APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1988, p. 1112-1116 Vol. 54, No. 5
0099-2240/88/051112-05$02.00/0

Ecological Indicators of Native Rhizobia in Tropical Soils†

PAUL WOOMER, PAUL W. SINGLETON, AND B. BEN BOHLOOL*
NifTAL Project, University of Hawaii, 1000 Holomua Avenue, Paia, Hawaii 96779-9744
Received 28 October 1987/Accepted 24 February 1988

The relationship between environment and abundance of rhizobia was described by determining the populations of root nodule bacteria at 14 diverse sites on the island of Maui. Mean annual rainfall at the sites ranged from 320 to 1,875 mm, elevation from 37 to 1,650 m, and soil pH from 4.6 to 7.9. Four different soil orders were represented in this study: inceptisols, mollisols, ultisols, and an oxisol. The rhizobial populations were determined by plant infection counts of five legumes *(Trifolium repens, Medicago saliva, Vicia sativa, Leucaena leucocephala,* and *Macroptilium atropurpureum)*. Populations varied from 1.1 to 4.8 $\log_{10}$ cells per g of soil. The most frequently occurring rhizobia were *Bradyrhizobium spp.*, which were present at 13 of 14 sites with a maximum of 4.8 $\log_{10}$ cells per g of soil. *Rhizobium trifolii* and *R. leguminosarum* occurred only at higher elevations. The presence of a particular *Rhizobium* or *Bradyrhizobium sp.* was correlated with the occurrence of its appropriate host legume. Total rhizobial populations were significantly correlated with mean annual rainfall, legume cover and shoot biomass, soil temperature, soil pH, and phosphorus retention. Regression models are presented which describe the relationship of legume hosts, soil climate, and soil fertility on native rhizobial populations.

Native rhizobia are adapted to their soil environments. However, the environmental factors which result in promotion or stress on natural populations are poorly understood. Most published work on the ecology of rhizobia deals with introduced organisms as affected by crop-induced change in soil factors such as moisture, temperature, pH, and soil toxicity (9, 16, 19). These studies are valuable in describing the important components of the soil environment which result in the successful establishment of introduced rhizobia in cropping systems.

Lawson et al. (15) recently developed a regression model that explains changes in the number of indigenous *Rhizobium trifolii* as a function of host legume height and solar radiation. However, rhizobial populations are not correlated with temperature, rainfall, soil moisture, or soil type. Other researchers find a positive correlation between the number of rhizobia in soils and annual rainfall (21).

Components of the soil environment are interrelated. The presence of a legume presupposes favorable conditions for that species's growth and establishment. For example, high rainfall results in an increase of acidity-related soil toxicity factors in many tropical soils. Consequently, increases in soil moisture, a soil condition which initially improves rhizobial survival, ultimately result in a less favorable, low-pH environment, disadvantageous to the host legume (10) and rhizobial persistence (14).

Yousef et al. (31) relate several environmental factors to the abundance of peanut rhizobia in 66 soil samples collected from various locations in Iraq. The abundance of *Bradyrhizobium spp. is* related to cropping history, inferring the importance of the legume rhizosphere and residues in maintaining rhizobial populations. Soil factors that relate to occurrence of peanut rhizobia are organic C (1% or less), electrical conductivity (2 mmoh/cm [2 mS/cm] or less), pH (7.6 to 8.1), lime content (20 to 30%), and cation-exchange capacity (CEC) (20 to 30 mEq/100 g of soil).

The numbers and effectiveness of indigenous rhizobia influence the inoculation response of a legume (12, 21), yet the same environment which affects the success or failure of introduced rhizobia is not necessarily responsible for the numbers and types of native rhizobia.

