# Environmental factors related to rhizobial abundance in kikuyugrass (*Pennisetum clandestinum*) pastures

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Population dynamics of legumes and their associated rhizobia were determined in kikuyugrass (*Pennisetum clandestinum* Hochst. *ex* Choiv.) pastures at four sites on the island of Maui, Hawaii, USA. Ranges in factors measured at the sites which represented three soil great-groups were mean annual rainfall (MAR) 810-1800 mm yr<sup>-1</sup>, pH 5.0-6.5 and total extractable bases 3.7-54 mEq 100 g<sup>-1</sup> soil. Competition from kikuyugrass, as estimated by mat thickness of the stolons, significantly reduced the abundance of white clover (*Difolium repens L.*) but not that of *Desmodium intortum cv*. Greenleaf (Mill.) Fawc. and Rendle. Soil populations of *Rhizobium leguminosarum* bv. *trifolii* and *Bradyrhizobium sp.* ranged 1.78-549 ( $\log_{10} g^{-1}$  soil) or MAR.

Keywords: Rhizobial populations; Trifolium repens; Desmodium intortum; Pennisetum clandestinum; Pasture ecology; Hawaii

The ability of rhizobia to colonize natural habitats can aid the establishment of pasture legumes (Chatel et al., 1968) and enhance their symbiotic N<sub>2</sub> fixation potential (Gibson et al., 1975; Singleton and Tavares, 1986). Recently. the correlation between selected environmental variables and rhizobial abundance has been examined (Lawson et al., 1987; Yousef et al., 1987; Woomer et al., 1988a). The results of these agree on the importance of the legume host (Lawson et al., ibid.; Woomer *et al.*, ibid.) and the cropping history (Mahler and Wollum, 1982; Weaver et al., 1987; Yousef et al., ibid.) in maintaining populations of rhizobia in soils. The role of soil physical and chemical parameters on the distribution and population size of saprophytic rhizobia in nature are confounded with host effects. The importance of specific abiotic co-variates which have been identified as regulators of saprophytic rhizobia is inconsistent (Lawson et al., ibid.; Lowendorf, 1980; Woomer et al., 1988a; Yousef et al., ibid.). This may be due to the ability of rhizobia to acclimatize to of site-specific stresses the environment (Pena-Cambriales and Alexander, 1983).

Despite the difficulty of developing predictive models of rhizobial population size, the benefits of understanding where and, in which circumstances when indigenous rhizobial populations are diminished, aids in the ability to predict the likelihood of a legume's response to inoculation with rhizobia.

On the island of Maui, kikuyugrass (*Pennisetum clandestinum* Hochst. ex Choiv.) is an important upland pasture grass adapted to a wide range of rainfall conditions (Whitney *et al.*, 1939). The suc-0041-3216/90/030217-04

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cess of legumes within these pastures varies considerably, but *Trifolium repens* is found throughout the ecological amplitude of kikuyugrass on the slopes of Maui's Haleakala volcano. To further describe the effect of vegetation, soil conditions and climate on rhizobial populations in natural habitats, *Rhizobium leguminosarum* bv. *trifolii* and *Bradyrhizobium sp*. were enumerated and the density of their host legumes measured in four kikuyugrass pastures.

## Materials and methods

## Site characterization

Four sites containing kikuyugrass swards with or without legumes were selected for this study. Soils at the four sites (Table 1) were classified by the Soil Conservation Service (1972, 1984) and detailed soil analyses were conducted (Ikawa *et al.*, 1985; SCS, ibid.). Mean annual rainfall (MAR) data were obtained from the Hawaii State Department of Land and Natural Resources (1982). Soil pH was measured in a 1:2 soil water paste.

## Vegetation measurement

Transects of 8 m length where legumes were present or absent were established at each of the four sites. Legume cover was obtained using a point intercept method at 10 cm intervals along the transects. Total shoot mass was determined from four 0.25 m<sup>2</sup> quadrats randomly located along each

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| Site       | Soil<br>classiciation | MAR <sup>a</sup><br>(mm yr <sup>-1</sup> ) | Elevation<br>(m) | pН  | Extractable<br>bases <sup>b</sup> | Base<br>saturation (%) |
|------------|-----------------------|--|------------------|-----|-----------------------------------|------------------------|
| Haleakala  | Humoxic tropohumult   | 1800                                       | 640              | 5.0 | 3.7                               | 11                     |
| Kekoa      | Typic dystrand        | 1060                                       | 884              | 6.4 | 43.1                              | 70                     |
| Olinda     | Entic dystrand        | 1200                                       | 1064             | 5.5 | 4.0                               | 12                     |
| Ulupalakua | Typic eutrand         | 810  | 700              | 6.5 | 54.0                              | 88                     |

