Number and effectiveness of cowpea rhizobia in soils of Guyana

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Soils in 15 fields in Guyana were sampled to determine the population size of cowpea rhizobia present and their effectiveness. The population size was influenced by the presence of cowpea in the cropping sequence and ranged from < 1 to 10^6 rhizobia gram^{-1} of soil. Most of the fields contained approximately 200 rhizobia g^{-1}. A total of 65 rhizobial isolates were tested for effectiveness and most were only moderately effective and ≈ 20% were ineffective. The results indicate that the indigenous cowpea rhizobial populations are low enough in number and effectiveness for a potential response to inoculation of cowpeas grown in Guyana.

Keywords: *Vigna unguiculata*; Nitrogen fixation; *Bradyrhizobium* sp.; Inoculation

The number and effectiveness of indigenous rhizobia in soil have a direct influence on the response of an introduced legume to artificial inoculation Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume in the tropics and is considered to be a promiscuous plant nodulated by many strains of rhizobia (Allen and Allen, 1939). However, inoculation does not guarantee adequate N provision nor does it guarantee a symbiotic association (Ahmad *et al.*, 1981; Sen and Weaver, 1981). Cowpea rhizobia isolated from soils in West Africa varied greatly in their effectiveness (Ahmad *et al.*, *ibid.*); these authors also determined the population size of cowpea rhizobia in three fields. The populations ranged from approximately 400 to 4 000 g^{-1} of soil. The positive response of cowpeas grown in West Africa to N fertilization and the low effectiveness of indigenous rhizobia indicated that inoculation of cowpeas in West Africa could potentially increase yield.

To obtain additional data on cowpea rhizobia in soils of the tropics, 15 fields in Guyana were sampled to determine the number of cowpea rhizobia present and their effectiveness.

Materials and methods

Number of rhizobia in soil

Composite soil samples from 15 sites were used in the determination of population size of indigenous cowpea rhizobia. They were chosen on the basis of their potential use for cowpea production in the coastal clay region of Guyana. Samples were collected from the soil surface to a depth of 15 cm immediately prior to the planting season during November 1982 and January 1983, when soil moisture levels were near field capacity. Soil samples were obtained by randomly collecting 20 core samples from within an area of 0.1 ha. All core samples from one site were placed in the same plastic bag and were later sieved (5 mm). To prevent contamination, the sieves were thoroughly washed in a 1:10 aqueous dilution of household bleach and were rinsed with rhizobia-free water for samples from different sites. Sieved samples were stored in a refrigerator at 4°C for at most two days before making dilutions for most probable number (MPN) counts of rhizobia.

For each site, duplicate 10 g sub-samples were carried through tenfold serial dilutions from 10^{-1} to 10^{-5} for MPN determinations (Weaver and Frederick, 1972a) and 1 ml aliquots were used to inoculate pre-germinated, surface-disinfected California Black Eye No. 5 cowpea seeds grown in plastic growth pouches. Five pouches were inoculated with each dilution. Uninoculated control plants were included with each determination to determine if cross contamination of pouches occurred.

Plants were grown for four weeks and were checked for presence or absence of nodules. Five tap root nodules were collected from the five plants from each site inoculated with the highest soil dilution that provided nodulation. Some plants in the highest dilution were sometimes not nodulated; in such cases more than one nodule was collected from the remaining plants of that dilution to obtain five nodules. The nodules were stored over silica gel for later use in isolation of rhizobia to be used in effectiveness tests. The highest dilution was selected since the nodules were likely to be formed by the most numerous rhizobia in the soil; rhizobia present in lower numbers would likely have been diluted to extinction.