The successful introduction of *Trifolium subterraneum* in Australia is related to the effectiveness of the indigenous *R. trifolii* present (12, 27) and the ability of introduced rhizobia to compete with existing populations (8). The failure of T. *subterraneum* to become established in certain soils is related to the failure of the microsymbiont to survive during the absence of the host legume, an annual plant (3, 27). Persistence of a soil microorganism is termed saprophytic competence and is a useful criterion for selecting superior *Rhizobium* strains (5). It is unlikely that saprophytically competent rhizobia would establish themselves in numbers greater than adapted native organisms in equilibrium with their environment. An understanding of the ecological determinants of native rhizobial populations is therefore important to the successful introduction and establishment of inoculant strains.

The island of Maui provides an excellent opportunity for the study of environment-plant-microbe interactions. The climate and soils vary as a function of elevation (from sea level to over 3,000 m) and orientation to the northeast tradewinds, which determine the amount of rainfall (from <300 to >9,000 mm annually). Soils are generally more highly weathered, phosphorus deficient, and acidic and have higher clay content with increasing rainfall. The University of Hawaii has a network of 22 characterized sites, along rainfall and temperature gradients, collectively known as the Maui Soil, Climate and Land Use Network (Maui Net) (23). Each site is equipped with instruments to monitor soil and climatic variables for a computerized multiuser data base.

To describe the ecological determinants of native rhizobial populations, a study was conducted along two transects (wet and dry) ranging from 37 to 1,600 m in elevation. These sites include four soil orders varying in soil pH from 4.9 to 7.9, organic matter content from 1.06 to 14.5%, clay contents of 2.5 to 66.7%, and available soil bases from 1.1 to 46.1 mEq/100 g soil. The vegetation of the dry transect is characterized by grass savannas with isolated leguminous shrubs

* Corresponding author.

† Publication no. 3209 of the Hawaii Institute of Tropical Agriculture & Human Resources.

and trees, the wet transect by legume-abundant short-grass pastures.

MATERIALS AND METHODS

Site description and sampling. Fourteen sites were chosen from the Maui Net on the island of Maui. Soils of these sites have been described by the Soil Conservation Service (22). Detailed soil analyses, except for soil pH, were conducted by the National Soil Survey Laboratory, Soil Conservation Service, Lincoln, Neb. (11, 23). The pH of the soil samples (1:1 in H_2O) was measured at the time of sampling. Mean and maximum soil temperatures at a 10-cm depth were monitored during 1985 and 1986 with a Fenwal Electronics thermistor and a Campbell Scientific CR21 Datalogger. Mean annual rainfall (MAR) (in millimeters) was taken directly from long-term records collected for 11 to 54 years (7). Soils from undisturbed areas adjacent to the weather stations were sampled with a 2.5-cm-diameter soil borer. Twelve samples (to a depth of 25 cm) were collected and formed into a composite at each site along an 8-m transect. The soil borer was cleaned and alcohol sterilized between sites. Site characteristics are given in Table 1.

Vegetation analysis. Vegetation was identified at 10-cm intervals along the 8-m transect, and the legume cover was estimated. A 0.25-m^2 section was randomly placed four times along the transect. Legumes were identified and removed at soil level, dry weights were recorded, and legume shoot mass per unit area was computed. Rare legumes not encountered along the transect but found immediately adjacent to the transect were assigned a cover value of 1%. Vegetation formation was described in the manner of Mueller-Dumbois and Ellenburg (17).

Rhizobial species determination. *Rhizobium spp., Bradyrhizobium* populations, and legume cover were determined twice, in October 1986 and April 1987, corresponding to the end of the dry and wet season, respectively. Values used in the analysis were the means of values for these two time points. To qualitatively determine the presence of *Rhizobium* and *Bradyrhizobium* spp. at the sites, 20 g of soil was mixed with 80 g (dry weight basis) of moist vermiculite, placed in 0.8-liter plastic pots, and watered with nitrogen-