Table 1 Site characterization of kikuyugrass pastures on Maui, Hawaii

<sup>a</sup> MAR, Mean Annual Rainfall, from Department of Land and Natural Resources, State of Hawaii  ${}^{b}$  mEq 100 g<sup>-1</sup> soil

Table 2 Relationship between the abundance of native *Rhizobium leguminosarum* bv. *trifolii*, white clover and kikuyugrass mat formation

|            | R. leguminosarum                  | White clover |                        | Total shoot                       | Kikuyugrass       |
|------------|-----------------------------------|--------------|------------------------|-----------------------------------|-------------------|
| Site       | $(\log_{10} g^{-1} \text{ soil})$ | (%)          | (kg ha <sup>-1</sup> ) | biomass<br>(kg ha <sup>-1</sup> ) | mat depth<br>(cm) |
| Haleakala  | 1.78                              | 0            | 0                      | 10 316                            | 10.8              |
|            | 5.00                              | 26.3         | 296                    | 3 980                             | tr <sup>a</sup>   |
| Kekoa      | 2.49                              | 0            | 0                      | 14 868                            | 12.8              |
|            | 4.76                              | 29.9         | 382                    | 6 675                             | 4.8               |
| Olinda     | 3.49                              | 0            | 0                      | 12 124                            | 21.0              |
|            | 5.49                              | 18.5         | 640                    | 6 750                             | 0.5               |
| Ulupalakua | 3.76                              | 0            | 0                      | 9 228                             | 7.0               |
|            | 5.23                              | 20.0         | 224                    | 4 214                             | 3.6               |

<sup>a</sup> tr, few stolons observed

**Table 3** Correlation (*r*) matrix of *R. leguminosarum* bv. *trifolii* and white clover with various factors measured at four sites in kikuyugrass pastures on Maui, Hawaii

|   | R. leguminosarum  | White clover   |   |
|---|---|--|---|
| Covariates  | $(\log_{10} g^{-1} \text{ soil})$   | (%)  | (kg ha <sup>-1</sup> )                    |
| <i>T. repens</i> (%)<br><i>T. repens</i> (kg ha <sup>-1</sup> )<br>Kikuyugrass mat (cm)<br>MAR (mm yr <sup>-1</sup> )<br>pH (1:2)<br>Extractable bases (mEq 100 g <sup>-1</sup> soil) | $\begin{array}{c} 0.82^{**} \\ 0.81^{**} \\ -0.76^{*} \\ -0.43 \\ 0.41 \\ 0.22 \end{array}$ | $\begin{array}{c} - \\ 0.79^{*} \\ -0.71^{*} \\ -0.09 \\ 0.24 \\ 0.12 \end{array}$ | 0.79*<br>-0.70*<br>-0.12<br>0.08<br>-0.10 |

\* P < 0.05; \*\* P < 0.01

transect. First, legume shoots were removed at soil level from each quadrat by hand clipping, then all remaining shoot material was removed with a rotary blade 'weed eater'. The legumes and grass samples were oven-dried at 65°C and weighed. The depth of the kikuyugrass mat was measured by placing a metre stick in the centre of each side of each quadrat (16 measurements per transect).

## Soil collection

Soil samples were collected to a depth of 25 cm at 0.5 m intervals along the 8 m transects with a 25 mm soil coring device. A subsample was used to measure soil moisture. Roots and, where legumes were present, nodules were removed from another subsample. The soil was diluted (1:10) on a dry weight basis for most probable number determination.

## Rhizobial enumeration

Soil populations of *Rhizobium* leguminosarum bv. *trifohi* and *Bradyrhizobium* sp. were measured using *Trifolium* repens cv. Regal Ladino and Macroptilium atropurpureum cv. Siratro, respectively, by plant infection procedures (Brockwell, 1963) modified by Woomer et al. (1988b).

## Data analysis

The data were analysed by the Stepwise and Rsquare regression procedures with adjusted  $R^2$  and correlation matrix options (SAS Institute Inc., 1985).

