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# Seasonal Fluctuation of Peanut Rhizobia In Coastal Plain Soils [1]

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ABSTRACT

This study was undertaken to observe the influence of seasonal fluctuation on native peanut rhizobia populations in Coastal Plain soils in cultivated fields with or without a peanut history, in pasture, and in forest environments. Soil samples were collected from Ap horizons every 30 days during a 16-month period and rhizobia populations were enumerated using a most probable number (MPN) technique with the peanut (*Arachis hypogaea* L.) as the host for plant infectivity tests.

Cultivated fields with a peanut production history had higher peanut rhizobia populations than cultivated fields without a peanut history, pastures, or forest environments. Seasonal fluctuation was evident in cultivated fields with a peanut history; peanut rhizobia populations were greatest in July whether peanuts or another crop was being grown that year and very low from September to May. Peak peanut rhizobia population reached 10,000 per gram of soil in peanut fields in July. Populations were generally less than 30 per gram of soil in cotton, corn, soybean, clover pasture, and alfalfa hay fields, and less than 10 per gram of soil in orchard and forest environments. Peanut rhizobia always constituted less than 0.01% of the total aerobic bacteria in all fields sampled.

Key Words: Cowpea rhizobia, microbial ecology, MPN, bacteria, sporeformers.

Native soil populations of peanut rhizobia have not been studied extensively. However, there is a need to understand the ecology of soil rhizobial populations to determine the opportunity for successful introduction of rhizobia of superior effectiveness. Seasonal variation of peanut rhizobial populations in soil has not been investigated nor has extensive numerical information been reported for members of the cowpea rhizobia group.

Numerical data for other rhizobia have been more widely reported as populations of *Rhizobium trifolii, Rhizobium meliloti,* and *Rhizobium japonicum* have been found to range from less than 10 to more than 1,000,000 per go of soil (5, 6, 8, 20). Highest populations are found in fields where the rhizobia were in the presence of the homologous host.

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Rhizobial populations have been negatively correlated with the average particle size of soils (6). Silt and clay probably offer more protection to the rhizobia against environmental stress than sand. Rhizobial numbers have not been often found to be correlated with pH or percent organic matter.

The absence of numerical data for soil populations of peanut rhizobia over sustained periods prompted this study. North Carolina, with its large peanut (*Arachis hypogaea* L.) hectarage, offered an ideal setting for a study of this type. The majority of North Carolina's 67,000 peanut hectares are on the Middle Coastal Plain in the northeastern part of the state where this study was located.

The objectives of this study were to: (1) compile population data on peanut rhizobia in fields of many different crops, and (2) to observe the influence of season of the year on native soil peanut rhizobial populations, and other microorganisms in cultivated fields with and without peanut histories, and in pasture and forest environments on the Coastal Plain of North Carolina.

## Materials and Methods

In June 1978 peanut rhizobia populations were determined in four to 15 peanut, cotton, corn, soybean, alfalfa hay fields, orchards, clover pastures, and forest environments in the Coastal Plain region of North Carolina. Counts were determined from composite soil samples of 20 soil cores from Ap horizons of each field sampled. Numbers of peanut rhizobia were determined on each composite soil sample using a plant infection and most porbably number (MPN) technique (2, 18). This procedure consisted of suspending 11 g of each moist composite soil sample in 90 ml of sterile, distilled water. Serial dilutions of 1:10 were prepared and 1 ml aliquots of the appropriate dilution were added to 1,000 ml plastic cups containing vermiculite. The cups contained five replicates of four Florigiant peanut seeds (18, 19). Inoculated seeds were grown in a glasshouse at temperatures between 24 and 30 C, and were watered every other day. A modified nitrogen-free Ahmed and Evans nutrient solution (1, 12) and sterile, distilled water alternated as watering solutions. At 21 days the root systems of all 20 plants were examined for nodule presence and MPN values calculated.

