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NATIVE ROOT-NODULE BACTERIAL POPULATIONS IN RUBBER GROWING SOILS AND FOREST SOILS

by

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SUMMARY

A knowledge of native rhizobial populations is essential for the proper understanding of their symbiotic performance and competition with introduced inoculant rhizobia. Rubber growing soils investigated in this study had varying numbers of resident rhizobial populations ranging from low (20 cells g^{-1} soil) to high (30,000 cells g^{-1} soil) depending on the cover crop history of the particular area. Soils from bare lands generally had the lowest rhizobial populations while soils from P. phaseoloides *rhizosphere had the highest numbers. Virgin forest soils were free from native rhizobial populations which could nodulate* Pueraria.

INTRODUCTION

To take advantage of inoculation and the potential to improve of nitrogen fixation by the introduction of efficient strains a knowledge of native rhizobial populations is essential. Furthermore, to explain the observations such as inoculation failures, resident rhizobial numbers are important as the competitiveness of introduced strains can be governed by background rhizobial populations. It is well-documented that in a population study we should pay more attention to the rhizobia of the host, subject to experimentation than to the total rhizobial population ; as competition is more likely to occur between introduced rhizobial strains and those of the same host species which are already established in the soil (Brockwell 1980, 1982).

Generally it is believed that a considerable native population of *Bradyrhizobium* of the cowpea complex is found in the humid tropics. This could be one of the critical factors which governs the competitive success and persistence of introduced rhizobia under those conditions.

Date (1982) found that native rhizobial populations in soils can be low ($<100\ g^{-1}$ soil), medium (100-10,000 g-1 soil) or high ($<10,000\ g^{-1}$ soil) and that these numbers are greatly influenced by several factors. Large numbers of rhizobia are often esti-

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mated in the soil from the immediate vicinity of the homologous host root systems due to the rhizosphere effect (Rovira, 1965 ; Macura, 1968 ; Parker et *al.*, 1977 ; Date, 1982). Further, nodule senescence may result in the entry of some nodule rhizobia into the soil thereby helping to increase soil population after the death of the host plant (Mahler and Wollum II, 1982).

Other factors affecting numbers of rhizobial populations include the complex of soil microorganisms, temperature, moisture, texture and soil reaction. Excellent reviews on these subjects are found in Trinick, (1970); Chowdhury, (1976) ; Alexander (1977) ; Parker *et al.* (1977) ; Lowendorf (1980) ; Salema (1983) ; Subba Rao (1984) and Eaglesham and Ayanaba (1984).

Estimates of native rhizobial populations have been carried out in a vast range of climatic conditions with different types of rhizobia including *R. trifolii* (Thompson and Vincent, 1967; Chatel and Parker, 1973); *R. meliloti* (Mahler and Wollum II, 1979), *R. leguminosarum* (Sorwli and Mytton, 1986), *B. japonicum* (Elkins et *al.*, 1976; Mahler and Wollum II, 1979, 1981, 1982; Brockwell et *al.*, 1975, 1985). *B. lupini* (Chatel and Parker, 1973) and *B. cowpea* complex (Mahler and Wollum II, 1979 ; Ikram and Broughton, 1980 ; Anon, 1980, 1981; Boonkerd and Weaver, 1982). However, we have found no reference to native rhizobial populations (cowpea complex) in Sri Lanka soils in the literature. Hence, the following investigations were carried out on soils of four different rubber growing localities and virgin forest soils to estimate *B. cowpea* complex populations.

The most widely used technology in estimation of native rhizobial populations is the plant infection technique (Brockwell, 1963). This method involves inoculating a aseptically grown legume with samples from a dilution series of soil in water to determine presence or absence of nodulation on the host. Estimations on the rhizobial populations are made by counting the number of nodulated plants at each dilution and multiplying by the dilution factor.

MATERIALS AND METHODS

The experimental procedure described by Brockwell (1982) was used in the study.