Effectiveness of rhizobia

Nodules collected from plants used in determining the size of rhizobial populations were rehydrated and the rhizobia isolated on yeast mannitol agar (YMA) plates (Weaver and Frederick, 1982). Single colony isolates were used. 65 isolates were obtained by selecting five isolates from each of 13 of the 15 sites. Only 13 sites were utilized because nodulation was not adequate on plants inoculated with soil

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from two of them. A check strain (THA 301) was used for comparative purposes and is the strain used for inoculant production in Guyana. Strain THA 301 was obtained from Dr. N. Boonkerd, Thailand Ministry of Agriculture. Inocula were prepared by scraping off five loopfuls of rhizobial growth from YMA plates and mixing them with 10 ml of sterile yeast mannitol broth (YMB). An aqueous suspension containing approximately 1×10^7 rhizobia ml^{-1} was utilized for inoculation. Surface-sterilized, pre-germinated cowpea seeds were planted into plastic containers of 20 cm length with a diameter of 4 cm. The containers were filled to within 2 cm of the top with coarse exploded vermiculite. Two pre-germinated cowpea seeds (California Black Eye-5) were sown in each container. Inoculation was achieved by dribbling 2 ml inoculum onto the seeds in each container. The seeds were then covered with vermiculite. Seedlings were thinned to one $container^{-1}$ soon after emergence.

Uninoculated plants were included and watered with either a complete nutrient solution or a N-free nutrient solution (Weaver and Frederick, 1982). Plants were grown in a glasshouse at Texas A&M University with maximum day temperature of 38°C and a minimum night temperature of 20°C. Plants were irrigated as needed with the N-free nutrient solution, added to the surface of the container and excess nutrient solution drained through holes in the bottom of the container into a tray. The quantity of nutrient solution added was regulated so that minimal quantities leached through the container. The tray was removed and washed between irrigations to help prevent cross-contamination.

A randomized complete block design with four replicates was utilized for the effectiveness test. Plants were grown for six weeks after which shoots were harvested and dried at 65°C to constant weight. Roots were examined for presence of nodules. Dry weight of tops was recorded and dry matter production was used as the indicator of effectiveness since N was the limiting factor for plant growth.

Effect of inoculation density on plant growth

An experiment was conducted in a glasshouse at Texas A&M University to determine the response of cowpeas, California Black Eye-5, to the quantity of inoculum added at sowing. The method for growing plants utilized 1000 ml plastic containers as described by Weaver and Frederick (1982). Three pregerminated seeds were sown into each container and inoculated with one of seven rates ranging from approximately one rhizobium to 10^6 rhizobia $container^{-1}$. Broth cultures of two strains of rhizobia, NifTAL 309 (Niftal Culture Collection, Univ. of Hawaii) and USDA 3281 (USDA Culture Collection, Beltsville, MD) were utilized as the inoculum. Populations of the cultures were initially determined by direct microscopic counts and were diluted as needed in sterile water containing 1 mg peptone ml^{-1}. Plate counts were made on YMA to confirm the population estimates and determine viability of cells.

The plants were grown for one month and the quantity of dry matter produced in shoots was determined. Nodules were picked off roots and their fresh weight recorded. The experiment was conducted using a completely randomized design with three replications for each inoculum level of each strain. Data were analyzed by analysis of variance and a response curve was fitted by regression analysis.

Results and discussion

Cowpea rhizobia were present in the soil at all sites sampled (Table 1). Generally, the size of the population at each site depended on whether cowpea had been grown previously. For areas where cowpeas were not known to have been grown, the rhizobial population size was 1–20 rhizobia g^{-1} soil; fields where cowpea had been grown but where inoculation was not practised contained 10–150 rhizobia g^{-1} of soil. Fields that had previously grown inoculated cowpea contained $2–10^7$ rhizobia g^{-1} of soil. Weaver *et al.* (1972) previously reported that the appearance of soybeans in the cropping history greatly influenced the number of soybean rhizobia in soil. The two low population sites (11 and 12) that had grown inoculated cowpeas (Table 1) were situated in an

Table 1 Population of cowpea rhizobia in soil collected from 15 sites in Guyana, information relating to the previous cropping history and if cowpea were inoculated when planted