For investigation of seasonal fluctuation of cowpea rhizobia, four locations on research farms operated by the North Carolina Agricultural Research Service were selected. Three were on the Middle Coastal Plain where peanuts are commonly grown. The fourth location was at Jackson Springs in the Sandhills section of Upper Coastal Plain. Four fields were selected at each location: (1) a cultivated field with a peanut history, (2) a cultivated field without a peanut history, (3) a pasture, and (4) a forest environment, except there was no pasture at two sites. A cultivated field with a peanut history was defined as one having been in peanut produc-

Table 1. Properties of soils used for evaluation of the seasonal variation of cowpea rhizobia in North Carolina soils.

| Location | Series | Texture | Sub Group | OM | Acidity | Extractable Bases | P | $NO_3N$ |
|---|---|---|---|---|---|---|---|---|
| Cultivated-Peanut History | | | | % | pH | meq/100 g | mg/cdm | |
| Lewiston | Goldsboro | Loamy Sand | Aquic Paleudult | 1.5 | 5.7 | 3.9 | 31 | 0 |
| Rocky Mt. | Duplin | Fine Sandy Loam | Aquic Paleudult | 1.1 | 6.0 | 6.4 | 68 | 4 |
| Clayton | Wagram | Loamy Sand | Arenic Paleudult | 0.9 | 6.1 | 2.6 | 108 | 0 |
| Jackson Springs | Lakeland | Sand | Typic Quartzipsamment | 1.0 | 5.6 | 1.6 | 70 | 0 |
| Cultivated-No Peanut History | | | | | | | | |
| Lewiston | Lynchburg | Sandy Loam | Aeric Paleudult | 1.8 | 6.0 | 6.3 | 68 | 15 |
| Rocky Mt. | Duplin | Sandy Loam | Aquic Paleudult | 1.6 | 6.0 | 6.5 | 49 | 8 |
| Clayton | Norfolk | Sandy Loam | Typic Paleudult | 1.7 | 6.1 | 5.2 | 53 | 8 |
| Jackson Springs | Lakeland | Sand | Typic Quartzipsamment | 1.2 | 5.2 | 1.8 | 83 | 4 |
| Pasture | | | | | | | | |
| Rocky Mt. | Duplin | Sandy Loam | Aquic Paleudult | 3.0 | 6.5 | 10.4 | 130 | 0 |
| Clayton | Norfolk Appling Complex | Clay Loam | -- | 2.6 | 6.2 | 10.1 | 86 | 0 |
| Forests | | | | | | | | |
| Lewiston | Lynchburg | Fine Sandy Loam | Aeric Paleaqult | 2.4 | 4.5 | 0.6 | 11 | 0 |
| Rocky Mt. | Coxville | Fine Sandy Loam | Typic Paleudult | 2.3 | 4.4 | 0.8 | 15 | 0 |
| Clayton | Norfolk Appling Complex | Clay Loam | -- | 2.3 | 4.3 | 0.9 | 22 | 2 |
| Jackson Springs | Lakeland | Sand | Typic Quartzipsamment | 1.1 | 4.4 | 0.5 | 6 | 0 |

tion during at least one of the previous three years.

Composite soil samples for preliminary characterization and analysis of soil type were collected from the Ap horizons of each sampling site. Samples were air-dried, crushed to pass through a 2 mm screen, and analyzed by the North Carolina Department of Agriculture using published methods (13, 14). The classification and relevant properties of the soils are shown in Table 1.

Sampling procedures used for the seasonal fluctuation study were as follows: Composite samples of 20 soil cores from the Ap soil horizon (depth about 15 cm) at each site were collected from 7 to 16 times during the study. The samples were placed on ice and kept cool until analysis. Analyses were usually begun within two days of collection (always within four days) using the plant infection and MPN technique previously described.

Plate counts of soil microorganisms were made in January 1979 and July 1979. Counts for total bacteria and aerobic sporeformers were made on soil extract agar (3, 4, 7) in replicates of four per dilution. Acid, rose bengal-streptomycin agar was used for determining fungal populations in the soil (11). Plates were maintained in an aerobic environment between 23 and 26 C. Counts were made on plates containing between 20 and 200 colonies at five days after plating.