TABLE 1. Characteristics of MauiNet sites used

Site	*MAR*[a] (mm)	*Soil*[b] (great group)	pH	Organic C (oho)	Elevation (m)	Legume cover	Vegetation[`]
Hashimoto Farm	322	Haplustolls	6.8	1.1	37	6.5	Short-grass savanna (Cenchrus ciliaris) with isolated deciduous shrubs (Leucaena leucocephala) and trees (Prosopis pallida)
Sugar Waiakoa	358	Haplustolls	7.9	1.6	187	3.0	Short-grass savanna (C. ciliaris) with isolated deciduous trees (P. pallida)
Sugar Maalaea	372	Haplustolls	7.4	1.2	38	2.0	Ephemeral short mixed grassland with forbs (Crotolaria sp.)
Kula Agricultural Park	375	Haplustolls	7.5	1.5	366	4.7	Short-grass savanna (C. ciliaris) with isolated deciduous shrubs (L. leucocephala)
Pasture Waiakoa	380	Haplustolls	7.0	2.0	366	1.0	Short-grass savanna (C. ciliaris) with isolated deciduous trees (P. pallida) and cacti
Sugar Paia	495	Torrox	6.8	1.5	61	4.5	Short-grass savanna (Chloris gayana) with shrubs (L. leucocephala)
Pulehu Station	565	Haplustolls	6.1	4.0	640	36.7	Tall-grass savanna (Panicum maximum) with forbs (Desmodium uncinatum, Vicia saliva), isolated shrubs (L. leucocephala), and trees (Acacia mearnsii)
Kula Station	845	Eutrandepts	6.8	8.7	945	22.1	Grassy pasture (Pennisetum clandestinum) with forbs (Trifolium repens, Medicago polymorpha, D. uncinatum, and V. saliva)
Pasture Kekoa	1,060	Dystrandepts	6.3	11.2	844	33.0	Grassy pasture (P. clandestinum) with forbs (T. repens, M. polymorpha, D. uncinatum, and V. saliva)
Pasture Puupahu	1,100	Dystrandepts	6.1	14.5	1,646	3.3	Mixed-grass pasture with forbs (T. repens)
NifrAL Project	1,130	Haplustolls	6.8	2.4	110	11.0	Mowed grass (Eremochloa ophiuroides) with forbs (Desmodium canam, Desmanthus virgatus, and M. polymorpha)
Olinda Prison Farm	1,200	Dystrandepts	5.2	7.5	1,067	50.2	Grassy pasture (P. clandestinum) with forbs (T. repens and V. saliva)
Haleakala Station	1,800	Tropohumults	5.3	3.6	640	39.5	Grassy pasture (P. clandestinum) with forbs (Desmodium intortum, T. repens)
Kuiaha Site	1,875	Tropohumults	4.9	2.9	287	26.8	Grassy pasture (Digitaria decumbens) with forbs (D. canam, Indigofera suffruticosa, Cassia leschanaultiana) and isolated trees (Acacia koa)

[a] From reference 7.
[b] From reference 22.
[`] From reference 17.

free nutrient solution (20). Plants inoculated with known rhizobia (10^9 live cells per pot) and uninoculated controls were included. The legumes were grown for 35 days in the glasshouse before nodule number and mass were measured. Test legumes were *Glycine max* cv. Lee; *Leucaena leucocephala* var. K8; *Macroptilium atropurpureum* cv. Siratro; *Medicago sativa* cv. Florida 77; *Trifolium repens* cv. Regal Ladino; and *Vicia sativa.*

Plant infection counts. The *Rhizobium* and *Bradyrhizobium* populations were determined for soils which tested positive in the glasshouse experiment. This was done by using the plant infection technique in a growth room. The plants were grown at 28°C with a 16-h photoperiod under 1,000-W metal halide lamps producing 350 microeinsteins m^{-2} s^{-1}.

Seeds of *L. leucocephala* and *M. atropurpureum* were treated with concentrated sulfuric acid for 25 and 5 min, respectively, followed by repeated rinses in sterile distilled water. Seeds of *G. max* and V. *sativa* were sterilized with 2% NaOCI for 2 min, followed by repeated sterile-water rinses. Seeds of T. *repens* and *M. sativa* were shown to be rhizobium free. All seeds were germinated on 1.5% water-agar petri dishes except for T. *repens* and *M. sativa,* which were planted directly into nutrient solution-agar tubes.