Sites	Number of rhizobia[a] g^{-1} soil	Previous history
1	<1	Not previously cropped to cowpea
2	2	Not previously cropped to cowpea
3	12	Not previously cropped to cowpea
4	11	Rice cultivation, then two crops of uninoculated cowpea the year prior to sampling
5	17	Rice cultivation, then two crops of uninoculated cowpea the year prior to sampling
6	7	Rice cultivation, then two crops of uninoculated cowpea the year prior to sampling
7	143	Rice cultivation, then two crops of uninoculated cowpea the year prior to sampling
8	27	Rice cultivation, then two crops of uninoculated cowpea the year prior to sampling
9	740	Commonly cropped to inoculated cowpea
10	270	Commonly cropped to inoculated cowpea
11	17	Commonly cropped to inoculated cowpea
12	2	Commonly cropped to inoculated cowpea
13	270	Commonly cropped to inoculated cowpea
14	7.5×10^6	Inoculated cowpea/corn rotation
15	5.6×10^4	Inoculated cowpea/corn rotation

[a] Figures are averages of two replications. Replications were in close agreement and within the precision expected for the method of enumeration

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area which is usually waterlogged during the wet season and this may explain the low populations, for some strains of cowpea rhizobia do not survive well under saturated soil conditions (Boonkerd and Weaver, 1982b). Rhizobial populations declined by two orders of magnitude during 45 days under saturated soil conditions and there were differences between strains in their ability to survive.

Ahmad *et al.* (1981) determined the population size of cowpea rhizobia in three soils in West Africa and found populations of 4.9×10^2 g^{-1}, 3.5×10^3 g^{-1} and 4.3×10^4 g^{-1} of soil. Information on previous cropping history was not provided. The size of rhizobial populations (Table 1) for Guyana soils probably should be increased by a factor of at least ten to reflect the actual number in the soil. According to Boonkerd and Weaver (1982a) and Wilson and Trang (1980) MPN counts on cowpea rhizobia underestimate the population size. Taking this into consideration, the populations of cowpea rhizobia were relatively similar to the size of soybean rhizobia populations in Iowa soils (Weaver *et al.*, 1972).

The number of rhizobia, in soil, required for maximal growth of cowpeas has not been determined. However, results from a controlled environment experiment indicated that dry matter production of cowpeas responded in a linear manner to the logarithm of the number of cowpea rhizobia provided as inoculum (Figure 1). Apparently, cowpea rhizobial populations of less than approximately 1×10^6 $seed^{-1}$ were not adequate for maximal growth of seedlings. Inoculant populations in the range of 10 to 100 rhizobia $seed^{-1}$, for either rhizobial strain, did not provide adequate nodulation (0.64 ± 0.28 g $plant^{-1}$) compared with 1.35 ± 0.24 $plant^{-1}$ when rhizobial populations in excess of 1×10^3 $seed^{-1}$ were utilized. When approximately 10 rhizobia were used to inoculate each seed, all plants grown in the pots formed at least one nodule but if inoculation was decreased to only one rhizobium $seed^{-1}$, plants were not nodulated.