## Results and Discussion

### Peanut Rhizobia Populations Under Different Crops

In the summer of 1978 fields under different crops were surveyed for peanut rhizobial populations. Cropping histories were ignored as the crop present at sampling served as the sole sampling criterium although no non-peanut fields had a history of peanut production.

Peanut rhizobial populations in Coastal Plain soils ranged from less than one to more than 1,500 per g of soil. The 15 peanut fields sampled averaged 500 cowpea rhizobia per g of soil. Cotton fields averaged in excess of 20 cells of cowpea rhizobia per g of soil while 10 cowpea rhizobia per g were common in soil samples of corn, soybean, clover pasture, and alfalfa hay fields on the Coastal Plain (Fig. 1). Counts of less than 10 cells per g of soil were found in orchard and forest sites.

Population trends of peanut rhizobia distribution un-

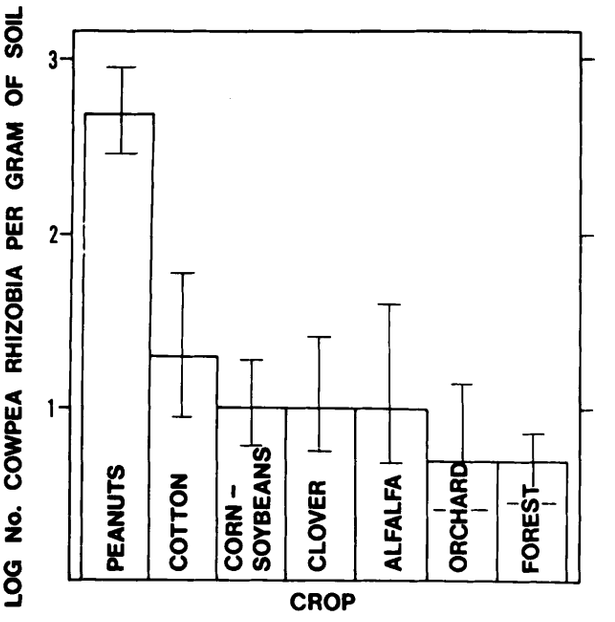


Figure 1. Cowpea rhizobia populations in June 1978 under different crops on the Coastal Plain of North Carolina.

der different crops are similar to those of the soybean, clover, and alfalfa rhizobia in that populations of the rhizobia are greatest in fields with the homologous host (8). Conversely, cowpea rhizobia populations are generally two log numbers lower in fields planted with other crops.

**Peanut Rhizobial Populations in Peanut Fields**

Populations of cowpea rhizobia in cultivated fields with a peanut history ranged from less than 2 to more than 10,000 per g of soil. The normalized monthly populations for the four sampling sites are shown as a percentage of the July 1979 populations (Fig. 2). Trends in seasonal distribution are evident. Populations of cowpea rhizobia apparently began to increase in the late spring and reached a peak in July. This pattern was observed in each of two years despite the fact that the fields were in the corn phase of the crop rotation in 1979. It appears that a peanut history is more important than the immediate presence of the peanut plant for maximum cowpea rhizobial populaitons in the midsummer months. The stimulation for growth of cowpea rhizobia may be a general rhizosphere stimulation requiring only an actively growing plant (15, 16, 17). Cowpea rhizobial populations started to decline in August 1978 and by October were undetectable. Similar trends prevailed in 1979.

