

ENRICHMENT OF *BRADYRHIZOBIUM* spp POPULATIONS IN SOIL DUE TO CROPPING OF THE HOMOLOGOUS HOST LEGUME

J. E. THIES,* P. L. WOOMER† and P. W. SINGLETON

University of Hawaii NifTAL Project, 1000 Holomua Ave, Paia, HI 96779, U.S.A.

Summary—Host legumes can enrich their immediate soil environment with rhizobia through rhizosphere effects. The extent to which this enrichment occurs, the specificity of the process and its interaction with soil management factors remain poorly described. In a series of field trials, we measured changes in the size of indigenous populations of *Bradyrhizobium* in response to cropping of host and non-host legumes under two N fertilizer regimes. Uninoculated cowpea (*Vigna unguiculata*) and soybean (*Glycine max*) were grown with or without applied urea (900 kg N ha⁻¹) at three field sites on the island of Maui, HI, USA, not previously cropped with legumes. Using the most-probable-number plant infection method and Siratro (*Macropodium atropurpureum*) as the host, the population density of *Bradyrhizobium* in the bulk soil at each site was measured at planting and at grain maturity and compared to the population density in adjacent fallow soil. When the size of the initial indigenous population was low (18 and 580 cells g⁻¹ soil), significant increases in the population density compared to fallow soil were observed only in soils cropped with cowpea receiving no applied urea. When the size of the initial indigenous bradyrhizobial population was high (5.8 × 10⁶ cells g⁻¹ soil), no significant increase in the population density was observed. These results suggested that enrichment of soil bradyrhizobial populations was host-specific, that symbiotic legumes can enrich their soil environment with microsymbionts up to a threshold level and that such enrichment can be curtailed by soil management practices that suppress nodulation.

INTRODUCTION

The population size of indigenous rhizobia affects the likelihood and magnitude of a legume yield response to inoculation (Singleton and Tavares, 1986; Weaver *et al.*, 1987; Thies *et al.*, 1991). Soybeans [*Glycine max* (L.) Merr.] frequently respond to inoculation with *Bradyrhizobium japonicum* when first introduced into an area, but increased yield due to inoculation is often not observed in subsequent crops (Dunigan *et al.*, 1984; Ellis *et al.*, 1984). Initial soybean yield responses in field soils typically occur where indigenous populations of *B. japonicum* are low or absent (Thies *et al.*, 1991). Lack of sustained inoculation response in soybean may be due to inoculant strains becoming naturalized in site soils, enrichment of indigenous populations due to cropping of the host legume or a combination of both phenomena (Herridge *et al.*, 1987). In contrast, yield and nodulation of cowpea [*Vigna unguiculata* (L.) Walp.] are seldom increased by inoculation (Kang, 1977). This is due, in part, to the typically high population sizes of indigenous bradyrhizobia in tropical soils (Danso and Owiredo, 1988). Indigenous populations of *Bradyrhizobium* can range from a few to > 10⁵ cells g⁻¹ soil, depending on soil

moisture, soil fertility and the extent of the homologous legume component of the vegetation (Woomer *et al.*, 1988). High specificity of soybean for its microsymbiont, *B. japonicum*, coupled with typically low populations of indigenous, homologous host legumes for *B. japonicum*, especially in tropical soils, may contribute to the differences observed between cowpea and soybean in the frequency and magnitude of yield responses to inoculation.

Host legumes can bring about increases in the number of rhizobia in their immediate soil environment through the stimulatory influence of the rhizosphere (Pena-Cabriaes and Alexander, 1983; Brockwell *et al.*, 1987, 1989; Bushby, 1993). However, the specificity and magnitude of any increase in soil rhizobial population size due to cropping of host and non-host legumes remain poorly described. If increases in population size are host-specific, intensive cropping of cowpea should serve to increase the size of local bradyrhizobial populations, whereas soybean cropping would have little effect. In our trials, changes in the size of indigenous populations of *Bradyrhizobium* in bulk soil were measured under two N fertilizer regimes in plots planted with a legume homologous (cowpea) or not homologous (soybean) with these bradyrhizobia. We sought to determine the specificity and magnitude of any increases in population size, assess their dependence on initial population size and N management and assess their effects on inoculation response.

*Author for correspondence, presently at: Centre for Legumes in Mediterranean Agriculture (CLIMA), University of Western Australia, Nedlands, WA 6009, Australia.