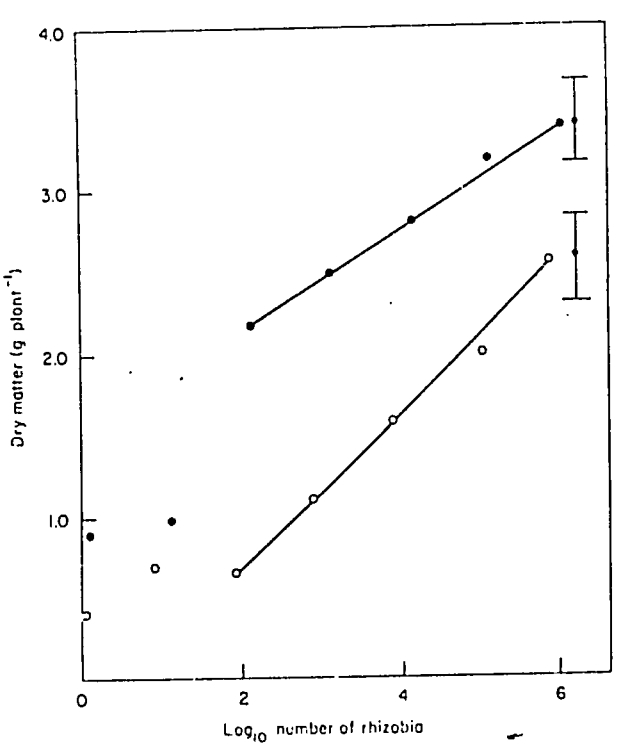

Figure 1 The influence of quantity of inoculum and strain of rhizobia on short-term dry matter production of cowpea shoots. Vertical bars indicate standard error of the means. ●, TAL 309 $y = 0.31x + 1.53$, $r = 0.99$; ○, USDA 3281 $y = 0.48x - 0.25$, $r = 0.99$

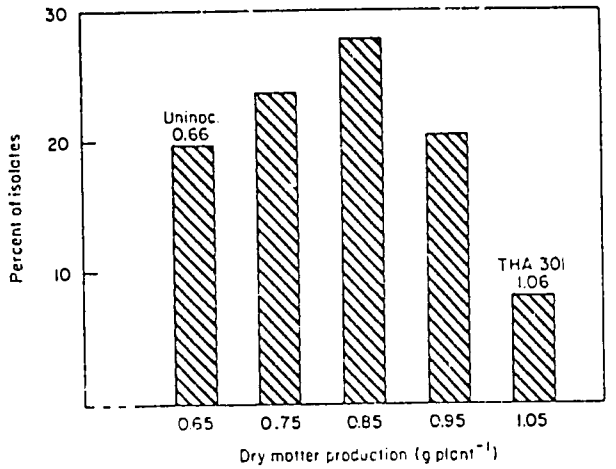

Figure 2 Frequency diagram of the effectiveness of 65 cowpea rhizobia isolates in influencing dry matter production of cowpea seedlings categorized into 0.1 g increments. S.E. = ± 0.11

Extrapolation of the results to the field situation is not straightforward but it is apparent that the rhizobial populations of some soils in Guyana may be too low to provide the numbers needed for maximum nodulation and plant growth. Our data indicate that plant response to inoculation was still occurring when approximately 10^6 rhizobia were provided $seed^{-1}$ (Figure 1). Dispersion of these rhizobia in 1 kg of soil in the root environment would result in 1 000 rhizobia g^{-1} of soil, a higher population density than was in soil at several sites (Table 1). Perhaps it is relevant that soybeans require approximately 1×10^3 rhizobia g^{-1} of soil for maximal numbers of nodules on seedling tap roots (Weaver and Frederick, 1974) and in excess of 1×10^3 rhizobia $seed^{-1}$ for maximal nodulation and N_2 fixation under controlled environmental conditions (Weaver and Frederick, 1972b).

The rhizobial isolates obtained from the plants used in MPN counts ranged widely in their ability to fix N_2 (Figure 2). Of the 65 isolates tested, only 8% were considered to be as effective in plant dry matter production as the recommended inoculum strain THA 301 (Figure 2). In comparison, 50% of the isolates were of moderate effectiveness (Figure 2) and 42% of the isolates were either ineffective or only slightly effective. It appears that there is considerable potential for increasing the effectiveness of rhizobia forming nodules on cowpeas grown in Guyana.

Ahmad *et al.* (1981) examined the effectiveness of 23 indigenous cowpea isolates from West African soils and found that 39% of the isolates were as effective in providing N as the control treatment. They also found that field-grown cowpeas responded positively to N fertilization, indicating a potential use for inoculants in Africa.

The results obtained in this study suggest that indigenous cowpea rhizobia in soils of Guyana are present in low enough numbers and are of low enough effectiveness for a potential response of cowpeas to inoculation to be obtained. It is now necessary to conduct research into the inoculum levels required to overcome indigenous cowpea

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rhizobia and increase the competitive advantage of the inoculum strain so that the potential for increasing yield of cowpea, through inoculation, can be realized.

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