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PERSISTENCE OF AN INTRODUCED *BRADYRHIZOBIUM JAPONICUM* AND OTHER SOYBEAN RHIZOBIA IN A SOYBEAN/MAIZE ROTATION

C. NKWIINE
Makerere University,
Department of Soil Science,
P.O. Box 7062, Kampala, Uganda

ABSTRACT

Streptomycin resistant mutant of *Bradyrhizobium japonicum* strain IRj 2114 was developed, tested for ability to nodulate and fix nitrogen, and introduced on seeds at the start of the field experiments. Soybean (*Glycine max* L.) and maize (*Zea mays* L.) were then grown in four cropping sequences namely soybean/soybean/soybean, soybean/soybean/maize (SSM), soybean/maize/soybean, and soybean/maize/maize. Percentage soybean nodulation by the introduced *rhizobium*, and seasonal changes in soybean rhizobial populations along and between crop rows were determined using the antibiotic resistance and most probable numbers methods, respectively. Results showed the number of nodules per plant and proportion of nodules due to introduced *rhizobium* strain varied significantly ($P \leq 0.01$) with cropping sequences. In the first season, nodule recovery due to the introduced *rhizobium* was low (15%). In the subsequent seasons, maize crop adversely affected soybean nodulation. As in the third season, occupancy of mutant *rhizobium* was 60% for continuous soybean cropping (SSS), and 42% for the soybean/maize soybean (SMS). Populations of soil soybean rhizobia were similarly affected by the cropping sequences. Rhizobia numbers were significantly ($P \leq 0,05$) higher when the first two crops were soybean (SS) than when maize followed soybean (SM). Throughout the sampling period, more rhizobia occurred along the crop rows (AR) than in the inter-row spaces (BR), indicating positive effects of rhizospheres on the rhizobial population. It was concluded therefore that for successful establishment of improved strains of *B. japonicum*, a second soybean crop should follow the first inoculated crop.

Key Words: Colonization, cropping, sequence, survival

RÉSUMÉ

Le mutant de *Bradyrhizobium japonicum* race IRj 2114 rebelle à la streptomycine a été développé, testé pour la capacité de noduler et fixer l'azote, et introduit dans les graines au commencement des expériences au champ. Le soja (*Glycine max* L.) et le maïs (*Zea mays* L.) ont alors été cultivés en quatre ordre de culture à savoir: soja/soja/soja, soja/soja/maïs (SSM), soja/maïs/soja, et soja/maïs/maïs. La nodulation du soja par le *Rhizobium* introduit en pourcentage, et les changements saisonniers en populations de rhizobia de soja le long de et entre les rangs de culture ont été déterminés au moyens de la résistance antibiotique et du nombres le plus probables, respectivement. Les résultats ont montré que le nombre de nodules par plante et la proportion de nodules dues à la race de *rhizobium* introduite a varié significativement ($P \leq 0,01$) avec les ordres de culture. Au cours de la première saison, le recouvrement de nodule due au *rhizobium* introduit était bas (15%). Dans les saisons ultérieures, la culture de maïs a affecté les nodulation de soja de façon défavorable. Comme dans la troisième saison, l'occupation du *rhizobium* mutant était 60% pour la culture de soja continue (SSS) et 42% pour les soja/maïs/soja (SMS). Les population des rhizobia de soja du sol ont été de la même façon affectées par les ordres de culture. Les nombres de rhizobia étaient significativement ($P \leq 0,05$) plus élevés quand les premières deux cultures étaient soja (SS) que quand le maïs suivait le soja (SM). Tout au long de la période d'échantillonnage, plus de rhizobia s'est produit le long des rangs de culture (AR) que dans les espaces entre les rangs (BR), indiquant des effets positifs des rhizosphères sur la population rhizobiale. On a conclu, par conséquent, que pour l'établissement fructueux de races de *B. japonicum* améliorée, une deuxième culture de soja devrait suivre la première culture inoculée.