L. leucocephala, M. atropurpureum, and T. *repens* were grown on N-free agar slants similar to those of Brockwell et al. (2). V. *sativa (1* seed per tube) and *M. sativa* were grown in similar tubes containing an oven-sterilized vermiculiteperalite (1:1, vol/vol) mixture and sterile nitrogen-free nutrient solution. During the April 1987 determination, V. *sativa* and *M. sativa* were grown on the agar slants.

Soil samples were thoroughly mixed, and rocks and large roots were removed. Serial dilutions were prepared as described in Somasegaran and Hoben (24). Tenfold dilutions were prepared for soils originating in the wet transect (MAR, >565 mm/year), and fivefold dilutions were made for areas in the dry transect (MAR, <565 mm/year). Portions (1 ml) of each dilution were applied to the various legumes.

The legume root systems were examined at regular intervals for nodulation. Results were recorded, and most probable number estimates were determined for the 5-fold (1) and 10-fold (29) dilution series. The lower limits of detection for the 5-fold dilution and 10-fold dilution series are 1.1 and 6.0 cells per g of soil, respectively.

Model development. A matrix of biotic and abiotic measurements of the environments was assembled, and stepwise regression was performed against the total rhizobial population with SAS regression analysis. Soil chemical and physical properties used in this analysis were organic carbon (percent); total nitrogen (percent); sum of the base nutrient ions (milliequivalents per 100 g of soil) in the CEC (Ca^2, Mg^{2+}, K^+, and Na^+); clay content (percent); phosphorus retention (percent); and soil pH. The average annual temperature, the mean of the monthly maximum temperatures, MAR, and legume cover values were also included in the analysis.

RESULTS AND DISCUSSION

The number of native *Rhizobium* and *Bradyrhizobium spp.* for five test species at 14 sites is presented in Table 2. All soils contained *Rhizobium* and/or *Bradyrhizobium* spp. The greatest variety of *Rhizobium* and *Bradyrhizobium* spp. was observed at the Pulehu, Pasture Kekoa, and Kula Station sites; each had four different species. One site had three, seven sites had two, and three sites had only one species. The presence of various legume hosts was stratified by moisture and elevation (Table 1), and a similar pattern was observed in the distribution of their microsymbionts (Table 2). Of the 70 site-by-species determinations, 34 were devoid of both host legume and the specific rhizobia; 32 were found to have both host and microsymbiont; at 2 sites rhizobia were found in the absence of their host; and at another 2 sites hosts were observed but their *Rhizobium* spp. could not be detected. There was a coincidence between the presence of legume hosts and rhizobial populations 94% of the time. This provides quantitative evidence for the "striking correspondence" between host and rhizobia proposed by Nutman and Ross (18).

In the initial greenhouse experiment, *Bradyrhizobium japonicum* was not found at any site. Host legumes associated with *B. japonicum* did not occur at any of the sites. Total rhizobial populations varied from 1.1 to 4.78 $\log_{10}$ cells per g of soil. Native populations of *R. trifolii* were present in the thousands per gram of soil when clover constituted a large part of the plant stand, whereas when it did not, populations were much smaller, e.g., at the Pulehu Experimental Farm.

R. mehloti was present at four sites but in low numbers even when its specific host legumes were present. *Medicago sativa is* capable of forming nodules with *R. meliloti* strains of other effectiveness subgroups (28), including *M. polymorpha,* which was found within the sample areas.

