosphatic fertilizer. The enhanced nodulation response, dry matter production, grain and fodder yield at the highest level of phosphorus application (75 kg/ha), indicated that next to nitrogen the most important limiting nutrient was phosphorus. Data

indicated that the present recommended dose of 50 kg P_2O_5 /ha could be enhanced to 75 kg P_2O_5 /ha with profit. Pelleting of inoculated seeds with either lime or bauxite improved survival of rhizobia on the seed. This resulted in enhanced infectivity of the inoculant strains in the nodule complement of the host.

As rhizobia are applied on the seed, they must necessarily move down and sideways as the roots of the growing plant spread, in order to form nodules on the younger roots. In the field, applied inoculum did not move sufficiently to cover the entire root system of the host. It was confined to the tap root, and to proximal ends of lateral and secondary roots around it. Cutting of plant tops tended to encourage spread of the inoculants downwards, to a limited extent. All the five experimental strains of Rhizobium were confined to an area 30 cm deep and 17 cm wide (on either side) from the crown of the plant, root system. Normal cultivation operations such as earthing up, weeding and hoeing aided in the lateral spread of inoculants. Ploughing after the harvest of the legume, brought about a uniform distribution of the surviving rhizobial cells of the inoculant strains in soil. The significance of this under field conditions has been discussed.

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VII. REFERENCES

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