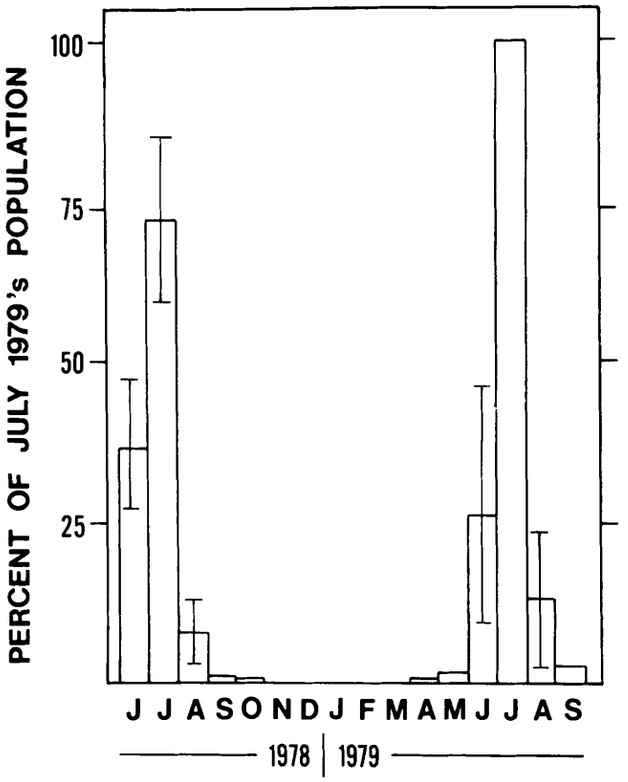


Figure 2. Cowpea rhizobia populations in cultivated fields with a peanut history as a percentage of the July 1979 population (July 1979 population - 3,400).

Environmental factors may play a major role for the short period of detection of measurable numbers of cowpea rhizobia in the soil. Soil temperatures probably reach an optimum level in June for rapid multiplicaiton of peanut rhizobia. However, sustained high maximum soil temperatures through July often reaching 40 C or greater at 10 cm (Munevar and Wollum, unpublished data), may contribute to the rapid decline observed in the peanut rhizobia population of August. Also, by mid-July prolonged periods of low soil water potentials (values less than -5 bars) are very common in coarse textured North Carolina Coastal Plain soils (9). Low soil water potentials coupled with high soil temperatures probably make the soil environment extremely harsh for cowpea rhizobia. Later in the fall, when soil temperatures and water potentials are more favorable, the absence of a actively growing plant to serve as a specific stimulant to the growth of peanut rhizobia may prevent the buildup observed in June and early July.

Peanut rhizobia populations were much lower in peanut fields than those of other rhizobia in fields planted with their respective hosts (8). In January 1979, cowpea rhizobia counts ranged from less than one to more than 100 cells per g of soil in peanut fields (Fig. 3). In contrast *R. japonicum* populations in soybean fields ranged from 100 to 100,000 per g of soil. Counts of *R. meliloti* and *R. trifolii* ranged from 3,000 to 4,000,000 and from 400,000 to 7,000,000 cells in alfalfa hay fields and clover pastures, respectively. In July 1979 peanut rhizobial populations were greater, ranging form 20 to 7,000 cells per g of soil (Fig. 3). However, at that time populations of *R. japonicum, R. trifolii, R. meliloti* were still greater in soybean, clover pasture, and alfalfa hay fields, respectively (Fig. 3).

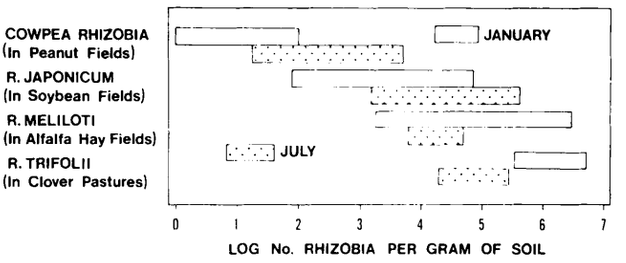


Figure 3. Population ranges of rhizobia in North Carolina soils in January 1979 and July 1979.