TABLE 2. Native rhizobial populations at 14 MauiNet sites

Site	Plant[a] infection count ($\log_{10}$/g of soil) with trap species:					
	T. repens	*M. sativa*	*V. sativa*	*L. leucocephala*	*M. atropurpureum*	Total
Hashimoto Farm	ND[b]	ND	ND	2.18	1.65	2.29
Sugar Waiakoa	ND	ND	ND	2.60	0.57	2.61
Sugar Maalaea	ND	ND	ND	2.73	1.51	2.76
Kula Ag Park	ND	ND	ND	1.30	1.34	1.62
Pasture Waiakoa	ND	ND	ND	ND	1.11	1.11
Sugar Paia	ND	ND	ND	1.49	1.50	1.80
Pulehu Station	0.48	ND	3.21	1.20	3.03	3.43
Kula Station	3.74	3.47	3.90	ND	4.00	4.42
Pasture Kekoa	3.13	1.36	3.77	ND	3.90	4.18
Pasture Puupahu	3.26	ND	ND	ND	ND	3.26
NifTAL Project	ND	1.78	ND	1.91	2.34	2.48
Olinda Prison Farm	3.77	ND	2.24	ND	1.78	3.78
Haleakala Station	3.46	ND	ND	ND	4.76	4.78
Kuiaha Site	ND	ND	ND	ND	4.50	4.50

[a] *Trifolium repens* cv. Regal Ladino, *Medicago sativa* cv. Florida 77, *Vicia sativa, Leucaena leucocephala* cv. K8, and *Macroptilium atropurpureum* cv. Siratro correspond to *Rhizobium trifolii, R. meliloti, R. leguminosarum* bv. *Rhizobium* sp. (*Leucaena*), and *Bradyrhizobium* sp., respectively.

[b] ND, No rhizobia detected.

Macroptilium atropurpureum is recommended as a promiscuous trap host of cowpea *Rhizobium* sp. (now *Bradyrhizobium* sp. [29]). Isolates from the root nodules of this legume were all *Bradyrhizobium* sp. These occurred at all sites except Puupahu, which lacked any associated host legume. Generally, *Bradyrhizobium sp.* occurred in low numbers (1.1 to 2.8 $\log_{10}$ cells per g of soil) along the dry transect and in high numbers (3.5 to 4.8 $\log_{10}$ cells per g of soil) along the wet transect. *Rhizobium sp. (L. leucocephala)* was recovered only from sites which included *L. leucocephala. Desmanthus virgatus,* another host legume associated with this type of *Rhizobium* (6), occurred at the NifTAL Project site. *Leucaena* was the dominant legume species at many of the study sites. However, as a test legume it was an inappropriate host for strains of *Rhizobium* sp. associated with other tropical legumes (25, 26). *Rhizobium sp. (L. leucocephala)* colonized soils only at lower elevations (0 to 640 m).

In a study of *Prosopis glandulosa* (13), slow-growing and fast-growing isolates were recovered from the nodules in the surface soil of the Sonoran Desert. At two of the sites in our study, *Prosopis pallida* was the only legume present. At Sugar Waiakoa, *Rhizobium* sp. and *Bradyrhizobium* sp. were recovered from the soil, yet at Pasture Waiakoa, only slow-growing *Bradyrhizobium sp.* was recovered (Table 2). *Rhizobium sp. (L. leucocephala)* was always present when *L. leucocephala* occurred. *Rhizobium sp. (L. leucocephala)* were able to colonize these dryland soils despite high fluctuations in moisture and nutrient availability and deciduous habit of leucaenae at the driest sites.

The rhizobial numbers observed in the tropohumults were among the highest of all sites. While the Kuiaha soil pH of 4.9 was near the lower limit for growth and survival for rhizobial species (16), the moisture status of the soil was highly favorable to host growth and resulted in rhizobial colonization of the sites.

Except for the sum of extractable bases in the soil, transformation of population estimates resulted in higher linear correlations than did nontransformed values (Table 3).

The clay contents of these soils did not correlate well with native rhizobial populations. The soils used in this study had kaolinitic, oxidic (22), and amorphous (30) clay fractions. The soils did not include montmorillonite, which protects *Rhizobium spp.* against dessication (4).

The influence of MAR on biotic and abiotic variables was considerable. The influences of soil pH, phosphorus retention, and temperature were difficult to distinguish in this experiment, as these abiotic variables covary with rainfall. A negative correlation between rhizobial populations and soil pH is indicative of this situation. The influence of soil moisture overrides the negative effect of low soil pH and resulted in native rhizobial populations in the thousands per gram of soil.