†Present address: Tropical Soil Biology and Fertility Program (TSBF), UNESCO Building, Nairobi, Kenya.

Table 1. Classification and characterization of site soils

Site*	Soil classification	Sand	Silt	Clay	Oct	CEC (cmol kg ⁻¹)	Mean soil temperature (°C at 10 cm)
I	Torroxial haplustoll (fine, kaolinitic isohyperthermic)	18	48	34	1.1	15.6	30.2
II	Torroxial haplustoll (kaolinitic, isohyperthermic)	25	49	26	2.0	26.4	22.1
III	Humoxial tropohumult (clayey, ferritic isothermic)	4	64	32	3.6	18.1	22.9

*Refer to Thies et al. (1991) for details.

tOrganic C.

MATERIALS AND METHODS

Field preparation and plant sampling

Three sites were prepared in fields not previously cropped with legumes and which had remained fallow for several years (Table 1). Soils were ripped, tilled and optimal amounts of P, K, Mg, Zn, Mo, B and lime were applied as described by Thies *et al.* (1991). Uninoculated seed of cowpea (cv. Knuckle purplehull) and soybean (cv. Clark, nodulating and non-nodulating isolines) were planted in a split-plot design with species assigned to main plots and N application assigned to subplots. Urea application rates were 0 and 100 kg N ha⁻¹ applied at weekly intervals during the cropping cycle for a total of 900 kg N ha⁻¹. Inoculated treatments (10⁷ rhizobia seed⁻¹) were included and used to assess the magnitude of any yield responses to inoculation [for list of strains used see Thies *et al.* (1991)]. There were 3 replications. Crops were grown under drip irrigation with the soil moisture tension maintained between 0.1–0.3 MPa from sowing to physiological maturity. The extent of nodulation was determined on 10 randomly selected plants at full bloom (approximately 35–45 days after planting). Crops were grown to grain maturity, shoots removed and seed yield determined as described by Thies *et al.* (1991).

Soil sampling and population determinations

Soil samples were taken immediately prior to planting and the most-probable-number of indigenous rhizobia determined on both cowpea and *Macroptilium atropurpureum* (DC.) Urb. cv. Siratro as described by Thies *et al.* (1991). Soil samples were taken again at grain maturity. This corresponded to 100 days after planting (DAP) at site I and 115 DAP at site III for both species. At site II, this corresponded to 113 DAP for soybean and 150 DAP for cowpea. Twelve 2.5 cm dia soil core samples to a depth of 20 cm were taken from each plot. Core samples were taken parallel to in-plot harvest rows, 12 cm from the base of the plants and stored at 4°C overnight. All large roots and nodules were removed and samples thoroughly homogenized prior to removal of subsamples for moisture and population size determinations. Siratro seeds were scarified and surface sterilized in concentrated H₂SO₄ for 5 min, rinsed thoroughly in sterile water, germinated on 1% water agar and planted into the troughs of growth pouches (Weaver and Frederick, 1972) containing N-free, but otherwise

complete plant nutrient solution (Singleton and Bohlool, 1983). Plants were selected for uniform growth prior to inoculation. Soil equivalent to 50 g (oven dry wt basis, 100°C) was placed in sterile buffered diluent for the first dilution step. A 10-fold dilution series was subsequently prepared. Diluted soil suspension (1 ml) was pipetted directly onto roots of each of 4 replicate plants per dilution level (Brockwell *et al.*, 1975). Uninoculated control pouches were inserted between each dilution treatment and subsequently inspected for nodulation. Nodulation results were recorded 25 days after inoculation.

Data analysis and mean separation

The most-probable-number enumeration system (MPNES; Woerner *et al.*, 1990) was used to estimate the population density of indigenous *Bradyrhizobium* in the soils. Site sample data were analysed as separate split-plot experiments by a two-way analysis of variance procedure (Wilkinson, 1988). Duncan's multiple range test was used for mean separation.