Collection of soil samples

Subsamples of soils were collected to a depth of 10 cm, using a pre-sterilized coring implement, from 20 randomly selected sites in the sampling area. These subsamples were thoroughly mixed in the laboratory and a sub-sample of 10 g was taken for the test.

Preparation of test plants

Pueraria phaseoloides was selected as the test plant, because it is a promiscuous host (Date, 1982). Acid-treated seeds (Waidyanatha and Ariyaratna, 1976) were germinated on water agar and when the radicles were 1-2 cm long, seedlings were transferred aseptically into nutrient agar (Jensen, 1942; Gibson, 1980) slants in 21 x 4 cm tubes.

Plant infection test

A 10 gram soil sample was suspended in 90 ml of physiological saline and shaken at 200 cycles per min for 10 min on a wrist action shaker. One ml of this suspension was then pipetted into 4 ml of physiological saline and shaken for a further 5 min. From this, four 1 ml aliquots were used to inoculate four test plants, the fifth ml being used to make the next dilution in the same way. This was repeated to provide six successive dilutions. Then the test plants were transferred to the glass house and observed for nodulation after 6 weeks.

Determination of rhizobial populations

The most probable number of rhizobia in the original sample was calculated from the number of nodulated plants at each dilution level using a modified version of Fisher and Yates(1963) tables as described by Brockwell (1982).

RESULTS

The estimated rhizobial populations for different rubber growing localities and virgin forest soils (Fig. 1) are presented in Table 1.

Soils from bare lands generally had the lowest rhizobial populations ranging from 20-162 cells g^{-1} soils. Lands with recent cover crop histories had a medium number of rhizobia numbers ranging from 328 to more than 1400 per gram of soil, depending on the locality. Rhizobial populations in *P. phaseoloides* rhizosphere varied markedly from medium (4100 bacteria g^{-1} soil) to high (30,200 bacteria g^{-1} soil) numbers in two experimental sites. It is interesting to note that virgin forest soils (Kottawa) were free from native rhizobial populations which could nodulate *P. phaseoloides*.