When only legume cover is considered, the power model [total *RhizobiumlBradyrhizobium* = *1.*25 (% legume cover) $^{0.33}$] significantly described rhizobial populations *(r = 0.*61). However, the model implies that rhizobia would not occur without host presence. An exception to this prediction was the case of *R. trifolii,* which occurred at low numbers at Pulehu Experimental Farm in the absence of T. *repens* (Tables 1 and 2).

The model of Lawson et al. (15) explaining fluctuation in *R. trifolii* populations throughout the year partially agrees with our findings. The *R. trifold* multiple linear model includes a legume component and solar radiation, but fails to include soil environmental parameters. This is due to the narrow range of environments used in developing the model (i.e., two fields in the same region). Solar radiation largely covaries with clover height, indirectly describing the rhizosphere.

Cropping history and soil parameters are included in a study of the abundance of *Bradyrhizobium* sp. nodulating peanut in Iraqi soils (31). While the results of our investigation and that of Yousef et al. (31) demonstrate the effect of legumes on rhizobial populations, correlations with other ecological determinants are not in agreement. Yousef et al. (31) report that peanut rhizobia prefer a pH range from 7.6 to 8.1, are not favored by increasing soil organic carbon over 1%, and are best correlated with soil CEC.

Our results show that high densities of *Bradyrhizobium sp.* occurred in low-pH soils (tropohumults) and in soils high in organic carbon (eutrandepts and dystrandepts). The correlation of rhizobial populations with CEC was not directly tested in our studies, but the poor correlation of rhizobial abundance with clay content *(r = -0.06),* organic carbon content *(r = 0.02),* and total extractable bases *(r = -0.25),* three soil conditions which directly affect CEC, suggests that the charge characteristics of soils influence rhizobial populations less than soil moisture *(r = 0.80).* The earlier studies (31) address a subgroup of *Bradyrhizobium* spp., those associated with peanut, in soils varying in pH between *7.1* and *8.4.* Our studies included a broader-host-range group of *Bradyrhizobium* sp., which nodulate the promiscuous legume *Macroptilium atropurpureum,* and a wider range of soil environmental conditions, pH *4.9* to *7.9.*

The importance of soil moisture and legume cover on the abundance of native rhizobia is presented in linear regression models (Table *4).* The linear model, which includes MAR, legume intensity, and the soil base nutrients, explained *90%* of the variation in rhizobial populations. The amount and distribution of rainfall directly affected soil moisture, which determined the survival and proliferation of rhizobia as well as growth of the host legume. Legumes served to maintain rhizobia in the soil through rhizosphere effects and senescence of nodules. The total extractable bases was a direct measurement of soil fertility as well as

TABLE 3. Correlation coefficients of biotic and abiotic factors influencing native rhizobial abundance[a]

Rhizobium values	*R*										
	Vegetation		Organic carbon (%)	N (%)	pH (1:1 in H_2O)	Clay (%)	Soil bases (mEq/100 g)	P retention (%)	Soil temp (°C)		MAR (mm)
	Legume cover (%)	Legume shoots (kg/ha)							Avg	Maximum	
Untransformed	0.49	0.41	0.02	−0.05	−0.58	−0.06	−0.25	0.16	−0.30	−0.50	0.80
Log_{10} transformed	0.75**	0.53*	0.47	0.33	−0.75**	−0.40	−0.01	0.66**	−0.65*	−0.75**	0.82***

[a] Significance of covariant parameter: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 4. Regression models describing native rhizobial populations at MauiNet sites[a]

Rhizobia (log_{10}/g of soil) + covariant determination	R
2.07 + 0.056 (% legumes)	0.75**
1.45 + 0.002 (MAR)	0.82***
1.33 + 0.030 (% legumes) + 0.0013 (MAR)	0.89***
0.48 + 0.033 (% legumes) + 0.0016 (MAR) + 0.028 (total extractable bases)	0.95***

[a] Significance of covariant parameter: **, $P < 0.01$; ***, $P < 0.001$.

being inversely related to soil acidity. Therefore, important climatic, biological, and soil fertility elements are included in the model.