Mots Clés: Colonisation, culture, ordre, survie

INTRODUCTION

Generally, grain legumes are grown as mixes, associated, relay and sole crops, and in crop rotations with cereals and other crops. They are utilized in several forms for food, animal feeds, soil cover and green manure (Rachie, 1977). Legumes also have special ability to grow in depleted soils and even contribute to the improvement of soil fertility through their unique symbiotic relationship with nitrogen fixing rhizobia. Nitrogen fixation takes place inside the root nodules through the action of the enzyme nitrogenase produced by the rhizobia. The rhizobia, therefore, provide fixed nitrogen to the plant and, in return, the plant supplies the rhizobia with carbohydrates, minerals and other nutrients. Nitrogen supply to succeeding crops is usually through decomposition of the legume crop residue.

In the tropics, however, the benefits from biological nitrogen fixation have been limited, mainly because effective *rhizobium* strains for introduced legumes such as soybean (*Glycine max* L. Merrill) are lacking in the soil (de Souza, 1969; Hamdi *et al.*, 1973; Ashley, 1973). Production of soybean in tropical agriculture, therefore, requires inoculation with appropriate rhizobia strains before planting (Freire, 1976). Soybean inoculation with rhizobia has been found necessary even with promiscuous soybean varieties that nodulate freely with native soil rhizobia (Pulver *et al.*, 1982). This is because indigenous strains are ineffective or poorly effective, and new more effective strains developed by genetic engineering and other means have to be introduced into soils.

In order that the introduced rhizobia are of long-term value, they must be able to survive, colonize, live saprophytically (outside the host) and compete with the indigenous rhizobial populations present in the soil. The persistence of introduced soybean inoculum rhizobia in tropical soils have, however, not been adequately investigated. *Bradyrhizobium japonicum* strains that nodulate soybean, have been reported to survive well in the field for more than five years, even in absence of the host legume (Nutman and Hearne, 1980). Crozat *et al.* (1982) also reported that the percentage of nodules formed by the inoculum strain increased with time, indicating a permanent establishment and a high competitive ability. In contrast, however, rapid decline in population when cotton was grown after soybean has been observed. This implied that soybean-cotton rotation had detrimental effects on *B. japonicum* strains. Legume and non-legume rotations are common in the tropics (Sanchez, 1976). The work reported here, therefore, involved determination of the effects of various soybean-maize cropping sequences on survival, colonization and establishment of an introduced soybean rhizobium in the soil.

MATERIALS AND METHODS

A field experiment was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, between June, 1984 and May, 1985. The Institute is located in the rain forest savanna transition zone of South West Nigeria at latitude 7° 30'N and longitude 30° 54'E. Average annual rainfall and temperatures range from 788 to 1884 mm and 21.3°C to 31.2°C, respectively. The soil is loamy with the following characteristics: pH (water) = 6.0%, C = 1.2, Bray 1 extractable P = 12.1 ppm, and CEC = 4 me 100 g^{-1}.

A mutant of *B. japonicum* 1Rj2114 was developed for spontaneous resistance level of 1,000 µg ml^{-1} streptomycin sulphate and was evaluated for nodulation and nitrogen fixation capacity under glass-house conditions. The mutant caused good soybean nodulation (74 nodules $plant^{-1}$) and high nitrogen fixation (158 mg N $plant^{-1}$).

In the first season, population of mutant 1Rj2114 was established in the field by planting soybean seeds inoculated with the mutant *rhizobium*. The seeds were planted in three plots measuring 27 x 9 m each, at a spacing of 75 x 10 cm (soybean) or 75 x 30 cm (maize). During the subsequent two seasons, plots were subdivided and soybean and maize were grown in the following cropping sequences; soybean-soybean - soybean (SSS), soybean-soybean-maize (SSM), soybean-maize-soybean (SMS), and soybean-maize-maize (SMM).

