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Effectiveness of Nodulation on Growth and Nitrogen Content of Legumes Grown on Several Hawaiian Soils With and Without the Use of Proper *Rhizobium* Strains

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Effectiveness of Nodulation on Growth and Nitrogen Content of Legumes Grown on Several Hawaiian Soils With and Without the Use of Proper *Rhizobium* Strains[1]

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INTRODUCTION

Legumes are important in pastures. Legumes provide better quality forage by increasing the protein level of the pasture and furnish their companion grasses with a free source of nitrogen which has been fixed in the legume through symbiotic association with the proper *Rhizobium*. The importance of proper legume nodulation cannot be overemphasized. Annual legumes will fix between 50 to 100 pounds of nitrogen per acre per year and perennial legumes will fix annually over 300 pounds of nitrogen per acre (Whitney, 1966). The ability of the legume to fix nitrogen in symbiotic association with proper *Rhizobium* strains is particularly important in the tropics where many of the soils are highly leached and depleted of available nitrogen. Grasses grown without companion legumes on these

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highly leached soils are rather low in dry matter production and in protein content unless supplied with nitrogen fertilizer. The best way to overcome this may be the use of properly inoculated, well-adapted legumes grown with the grass.

Much of Hawaii's vegetation is composed of tropical and semitropical legumes. When the land is cleared for agricultural use, the assumption is often made that there is no need to provide inoculum for the pasture legume since the native inoculum in the soil will be effective. This is a fallacy which often has led to difficulties in legume establishment and poor legume performance. No wonder the idea has developed that tropical pasture legumes are inefficient nitrogen fixers! Moore (1960), Norris (1959), Norris and 'T Mannetje (1964), and Whitney (1966) have emphasized that certain tropical pasture legumes inoculated with an appropriate strain of *Rhizobium* are effective nitrogen fixers. Those uninoculated or improperly inoculated legume plants which do grow may be competing directly with the associated grasses for available nitrogen instead of providing a source of nitrogen for the grass.

The purpose of this study was to determine the effect of nodulation on the dry matter yield and nitrogen content of inoculated and uninoculated legumes grown on several soils selected from six of the great soil groups.

MATERIALS AND METHODS

The following soils were used:

Great Soil Group	*Soil Series*
Red Desert	Kawaihae
Reddish Prairie	Pahala
Low Humic Latosol	Waimanalo
Latosolic Brown Forest	Maile
Hydrol Humic Latosol	Manu
Hydrol Humic Latosol	Honokaa
Aluminous-Ferruginous Humic Latosol	Kapaa

Soil pH was adjusted to pH 6–7 using $Ca(OH)_2$.

The following legume species were used:

Alfalfa	*Medicago sativa* L.
Centro	*Centrosema pubescens* Benth.
Kaimi clover	*Desmodium canum* (Gmel.) Schintz and Thellung
Siratro	*Phaseolus atropurpureus* DC.

Commercial strains of the appropriate *Rhizobium* were used.

A factorial design was used in the experiment with the following as main effects:

plant species (four species)
plant treatments (inoculated and uninoculated plants)
soils (seven soils)
blocks (three pots per plant treatment)

The pots were segregated into two groups—inoculated and uninoculated—on the greenhouse bench in order to prevent contamination. Pots within each group were randomized at planting time and at 6 weeks after planting. After seedling emergence the plants were thinned to two plants per pot. All treatments received a nutrient solution lacking nitrogen each week.

Plants were harvested as soon as the first flower buds were observed. This time varied according to the species; alfalfa, 65–70 days; Kaimi clover, 80–90 days; Siratro, 60–65 days; and Centro, 70–80 days. The whole plant was separated from the soil, and tops were removed for yield and total nitrogen determination by the Kjeldahl method. Roots were visually scored for numbers, size, and distribution of nodules as 3 (best), 2 (intermediate), and 1 (poor). All results were recorded on a pot basis.

RESULTS AND DISCUSSION

The dry weight of the tops of each plant species grown on the different soils is presented in Table 1. It should be noted that the dry weights of one plant species cannot be directly compared with those of other species, because of differences in their morphology and in the length of growing period. They are comparable within species grown in any of the different soils. The response to inoculation is remarkable for each species in each soil in which they were grown. There was an average increase of 46.74 percent in dry matter production with a range of 35.71 to 50.88 percent. Kaimi clover and Siratro plant yields were low because of African snail damage.