A close relationship exists between legumes and the occurrence and proliferation of rhizobial populations. Rhizobia are facultative symbionts and, in the saprophytic state, are independent of their host legumes. Likewise, under certain nitrogen conditions, legumes are not dependent on symbiosis. However, nodulation has developed as an ecologically convenient mechanism in which the occurrence of one symbiont frequently accounts for the presence of the other.

ACKNOWLEDGMENTS

This research was supported in part by National Science Foundation grant BSR-8516822 and the Agency for International Development Cooperative Agreement DAN-4177-A-00-6035-00 (NifTAL Project).

We thank H. Ikawa for the use of the MauiNet sites, Susan Hiraoka for manuscript preparation, and Wendy Asano for carrying out the plant infection tests.

LITERATURE CITED

1. Brockwell, J. 1982. Plant infection counts of rhizobia in soils, p. 41-58. *In* J. M. Vincent (ed.), Nitrogen fixation of legumes. Academic Press, Ltd., Australia.
2. Brockwell, J., A. Diatloff, A. L. Grassia, and A. C. Robinson. 1975. Use of wild soybean *(Glycine ussuriensis* Regel and Maack) as a test plant in dilution-nodulation frequency tests for counting *Rhizobium japonicum*. *Soil* Biol. Biochem. 7:305-311.
3. BrockweP, J., W. F. Dudman, A. H. Gibson, F. W. Hely, and A. C. Robinson. 1968. An integrated programme for the improvement of legume inoculant strains. Trans. 9th Int. Cong. Soil Sci. Soc. Adelaide 2:103-114.
4. Bushby, H. V. A., and K. C. Marshall. 1977. Some factors affecting the survival of root nodule bacteria on dessication. Soil Biol. Biochem. 9:143-147.
5. Chatel, D. L., R. M. Greenwood, and C. A. Parker. 1968. Saprophytic competence as an important character in selection of *Rhizobium* for inoculation. Trans. 9th Int. Cong. Soil Sci. Soc. Adelaide 2:65-73.
6. Davis, P. E. 1982. *Desmanthus virgatus* as a test plant for dilution-frequency infection tests using *Leucaena leucocephala* rhizobia. Soil Biol. Biochem. 14:313-314.
7. Department of Land and Natural Resources. 1982. Median rainfall. Circular C88, Department of Natural Resources, Division of Water and Land Development, Honolulu, Hawaii.
8. Gibson, A. H., B. C. Curnow, F. J. Bergerson, J. Brockwell, and A. C. Robinson. 1975. Studies of field populations of *Rhizobium:* effectiveness of strains of *Rhizobium trifolii* associated with *Trifolium subterraneum* L. pastures in southeastern Australia. 1975. Soil Biol. Biochem. 7:95-102.
9. Ham, G. E. 1980. Inoculation of legumes with *Rhizobium* in competition with naturalized strains, p. 131-138. *In W. E.* Newton and W. H. Orme-Johnson (ed.), Nitrogen fixation, vol. II. University Park Press, Baltimore, Md.
10. Hosaka, E. Y., and J. C. Ripperton. 1944. Legumes of the Hawaiian ranges. Hawaii Agricultural Experiment Station Bulletin 93. University of Hawaii, Honolulu.
11. Ikawa, H., H. H. Sato, A. K. S. Chang, S. Nakamura, E. Robello, Jr., and S. P. Periaswamy. 1985. Soils of the Hawaii Experiment Station, University of Hawaii: soil survey, laboratory data and soil description. BSP Technical Report 4, HITAHR Research Extension Series 022, University of Hawaii, Honolulu.
12. Ireland, J. A., and J. M. Vincent. 1968. A quantitative study of competition for nodule formation. Trans. 9th Int. Cong. Soil Sci. Soc. Adelaide 2:85-93.