At the end of each season, mature crops were cut at ground-level using cutlasses. Crop trash was raked off the plots and soil samples taken before preparing the land for the next planting. Uniform broadcast of potassium (50 kg k ha^{-1}), phosphorus (80 kg P ha^{-1}) and molybdenum (1 kg Mo ha^{-1}) fertilizers was done before every planting. Crops were kept weed free by regular weeding using hand-hoes. During dry spells, crops were irrigated using over-head sprinklers. Leaf pests on soybean were controlled with nuvacron (dichlorovos), applied only when necessary, using an electrodyn sprayer that minimized any possible effects of pesticide residues on soil rhizobia.

Soil samples were taken from a depth of 0-15 cm using a soil auger of 6.0 cm diameter. Samples were randomly obtained from two sampling positions: along the crop rows (AR) within 15 cm from the planting row, and between the rows (BR) within the remaining area between two planting rows (45 cm). For each sampling position, 20 samples per plot were collected and composited. Sub-samples taken from these composite samples were then used to enumerate soybean rhizobia.

Assessment of soybean nodulation was done on twenty plants from middle rows of each plot, sub-plot or sub-subplot during the three seasons. The plants were carefully uprooted using a hand shovel and the nodules collected, washed, and counted. Monitoring of 1Rj2114 establishment in the field was done by typing thirty nodules per sample for antibiotic resistance using the procedures described by Obaton (1973). Percentage nodulation caused by 1Rj2114 mutant *rhizobium* was calculated using the formula: $x = (y/m)100$ where x = percent nodulation due to mutant *rhizobium*, y = positive scores on plate with streptomycin, and m = positive scores on plate with plain yeast mannitol agar.

Effect of soybean-maize cropping systems on soybean nodulation and nodule occupancy by 1Rj2114 was determined for SSS and SMS only because they were composed of soybean crop in the third season. When enumerating rhizobia in the soil or on seeds, the "most probable number" (MPN) technique (Weaver and Frederick, 1982) was used catering for a soil suspension dilution range of 10^{-1} to 10^{-10} at 10 fold dilution intervals. Negative control pouches containing non-inoculated plants were established as test for cross contamination during growth, while postive control pouches with *rhizobium* inoculated plants helped to monitor the suitability of plant growth conditions for nodulation.

Although positive controls showed nodulation within three weeks, test plants were kept for four weeks to ensure adequate time for nodulation, after which roots of all plants were examined and scored. From the most probable number table (Vincent, 1970), the most likely number of rhizobia corresponding to a particular number of positive scores was obtained for the least dilute number of the series, and the estimate number of rhizobia occuring per gramme of soil calculated, in accordance with the formula given by Vincent (1970):

$$y = \frac{m \times d}{v \times g} \times 100$$

Where y = number of rhizobia per gramme of soil, m = most likely number from the MPN table for the lowest dilution of the series, d = lowest dilution, v = volume of aliquot applied to plants, and g = weight of soil sample.

The most probable numbers (MPN) of soybean rhizobia per gramme of soil were transformed to $\log_{10}$ MPN or $\log_{10}$ (MPN + 1) to ensure detection of differences in populations of the rhizobia (Russek and Caldwell, 1983). Data collected were subjected to analysis of variance (ANOVA) and means for the first and second seasons compared using Least Significant Difference (LSD) at 5% level.

RESULTS

Data on soybean nodulation under continuous soybean cropping (SSS) are presented in Table 1. Both total number of nodules per plant and percentage of nodules formed by mutant *rhizobium* (nodule occupancy) increased significantly (P=0.05) with successive cropping seasons. However, the increase in nodule occupancy was much higher (4 times by third season) than that in nodule numbers.

TABLE 1. Soybean nodulation and percentage of nodules occupied by IRj 2114 mutant rhizobium in continuous soybean cropping during three seasons

Season	Mean number of nodules plant^{-1}	% nodules due to IRj 2114
1	29	15
2	39	38
3	45	60
LSD (5%)	**7**	**10**

Statistical analysis showed that types of cropping sequences significantly (P=0.01) influenced nodulation of soybean. When soybean was grown in rotation with maize (SMS), maize grown during second season adversely affected nodulation of the subsequent soybean crop (Table 2).