RESULTS

Populations of indigenous *Bradyrhizobium* prior to planting ranged from 1.8 × 10¹ to 5.8 × 10¹ cells g⁻¹ soil (Table 2) at the 3 sites. Initial counts made on Siratro did not differ from those made on cowpea. Cropping cowpeas without applied N but with otherwise intensive inputs of other nutrients, increased the size of the populations of indigenous *Bradyrhizobium* over the initial counts 61-, 94- and 19-fold for sites I, II and III, respectively and 275-, 262- and

Table 2. Initial population densities of *Bradyrhizobium* in site soils and those resulting from cropping to host and non-host legumes

Crop management	Population density (bradyrhizobia g ⁻¹ soil)		
	Site I	Site II	Site III
Initial population:			
<i>M. atropurpureum</i>	1.8 × 10 ¹	5.8 × 10 ¹	5.8 × 10 ¹
<i>V. unguiculata</i>	5.4 × 10 ¹	2.8 × 10 ²	3.6 × 10 ⁴
After cropping (kg N ha⁻¹):			
<i>V. unguiculata</i>			
0	1.1 × 10 ^{3a} *	5.5 × 10 ^{4a}	1.1 × 10 ^{6NS}
900	7.5 × 10 ^{3b}	1.3 × 10 ^{5b}	6.5 × 10 ^{6NS}
<i>G. max</i>			
0	8.0 × 10 ^{0b}	2.9 × 10 ^{2b}	2.1 × 10 ^{2NS}
900	3.0 × 10 ^{0b}	1.4 × 10 ^{2b}	1.2 × 10 ^{2NS}
Fallow (no legumes)	4.0 × 10 ^{0b}	2.1 × 10 ^{2b}	2.6 × 10 ^{2NS}

*Letters indicate significant differences between treatments within a column by Duncan's multiple range test ($P = 0.05$); NS = not significant.

Table 3. Nodulation and seed yield of *V. unguiculata* and *G. max* at the three sites

Crop management	Nodule mass (mg plant ⁻¹)*			Seed yield (kg ha ⁻¹)		
	Site I	Site II	Site III	Site I	Site II	Site III
<i>V. unguiculata</i> (kg N ha ⁻¹)						
0	133a†	115a	175a	2179b	1910a	2884a
900	23b	6b	44b	2839a	1764a	2923a
<i>G. max</i> (kg N ha ⁻¹)‡						
0	0	0	0	627b	1356a	1711b
900	0	0	0	3024a	1983a	4596a

*Sampled at full bloom (35–45 days after planting).

†Letters indicate significant differences between treatments within a species in the same column by Duncan's multiple range test.

‡Non-nodulating isolate of soybean.

4-fold, respectively, over the fallow condition. The enrichment effect on bradyrhizobial populations in soil due to cropping is more apparent when the population densities of the fallow plots are subtracted from those of the cropped treatments. For soils cropped with *V. unguiculata* without applied urea, these values are 1.1×10^4 , 5.4×10^9 and 8.4×10^5 , for sites I, II and III, respectively. While the absolute value of the increase in the bradyrhizobial population at site III was greater than at the other two sites, the increase was not statistically significant ($P > 0.05$). Significant enrichment in population size occurred only in the 2 Torroxic haplustolls (sites I and II) after cropping with cowpea without applied urea and where the initial populations were $< 10^4$ rhizobia g⁻¹ soil (Table 2).

No significant enrichment of indigenous bradyrhizobial populations resulted from the rhizosphere influence of the non-host legume, soybean (Table 2). Although no indigenous *B. japonicum* were present at any of the sites, soybean did not nodulate due to the use of a non-nodulating isolate.

N application significantly decreased nodulation in cowpea, increased growth and yield in both crops (Table 3), but resulted in no change in the population density of indigenous *Bradyrhizobium* regardless of legume species (Table 2).

A significant response to inoculation was obtained only for soybean grown at sites I (Hashimoto Farm) and III (Haleakala Station), as reported by Thies *et al.* (1991).

DISCUSSION

Significant increases in bradyrhizobial population densities were observed only in response to cropping with the homologous host legume, which suggests that enrichment of soil bradyrhizobial populations is host-specific. Host-specific enrichment has been demonstrated in fast-growing species of *Rhizobium* (Wilson, 1930, 1931; Krasil'nikov, 1958; Tuzimura and Watanabe, 1962; Jones, 1966). Our results agree with those of Wilson (1930, 1931), who showed that *Rhizobium leguminosarum* bv. *trifolii* and *R. leguminosarum* bv. *viciae* populations were stimulated more

by their specific hosts than by non-hosts and that the number of rhizobia in rhizosphere soil was increased by cropping of the homologous host.