Unlike peanut rhizobia populations, *R. japonicum, R. meliloti, and R. trifolii* were detected throughout the year in North Carolina Coastal Plain soils. Even when peanut rhizobial populations were detectable, numbers were substantially below clover, alfalfa, and soybean rhizobial populations due to a lower level of host stimulation or due to coarse soil texture that prevents a large population buildup (10). The latter explanation appears more viable, as peanuts are usually grown on sandier soils than the other legumes. It is believed that bacteria populations are lower on coarser textured soils due to the fact that less organic substances are present for bacterial growth and that environmental stresses are magnified; therefore, it is possible that the cowpea rhizobia are reacting like the other bacteria present in sandy soils. Furthermore, if peanuts were grown on finer textured soils larger cowpea rhizobia populations may be seen.

**Peanut Rhizobial Populations in Cultivated Fields Without Peanut Histories**

Cowpea rhizobial populations in cultivated fields without a peanut cropping history ranged from less than one to

more than 200 cells per g of soil. Comparison of the monthly populations for the four sampling sites revealed no definite seasonal distribution in cowpea rhizobia.

### Peanut Rhizobial Populations in Pastures and Forests

Cowpea rhizobia populations in pastures and forest environments were much lower than those observed in cultivated fields with a history of peanut production. There was no distinct seasonal fluctuation in cowpea rhizobial populations in pastures and forests. Peanuts had never been grown in any of these fields. Pastures were assumed to have low cowpea rhizobial populations due to the lack of introduced rhizobia, while the highly acid conditions in combination with the lack of introduced cowpea rhizobia contributed to the absence of cowpea rhizobia in the forest soils.

### Enumeration of Non-Rhizobia Organisms

Average bacterial counts for January 1979 and July 1979 are shown in Table 2. Generally, aerobic bacteria counts were largest in nonpeanut fields, intermediate in peanut fields, and lowest in forest environments. There did not appear to be any seasonal effect on total soil bacteria as enumerated in soil extract agar.

The average number of aerobic sporeformers in peanut fields was lower than that observed in nonpeanut fields (Table 2). On the other hand, no trends were noted for fungal populations (Table 2).

Table 2. Average bacteria, sporeformer, and fungi populations in North Carolina soils with different cropping histories.

| Date | Field | Bacteria | Sporeformers | Fungi |
|---|---|---|---|---|
| | | ——————x10$^5$/g—————— | | |
| January 1979 | Peanut | 118.3 | 4.3 | 2.5 |
| | Non-Peanut | 301.0 | 8.3 | 4.5 |
| | Forest | 48.2 | 6.4 | 3.4 |
| July 1979 | Peanut | 90.0 | 3.9 | 7.3 |
| | Non-Peanut | 308.0 | 16.6 | 6.6 |
| | Forest | 33.2 | 0.8 | 9.0 |

### Peanut Rhizobia Populations as a Portion of Total Bacteria Population

Cowpea rhizobia were found to constitute less than 0.01% of the total bacteria population in cultivated fields with a peanut history, pastures, and in forest environments. Overall, one out of every 15,000 aerobic bacteria in cultivated fields with a peanut history was a cowpea *Rhizobium*, while less than one out of every 100,000 was a cowpea *Rhizobium* in cultivated fields without a peanut history, pastures, and forest environments. In contrast, *R. japonicum, R. meliloti, and R. trifolii* have been found to constitute up to 9.1, 1.7, and 8.5% of the total aerobic bacteria population in fields with homologous hosts, respectively (8). Also, *R. japonicum, R. meliloti, and R. trifolii* have been found to constitute 0.01, 0.01, and 0.12% of the total aerobic bacteria population in fields planted with crops other than the homologous host, respectively (8). It may be concluded, therefore, that cowpea rhizobia play a lesser role than *R. japonicum, R. meliloti, and R. trifolii* in the soil microbial environment.

Data presented in this paper confirm assumptions that cowpea rhizobia numbers are greatest in soils with peanut histories and provides information on the seasonality of cowpea rhizobia populations in soils on the Coastal Plain of North Carolina. Additional field and laboratory studies are desirable to find reasons for seasonal changes in populations of cowpea rhizobia and how these changes may affect inoculation practices.

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