TABLE 2. Effect of continous soybean (SSS) and soybean-maize rotation (SMS) systems on nodulation of soybean and nodule occupancy by 1Rj2114 mutant during third season

Types of cropping sequence	Mean number of nodules plant^{-1}	% nodules due to IRj 2114
SSS	45	60
SMS	26	42
LSD (%)	**8**	**n.s**

n.s = not significant

Mean number of nodules per plant ranged from 45 for continuous soybean (SSS) to 26 for the soybean-maize rotation (SMS). Although nodule percentages due to the mutant *rhizobium* obtained under the SSS and SMS cropping sequences were not significantly different, lower recovery of nodules of the introduced *rhizobium* was obtained under soybean-maize rotation.

Data on rhizobial count in soil from the third season SSS, SSM, SMS and SMM cropping sequences are represented in Figure 1. Soybean rhizobia (introduced and native types) counts along crop rows (AR) of the third season crops showed that significantly (P=0.05) higher population of rhizobia occurred in soil when both soybean and maize followed two consecutive soybean crops (SS) than when maize was grown as the second crop. There was no significant difference in soybean rhizobia counts between rows (BR) under SSS, SSM and SMS cropping sequences, but significantly low soybean rhizobial populations were obtained under SMM.

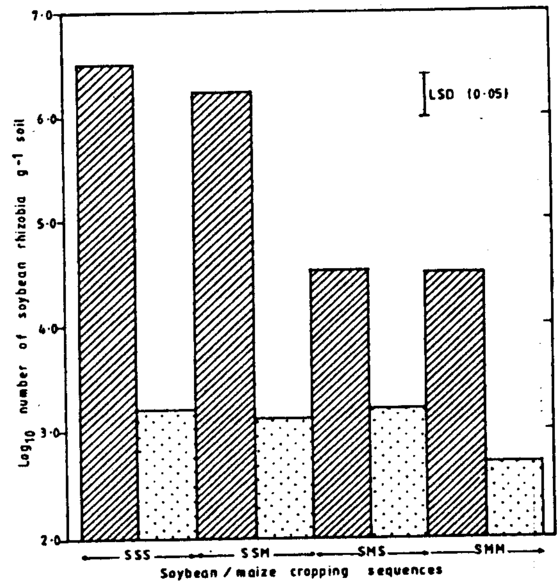

FIGURE 1. Effects of four soybean/maize cropping sequences on $\log_{10}$ numbers of soybean rhizobia per gram of soil sampled from along (▨) and between (⬚).

DISCUSSION

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

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

Although non-legume rhizospheres are known to stimulate rhizobia growth, their effects are generally smaller than stimulation caused by the legume rhizopheres (Pena-Cabriales and Alexander, 1983). This low stimulation affects of non-legume crops means, therefore, that careful consideration should be given to the type of cropping sequences to be followed when introducing new highly effective rhizobial strains.

Data collected also showed that populations of soybean rhizobia were consistently higher along crop rows (AR) than in the spaces between the rows (BR) (Fig.1). Moerman (1965) also found, after soybean harvest, that while there were virtually no soybean rhizobia between crop rows, soil near the crowns of the old plants (about 15 cm radius) was heavily infected. It is reported that rhizospheres of both host and non-host plants significantly influence the growth of soil rhizobia populations (Diatloff, 1969; Jensen and Sorensen, 1987), in response to available nutrients (carbohydrates, amino acids and vitamins) contained in root exudates (Date and Brockwell, 1978). The high density of roots found along crop rows (AR) and the release of rhizobia into soil by the decaying nodules were, therefore, responsible for high populations of rhizobia found in this region as compared to the inter-row spaces.

From this study, it can be concluded that there should be a second soybean crop before introducing a maize crop in the rotation to ensure adequate establishment and build-up of the microsymbiont population. This is even more important where inoculation with *rhizobium* has been done.

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