Our results suggest that enrichment of bradyrhizobia in bulk soil is related to nodulation rather than plant growth, since application of urea increased plant growth but depressed nodulation and dampened the stimulatory effect of cowpea cropping on soil bradyrhizobial populations. In addition, there were no increases associated with non-nodulating soybean. Only soils in which plants were well nodulated became significantly enriched with rhizobia. Brockwell *et al.* (1987, 1989) also reported increases in the size of established populations of inoculant-derived *B. japonicum* in the bulk soil following soybean cropping. These populations continued to increase in size in the months following cropping and were attributed mainly to release of bradyrhizobia from decaying nodules. While some of the enrichment results from multiplication of bradyrhizobia in the homologous host rhizosphere, the bulk of the increase is likely due to release from senescing nodules.

The size of indigenous bradyrhizobial populations appeared to be related to soil and climatic factors, as larger populations were supported in soils with higher organic matter content and lower soil temperature regimes (Tables 1 and 2). Whereas, the degree of enrichment may be a function of the initial size of the indigenous population. There appears to be a threshold level above which population density does not increase due to cropping as *Bradyrhizobium* spp populations were not increased by more than a factor of 102, regardless of soil type, climate or extent of nodulation. This interpretation is supported by Mahler and Wollum (1982), who observed a 194-fold higher population of *B. japonicum* in North Carolina soils with soybean cropping histories as compared to those without. This also agrees with results of Krasil'nikov (1958), who measured the rhizosphere population of *R. l. bv. trifolii* of clover during the growing season and observed a population increase from 105 to 10⁶ cells g⁻¹ soil over a 3-week period. Bushby (1993) reported that rhizosphere bradyrhizobial populations of a number of legume genotypes tended to reach a maximum size during crop growth. These observations support the concept of a maximum carrying capacity of an environment for these microorganisms.

We tested the equation developed for estimation of rhizobial numbers in natural ecosystems (Woomer *et al.*, 1988) to see if it could be applied to legume cropping systems. When model input variables were set to reflect legume monoculture under irrigated conditions, the soil population resulting from cropping was estimated at 7.0×10^4 cells g⁻¹ soil, which greatly overestimates the observed enrichment. Thus, models developed from long-term unmanaged ecosystem data may not reflect adequately the rhizobial population dynamics in highly managed systems.

Although a significant increase in yield was achieved by applying N to cowpea at site I, no inoculation response was obtained for this species at any site, even when the initial indigenous population estimate was only 18 cells g⁻¹ soil. Enrichment of soil bradyrhizobial populations observed in these trials may help to explain why so few rhizobia g⁻¹ soil are able to nodulate crops and fix sufficient N to produce yields not significantly different from those of inoculated crops (Thies *et al.*, 1991). Depending on the survival of bradyrhizobia between croppings, enrichment of specific bradyrhizobia by cropped host legumes can lead to soil populations of sufficient size to make later positive responses to applied rhizobia unlikely (Brockwell *et al.*, 1987; Thies *et al.*, 1991). In addition, where significant enrichment of bradyrhizobial populations occurred (sites I and II), bradyrhizobial numbers increased to a level which Weaver and Frederick (1974) have suggested may represent a significant competition barrier to introduced strains (10⁷ cells g⁻¹ soil). The influence of indigenous rhizobial population characteristics (numbers, saprophytic competence, diversity, effectiveness and competitive ability) should be carefully considered when trying to manipulate soil rhizobial population dynamics to agricultural advantage.

Acknowledgements—This research was supported by the National Science Foundation and the United States Agency for International Development Cooperative Agreement (NifTAL Project). We appreciatively acknowledge use of the non-nodulating isolate of *G. max* cv. Clark IV provided by P. Cregan (USDA Nitrogen Fixation Laboratory, Beltsville, MD), use of the Maui Soil, Climate and Land Use Network developed by H. Ikawa (Department of Agronomy and Soil Science, University of Hawaii) and technical assistance by Wendy Asano and Geoffrey Haines. This paper is Journal series No. 4032 of the Hawaii Institute of Tropical Agriculture and Human Resources.