## Results

In the absence of a legume host, *Rhizobium* kguminosarum bv. *trifolii* densities ranged from  $log_{10}$  1.78 to  $log_{10}$  3.76 cells g<sup>-1</sup> soil, compared with  $log_{10}$  4.76 to  $log_{10}$ 5.49 cells g<sup>-1</sup> soil when *T. repens* was present in the *P. clandestinum* pasture (Table 2). Increases in total shoot biomass which consisted primarily of *P. clandestinum*, and *P. clandestinum* stolon mat thickness coincided with decreases of both clover shoot biomass and *Rhizobium leguminosarum* bv. *trifolii* populations in the soil.

Significant linear correlations were observed between *R. leguminosarum* bv. *trifolii* abundance, increases in host legume cover and decreases in kikuyugrass mat thickness (Table 3). The linear correlation was higher when using rhizobial population sizes expressed as  $\log_{10}$  cells g<sup>-1</sup> soil than with the non-transformed data (data not shown).

|  | Site 1         | Site 2               | Site 3 | Site 4 |
|--|----------------|----------------------|--------|--------|
| Bradyrhizobium spp. $(\log_{10})$          | 1.78           | 2.764.210 31610.84.9 | 4.00   | 5.00   |
| D. intortum (%)                            | 0 <sup>a</sup> |                      | 45.9   | 94.6   |
| Total shoot biomass (kg ha <sup>-1</sup> ) | 4000           |                      | 12 400 | 5 708  |
| Vegetation mat depth (cm)                  | 0              |                      | 28.8   | 4.5    |
| Soil pH (1:2)                              | 5.2            |                      | 5.0    | 4.9    |

**Table 4** Bradyrhizobium spp., Desmodium intortum cv. Greenleaf and selected measurements at four sites within the Haleakala Pasture, Hawaii

<sup>a</sup> No *D. intortum* was observed in any of the four 0.25 m<sup>2</sup> guadrats along the 8 m transect

Table 5 Linear regression models relating the abundance of Rhizobium leguminosarum bv. trifolii and Bradyrhizobium SDD.

| Rhiz | zobia (cells $g^{-1}$ soil)                | Regression model  | R <sup>2 c</sup>                  |
|------|--|---|-----------------------------------|
| A.   | bv. <i>trifolii</i> ª (log <sub>10</sub> ) | 2.9 + 0.0005 <i>T. repens</i> (kg ha <sup>-1</sup> )<br>0.005 <i>T. repens</i> (kg ha <sup>-1</sup> ) + 0.73 (pH) - 1.3<br>0.17 + 0.08 <i>T. repens</i> (%) + 0.47 (pH)<br>1.50 + 0.06 <i>T. repens</i> (%) + 0.35 (pH) - 0.04 kikuvugrass mat (cm) | 0.78*<br>0.84**<br>0.80*<br>0.80* |
| В.   | Bradyrhizobium sp. <sup>b</sup>            | 218.7 Desmodium intortum (%) – 63.3   | 0.99***                           |
|      |  |   |                                   |

 ${}^{a}_{\ b} \log_{10} \text{ cells g}^{-1}_{\ cells g}$  soil  ${}^{c}_{\ cells g} = {}^{1}_{\ soil}_{\ cells g} = {}^{2}_{\ soil}_{$ 

The relationship between Bradyrhizobium sp., vegetation and soil pH at one of the sites, Haleakala pasture, is presented in Table 4. Desmodium intortumis a stoloniferous trailing legume able to compete with the mat formation of P. *clandestinum*. Large population sizes of Bradyrhizobium sp. were observed in the

presence of the host legume at pH 4.9-5.0. Single and multiple linear regression models cor-relating rhizobial abundance with vegetation, climate and soil variables are presented in Table 5. Linear and multiple linear models predicting the population size of rhizobia were constructed on the significance of the adjusted coefficient of determination and the appropriateness of the y intercept.

## Discussion

The probable role of host legumes in maintaining rhizobia in natural habitats was demonstrated by the existence of a relationship between microsymbiont population sizes and legume host densities at four different kikuyugrass pastures in upland Maui.