13. Jenkins, M. B., R. A. Virginia, and W. M. Jarrell. 1987. Rhizobial ecology of the woody legume mesquite *(Prosopis glandulosa)* in the Sonoran Desert. Appl. Environ. Microbiol. 53:36-40.
14. Keyser, H. H., and D. N. Munns. 1979. Tolerance of rhizobia to acidity, aluminum and phosphate. J. Soil Sci. Soc. Am. 43:519523.
15. Lawson, K. A., Y. M. Barnet, and C. A. McGilchrist. 1987. Environmental factors influencing numbers of *Rhizobium leguminosarum* biovar *trifolii* and its bacteriophages in field soils. Appl. Environ. Microbiol. 53:1125-1131.
16. Lowendorf, H. S. 1980. Factors affecting survival of *Rhizobium* soils, p. 87-123. *In* M. Alexander (ed.), Advances in microbial ecology. Plenum Publishing Corp., New York.
17. Mueller-Dumbois, D., and H. Ellenburg. 1974. Aims and methods of vegetation ecology, p. 466-488. John Wiley & Sons, Inc., New York.
18. Nutman, P. S., and G. J. S. Ross. 1970. *Rhizobium* in the soils of the Rothamsted and Woburn farms, p. 148-167. Rep. Stn. Rothamsted 1969. Harpenden, Herts, England.
19. Schmidt, E. L. 1978. Ecology of the legume root nodule bacteria, p. 269-303. In Y. R. Dommergues and S. V. Krupa (ed.), Interaction between non-pathogenic soil microorganisms and plants. Elsevier Scientific Publications Co., Amsterdam.
20. Singleton, P. W. 1983. A split-root growth system of evaluating components of the soybean-Rhizobium japonicum symbiosis. Crop Sci. 23:259-262.
21. Singleton, P. W., and J. W. Tavares. 1986. Inoculation response of legumes in relation to the number and effectiveness of indigenous *Rhizobium populations*. Appl. Environ. Microbiol. 51:1013-1018.
22. Soil Conservation Service. 1972. Soil survey of the islands of Kauai, Oahu, Maui, Molokai and Lanai; State of Hawaii. Soil Conservation Service, U.S. Department of Agriculture, Washington, D. C.
23. Soil Conservation Service. 1984. Soil survey, laboratory data and descriptions for some soils of MauiNet. Soil Conservation Service, U.S. Department of Agriculture, Washington, D.C.
24. Somasegaran, P., and H. Hoben. 1985. Methods in legume*Rhizobium* technology. University of Hawaii NifTAL Project, Paia, Hawaii.
25. Trinick, M. J. 1968. Nodulation of tropical legumes: Specificity of the *Rhizobium* symbioses of *Leucaena leucocephala*. Exp. Agric. 4:243-253.
26. Trinick, M. J. 1980. Relationships amongst the fast-growing rhizobia of *Lablab purpureus, Leucaena leucocephala, Mimosa spp, Acacia farnesiana* and *Sesbania grandifiora* and their affinities with other rhizobial groups. J. Appl. Bacteriol. 49:3953.
27. Vincent, J. M. 1954. The root-nodule bacteria as factors in clover establishment in the red basaltic soils of the Lismore District, N.S.W. I. A survey of "native" strains. Aust. J. Agric. Res. 5:55-60.
28. Vincent, J. M. 1954. The root nodule-bacteria of pasture legumes. Proc. Linn. Soc. N.S.W. 79:1-32.
29. Vincent, J. M. 1970. A manual for the practical study of the root nodule bacteria. Blackwell Scientific Publications, Oxford.
30. Wada, K., Y. Kakuto, and H. Ikawa. 1986. Clay minerals, humus complexes and classification of four "andepts" of Maui, Hawaii. J. Soil Sci. Soc. Am. 50:1007-1013.
31. Yousef, A. N., A. S. AI-Nassiri, S. K. AI-Azawi, and N. AbdulHussain. 1987. Abundance of peanut rhizobia as affected by environmental conditions in Iraq. Soil Biol. Biochem. 19:319396.