An analysis of variance of dry matter yields is presented in Table 2. Main effects and their interactions except for the block effect were statistically significant or statistically highly significant. Some of the plant

TABLE 1. The average dry weight in grams of the tops of inoculated and uninoculated plants of four legume species grown in seven different soils*

Soil Series	Alfalfa		Siratro		Centro		Kaimi clover		Average	
	In.[1]	Un.[2]	In.	Un.	In.	Un.	In.	Un.	In.	Un.
Kawaihae	3.20	2.00	2.16	1.53	2.06	0.93	1.26	0.76	2.17	1.31
Pahala	2.20	1.20	3.01	1.43	1.03	0.66	0.66	0.63	1.72	0.98
Waimanalo	2.16	1.60	2.10	1.40	0.74	0.70	0.90	0.73	1.47	1.11
Maile	1.00	0.70	1.03	0.73	0.76	0.66	0.66	0.66	0.86	0.69
Manu	1.10	0.83	0.83	0.70	1.50	1.06	0.80	0.60	1.06	0.81
Honokaa	1.06	0.70	0.83	0.66	0.86	0.63	0.93	0.66	0.92	0.66
Kapaa	1.33	0.93	1.76	1.26	0.73	0.63	1.43	0.86	1.31	0.92
Average	1.72	1.14	1.67	1.10	1.10	0.75	0.95	0.70	1.36	0.92
Percentage of dry matter *increase* (inoculated over uninoculated)	50.88		51.81		46.67		35.71		47.82	

*The weights of any one species are not directly comparable with those of another because of differences in the length of growing periods and in growth habit. They are comparable, however, within any one species over all soils in which they were grown.

[1] Inoculated

[2] Uninoculated

TABLE 2. Analysis of variance of the dry weight of the tops of inoculated and uninoculated plants of four legumes grown in seven different soils

Source of Variance	d.f.	Mean Squares
Blocks	2	0.215
Soils	6	2.93**
Plant species	3	4.16**
Treatments	1	7.88**
Soil x Plant species	18	0.845**
Soil x Treatment	6	0.4316**
Plant species x Treatment	3	0.33**
Soil x Plant species x Treatment	18	0.1427*
Error	110	0.074

*Significant at 5% level
**Significant at 1% level

species were unadapted to some of the soils. Not all of the plant species responded similarly to inoculation in the different soils. There is also the effect of soil on plant growth. The soils which were weathered the least produced the greatest amount of plant growth. The soils in their approximate order of increasing weathering are Kawaihae, Pahala, Waimanalo, Maile, Manu, Honokaa, and Kapaa. The important feature to note is the increase in dry matter yields of the inoculated plants over the uninoculated (Table 1).

The average score for root nodulation and the percentage nitrogen in the tops for each plant species grown in the different soils are presented in Table 3. Inoculated plants had a higher percentage of nitrogen than uninoculated plants. The average increase of percent nitrogen in the tops was 37.13 percent. The average overall percentage increase (40.39 percent) was greatest for Kaimi clover. The lowest (29.15 percent) was for Siratro. It should be pointed out that the Waimanalo soil was obtained from a field which had been previously planted to *Desmodium* species. Although there were no great differences in yield (Table 1) between inoculated and uninoculated Kaimi clover grown in Waimanalo soil, there was a large increase in the percentage of nitrogen in the inoculated Kaimi plant over the uninoculated plants (Table 3).

TABLE 3. The average score for nodulation and average percentage of nitrogen in the tops of inoculated and uninoculated legume species grown in seven different soils

Soil Series	Alfalfa				Siratro				Centro				Kaimi clover			
	Nodules*		% N		Nodules		% N		Nodules		% N		Nodules		% N	
	In.[1]	Un.[2]	In.	Un.	In.	Un.	In.	Un.	In.	Un.	In.	Un.	In.	Un.	In.	Un.
Kawaihae	3.0	2.0	3.32	2.68	3.0	1.0	3.03	2.00	3.0	1.3	2.03	1.86	3.0	2.0	2.88	2.40
Pahala	3.0	1.6	3.03	2.53	3.0	2.0	3.32	2.57	3.0	1.3	2.75	2.02	3.0	1.5	2.76	2.34
Waimanalo	3.0	1.6	3.14	1.59	3.0	2.6	3.43	3.08	3.0	2.0	2.76	1.85	3.0	1.3	3.00	1.49
Maile	3.0	2.0	2.84	2.23	2.3	1.0	2.70	1.85	3.0	2.0	2.95	2.06	3.0	2.0	2.70	2.09
Manu	3.0	1.3	2.83	1.94	3.0	2.0	3.29	2.56	3.0	2.0	2.70	1.99	3.0	2.0	2.94	2.03
Honokaa	3.0	2.0	2.72	1.92	3.0	3.0	3.07	2.83	3.0	1.6	2.88	2.13	3.0	1.3	2.91	1.44
Kapaa	3.0	2.0	3.34	2.97	3.0	2.0	3.47	2.43	3.0	1.0	3.25	1.97	3.0	2.5	2.78	2.40
Average	3.0	1.8	3.03	2.27	2.9	1.9	3.19	2.47	3.0	1.6	2.76	1.98	3.0	1.8	2.85	2.03
Percentage of nitrogen *increase*	33.48				29.15				39.39				40.39			
Average percentage of nitrogen *increase*—35.60																

*Nodule score: 3—Best, 2—Intermediate, 1—Poor

[1] Inoculated

[2] Uninoculated

The nodulation scores for inoculated and uninoculated plants indicate that the use of recommended commercial inoculum gave the best nodulation (Table 3) in terms of number, size, and distribution. Depending on the soil to furnish the inoculum resulted in much lower nodulation scores, which in turn resulted in a decrease of percentage nitrogen in the plants and lower dry matter yields.