REFERENCES

- Brockwell J., Diatloff A., Grassia A. and Robinson A. C. (1975) Use of wild soybean (*Glycine ussuriensis* Regal and Maack) as a test plant in dilution-nodulation frequency tests for counting *Rhizobium japonicum*. *Soil Biology & Biochemistry* 7, 305-311.
- Brockwell J., Roughley R. J. and Herridge D. F. (1987) Population dynamics of *Rhizobium japonicum* strains used to inoculate three successive crops of soybean. *Australian Journal of Agricultural Research* 38, 61-74.
- Brockwell J., Gault R. R., Morthorpe L. J., Peoples M. B., Turner G. L. and Bergersen F. J. (1989) Effects of soil nitrogen status and rate of inoculation on the establishment of populations of *Bradyrhizobium japonicum* and on the nodulation of soybeans. *Australian Journal of Agricultural Research* 40, 753-762.
- Bushby H. V. A. (1993) Colonization of rhizospheres by *Bradyrhizobium* sp. in relation to strain persistence and nodulation of some pasture legumes. *Soil Biology & Biochemistry* 25, 597-605.
- Danso S. K. A. and Owiredo J. D. (1988) Competitiveness of introduced and indigenous cowpea *Bradyrhizobium* strains for nodule formation on cowpeas [*Vigna unguiculata* (L.) Walp.] in three soils. *Soil Biology & Biochemistry* 20, 305-310.
- Dunigan E. P., Bollich P. K., Hutchinson R. L., Hicks P. M., Zaunbrecher F. C., Scott S. G. and Mowers R. P. (1984) Introduction and survival of an inoculant strain of *Rhizobium japonicum* in soil. *Agronomy Journal* 76, 463-466.
- Ellis W. R., Ham G. E. and Schmidt E. L. (1984) Persistence and recovery of *Rhizobium japonicum* inoculum in a field soil. *Agronomy Journal* 76, 573-576.
- Herridge D. F., Roughley R. J. and Brockwell J. (1987) Low survival of *Rhizobium japonicum* inoculant leads to reduced nodulation, nitrogen fixation and yield of soybean in the current crop but not in the subsequent crop. *Australian Journal of Agricultural Research* 38, 75-82.
- Jones D. G. (1966) The contribution of white clover to a mixed upland sward. II. Factors affecting the density and effectiveness of *Rhizobium trifolii*. *Plant and Soil* 24, 250-260.
- Kang B. T. (1977) Effects of inoculation and nitrogen fertilizer on soybean in western Nigeria. *Experimental Agriculture* 11, 23-31.
- Krasil'nikov N. A. (1958) *Soil Microorganisms and Higher Plants*. Academy of Science, U.S.S.R.
- Mahler R. L. and Wollum A. G. II (1982) Seasonal fluctuation of *Rhizobium japonicum* under a variety of field conditions in North Carolina. *Soil Science* 134, 317-324.
- Pena-Cabral J. J. and Alexander M. (1983) Growth of *Rhizobium* in unamended soil. *Soil Science Society of America Journal* 47, 81-84.
- Singleton P. W. and Bohlool B. B. (1983) A split-root growth system for evaluating the effect of salinity on components of the soybean *Rhizobium japonicum* symbiosis. *Crop Science* 23, 259-262.
- Singleton P. W. and Tavares J. W. (1986) Inoculation response of legumes in relation to the number and effectiveness of indigenous *Rhizobium* populations. *Applied and Environmental Microbiology* 51, 1013-1018.
- Thies J. E., Singleton P. W. and Bohlool B. B. (1991) Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Applied and Environmental Microbiology* 57, 19-28.
- Tuzimura K. and Watanabe I. (1962) The effect of rhizosphere of various plants on the growth of *Rhizobium*. Ecological studies of root nodule bacteria (Part 3). *Soil Science and Plant Nutrition* 8, 153-157.
- Weaver R. W. and Frederick L. R. (1972) A new technique for most probable number counts of rhizobia. *Plant and Soil* 36, 219-222.
- Weaver R. W. and Frederick L. R. (1974) Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. I. Greenhouse studies. *Agronomy Journal* 66, 229-232.
- Weaver R. W., Morris D. R., Boonkerd N. and Sij J. (1987) Populations of *Bradyrhizobium japonicum* in fields cropped with soybean-rice rotations. *Soil Science Society of America Journal* 51, 90-92.
- Wilkinson L. (1988) *SYSTAT: The System for Statistics*. SYSTAT Inc., Evanston, IL.
- Wilson J. K. (1930) Seasonal variation in the number of two species of *Rhizobium* in soil. *Soil Science* 30, 289-296.
- Wilson J. K. (1931) Relative numbers of two species of *Rhizobium* in soils. *Journal of Agricultural Research* 43, 261-266.
- Woomer P., Singleton P. W. and Bohlool B. B. (1988) Ecological indicators of native rhizobia in tropical soils. *Applied and Environmental Microbiology* 54, 1112-1116.
- Woomer P., Bennett J. and Yost R. (1990) Overcoming the inflexibility of most-probable-number procedures. *Agronomy Journal* 82, 349-353.