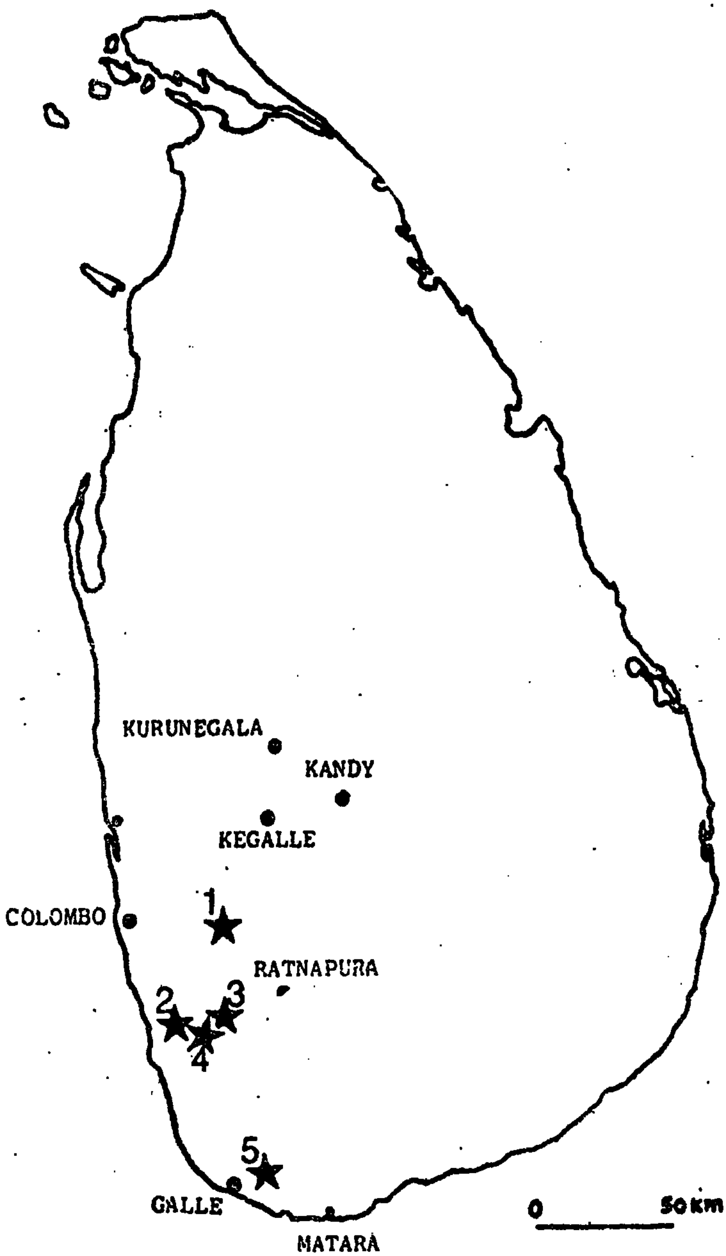

Figure 1. Sampling locations in Sri Lanka for native *Bradyrhizobial* populations.

1. Awissawella
2. Dodangoda
3. Matugama
4. Agalawatta
5. Kottawa

Table I. Bradyrhizobial *population in rubber soils and forest soils*

Locality and description		No. of *Bradyrhizobia* * g^{-1} soil	Confidence limits 95%
Agalawatta			
Site I (bare land)		162	6.2 - 42.4x10^1
Site 2 (bare land)	...	65	2.3 - 18.0x10^1
Site 3 (land with recent *Pueraria* history)	...	1410	5.3 - 38.1x10^2
Site 4 (,, ,, ,, ,, ,,)	...	328	12.2 - 87.9x10^1
Site 5 (soils from *Pueraria* rhizosphere)	...	4100	1.6 - 10.6x10^3
Awissawella	...		
Site I (land with recent *Pueraria* history)	...	1080	4.0 - 28.7x10^2
Kottawa (virgin rain forest)			
Site I (under forest vegetation)	...	<I	
Site 2 (under forest vegetation)	...	<I	
Matugama			
Site I (bare land)	...	40	1.2 - 12.8x10^1
Site 2 (soils from *Pueraria* rhizosphere)	...	30200	11.2 - 81.2x10^3
Dodangoda			
Site I (bare land)	...	23	0.6 - 9.6x10^1
Site 2 (land with recent *Pueraria* history)	...	436	16.6 - 114.2x10^1

*most probable number estimated by a soil dilution/plant infection test.

DISCUSSION

It has been reported that, in Malaysian soils rhizobial number vary from 4—700 cell g^{-1} soil when estimated using *Psopocarpus tetragonolobus* as the test plant (Ikram and Broughton, 1980). However, reports of similar studies with *Bradyrhizobium cowpea* complex under tropical conditions are limited.

Mahler and Wollum II (1982) carried out a detailed study on the population dynamics of *B. japonicum* in two sites of North Carolina and reported that numbers ranged from fewer than 1 to more than 100,000 g^{-1} of soil, depending on the crop present. *B. japonicum* populations in soybean, corn, wheat, and tobacco fields averaged in excess of 10,000 g^{-1} of soil, while counts of fewer than 10 g^{-1} occurred in the forest environments.

The high numbers of rhizobia detected in the immediate vicinity of host roots are due to the rhizosphere effect as discussed in the introduction. The considerable numbers present in the soils with recent *Pueraria* histories may have resulted from rhizosphere populations and senescenced nodules of the previous crop.

In certain assays we experienced the occurrence of 'Skips' which presented a difficult situation in determining the most probable numbers as tables do not provide for such non random occurrences. As pointed out by Brookwell (1982) these complications could have been caused by various causes. For instance, failure of rhizobia to survive or multiply within the tube, poor health of the test plant, or antagonism by other soil microorganisms.

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