Analysis of variance for percentage nitrogen in the tops of inoculated and uninoculated plants grown in the different soils is presented in Table 4. Statistically significant or statistically highly significant differences were obtained for species, treatment, soils, and for their interactions.

TABLE 4. Analysis of variance of the percentage of nitrogen found in the tops of inoculated and uninoculated plants of four legume species in seven different soils

Source of Variance	d.f.	Mean Squares
Blocks	2	0.3**
Soils	6	0.394**
Plant species	3	1.673**
Treatments	1	25.889**
Soil x Plant	18	0.370**
Soil x Treatment	6	0.203**
Soil x Plant x Treatment	18	0.143*
Error	110	0.00079

*Significant at 5% level
**Significant at 1% level

In order to obtain a measure of the amount of nitrogen obtained by the use of proper inoculum, the average nitrogen content of the plants expressed as milligrams per pot is presented in Table 5. The amount of nitrogen per pot for inoculated plants averaged over all soils was 40.4 mg as compared to 20.6 mg for uninoculated plants averaged over all soils. The amounts of available nitrogen present in the soils at the beginning of the experiment and of nitrogen released during the growth period were not determined. There is no direct measure available, therefore, of the amount

TABLE 5. The nitrogen content in milligrams per pot from plants grown from uninoculated and inoculated seeds in seven different soils

Soil Series	Alfalfa		Siratro		Centro		Kaimi clover		Average	
	In.[1]	Un.[2]	In.	Un.	In.	Un.	In.	Un.	In.	Un.
Kawaihae	106.2	53.6	65.4	30.6	41.8	17.3	36.3	18.2	62.4	29.9
Pahala	66.7	30.4	99.9	36.7	28.3	13.3	18.2	14.7	50.3	23.8
Waimanalo	67.8	25.4	72.0	43.1	20.1	12.9	27.0	10.9	46.7	23.0
Maile	28.4	15.6	27.8	13.5	22.4	13.6	17.8	13.8	24.1	14.1
Manu	31.1	16.1	27.3	17.9	40.5	21.1	23.5	12.2	30.6	16.8
Honokaa	28.8	13.4	25.5	18.7	24.8	13.4	27.1	9.5	26.5	13.7
Kapaa	44.4	27.6	61.1	30.6	23.7	12.4	39.8	20.6	42.2	22.8
Average	53.3	26.0	54.1	27.3	28.8	14.9	27.1	14.3	40.4	20.6

[1] Inoculated

[2] Uninoculated

TABLE 6. Percentage crude protein equivalent (percent nitrogen x 6.25) of four legume species grown with and without the use of commercial inoculum on seven Hawaiian soils

Soil Series	Alfalfa		Siratro		Centro		Kaimi clover		Average	
	In.[1]	Un.[2]	In.	Un.	In.	Un.	In.	Un.	In.	Un.
Kawaihae	20.75	16.75	18.94	12.50	12.69	11.62	18.00	15.00	17.59	13.97
Pahala	18.94	15.81	20.75	16.06	17.19	12.62	17.25	14.63	18.53	14.78
Waimanalo	19.62	9.94	21.44	19.25	17.25	11.56	18.75	9.31	19.26	12.51
Maile	17.75	13.94	16.87	11.56	18.44	12.87	16.87	13.06	17.48	12.86
Manu	17.69	12.12	20.56	16.00	16.87	12.44	18.37	12.69	18.37	13.31
Honokaa	17.00	12.00	19.18	17.69	18.00	13.31	18.19	9.00	18.09	13.00
Kapaa	20.87	18.56	21.69	15.19	20.31	12.31	17.37	15.00	20.06	15.26
Average	18.95	14.16	19.92	15.46	17.25	12.39	17.83	12.68	18.48	13.67

[1] Inoculated

[2] Uninoculated

of nitrogen fixed by the plants. There is, however, at least a twofold increase in the amount of nitrogen per pot of inoculated plants as compared to uninoculated plants (Table 5). An increase of this magnitude was obtained for each plant species averaged over all soils. The increase was much greater than this for individual species on individual soils.

The percentage of nitrogen (Table 3) was converted to give a measure of the percent crude protein equivalent (percent nitrogen x 6.25) in each of the plant species studied (Table 6). Among inoculated plants averaged over all soils, Siratro had the highest, 19.92 percent, and Centro had the lowest, 17.25 percent. Among uninoculated plants averaged over all soils, Siratro had the highest, 15.46 percent, and Centro had the lowest, 12.39 percent. For any of the four species averaged over all soils, there was at least a 4 percent difference in crude protein equivalent between inoculated and uninoculated plants.

These changes in percentage of crude protein equivalent may mean the difference between a pasture which is supplying an adequate maintenance level for the grazing animal or one which requires a protein supplement. The cost of obtaining this benefit is cheap—a dollar-sized package of commercial inoculum—enough for one bushel of seed, which seeded at the rate of 5 pounds per acre will cover 12 acres of land.

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