The negative linear correlation between the thickness of the P. clandestinum mat and the abundance of both host legume (T. repens) and rhizobia of R. *leguminosarum* bv. *trifolii* in these pastures (Table 3) further indicated the important role that vegetation characteristics (*e.g.*, species mixtures) play in rhizobial population sizes. The results suggest that the morphology of a legume determine its persistence in kikuyugrass swards, particularly overgrown stands with pronounced stolon mats. *Desmodium intortum*, a vigorously stoloniferous legume, was well established in P. clandestinum with sticker stolon mats, and the soils at these sites supported populations of *Bradyrhizobium sp.* in the range log<sub>10</sub> 4.0 –log<sub>10</sub> 5.0 cells g-1 soil (Table 4).

Previous work on Maui across a wide range of environments indicates that legume density and mean annual rainfall are major determinants of Rhizobium and *Bradyrhizobium spp.* colonization across a wide range of vegetation formations and

climates (Woomer et al., 1988a). This study examines the legume and rhizobial population densities of the upland kikuyugrass communities; consequently, the range of abiotic parameters is lessened and mean annual rainfall did not determine rhizobial abundance. This is in agreement with and places the findings of Lawson et al. (ibid.) for R. leguminosarum bv. trifolii in Australia and of Yousef *et al.* (*ibid.*) for *Bradyrhizobium* associated with peanut in drylands in Iraq into context as studies conducted across a narrow range of soil moisture conditions.

Multiple linear models which predict the densities of rhizobia in natural habitats necessarily include a measurement of host legume cover or biomass (Table 5). Models using  $\log_{10}$  rhizobial populations and having low y intercept values include the addition of soil pH to host legume term (Table 5A). As soil pH and soil extractable bases (mEq 100 g<sup>-1</sup> soil) co-varied (r = 0.93), it was not appropriate to include both terms within the model of rhizobial abundance. Addition of kikuyugrass mat thickness (cm) did not increase the level of significance in the model (Table 5B) but the y intercept remained accurate, and an easily measured, although not an entirely independent element of vegetation formation was included in the model.

The multiple regression model which predicts the population size of *R. leguminosarum* bv. *trifolii* in the soil, based on the quantity of host legumes and the pH of the soil, agrees with the results obtained by adjusting the pH of a single site (Richardson and Simpson, 1988).

When these authors experimentally controlled the pH of a soil, numbers of R. leguminosarum by. trifolii associated with subterranean clover (Trifolium subterraneum L.) were influenced by the presence of host plants and the pH of the soil as well as by the seasonal influences of soil moisture. Thorton and Davey (1983) demonstrated a range of symbiotic effectiveness by strains of *R. leguminosarum* by. trifolii in association with subterranean clover growing in an acid soil (pH 4.4).

The linear model describing the relationship of *Bradyrhizobium sp.* to the cover of *Desmodium* 

*intortum* (Table 5B) was highly significant (r = 0.99).

Assuming that nitrogen availability of a soil is limiting legume growth, the magnitude of the response of that legume to inoculation is regulated by the population size and effectiveness of the rhizobia indigenous to the soil. Even though large quantities of nitrogen are present in primary and fallow vegetation, agricultural use of lands generally results in a steady decline of nitrogen content of soils, causing widespread of nitrogen (Bartholomew, 1977). deficiencies Consequently, the infection and symbiotic processes are seldom prevented by naturally occurring levels of soil nitrogen. The response to inoculation of legume seed with rhizobia when no infective or effective rhizobia are present is dramatic and predictable. However, where rhizobial populations of various sizes and effectiveness are present, agriculturists are unable to predict the probability and magnitude of the response to applied rhizobia. Assays have been developed which evaluate the "whole-soil" populations of rhizobia in terms of size and effectiveness for small-seeded legumes (Brockwell et al., 1988). These assays are less labour- and time-consuming than previous efforts and can serve as a site-specific guide to the need for inoculation. The prediction of rhizobial population size based on measurable ecological factors complements these efforts, acting in preliminary identification of soils deficient in rhizobia.

In conclusion, the abundance of R. *leguminosarum* bv. *trifolii* and *Bradyrhizobium spp*. in kikuyugrass pastures of East Maui were legume-host dependent. Populations of rhizobia and bradyrhizobia in soil samples from pasture areas devoid of host legumes ranged 1.78-3.76 loglo g-1 soil. Populations increased between 30- and 1600-fold when legumes were present in the pastures. The inability of T. *repens* to become established in certain kikiyugrass pastures was related to legume shoot competition with grass mat formation and not to a failure of the microsymbiont to maintain a presence in pasture systems devoid of their appropriate host legumes.

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