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# INFLUENCE OF LIME ON NITROGEN FIXATION BY TROPICAL AND TEMPERATE LEGUMES*

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## SUMMARY

A nitrogen deficient Oxisol which had been fertilized with P, K, Zn and Mo received $CaCO_3$ at rates which increased continuously from zero to 22 tons/ha. Liming produced a range of pH in the saturation paste from 4.7 to 7.1; a range of calcium in the saturation extract from 0.3 to 3 meq/l; and a significant decline in available manganese. Responses of 9 tropical and 7 temperate legumes were compared across the pH variable.

For Stylosanthes spp. nodule numbers and weight and plant yield declined above pH 5.5. *Arachis hypogaea* and *Vigna sinensis*, whose yield increased by only 30%, showed no clear improvement in nodulation or nodule effectiveness (acetylene reduction rate per unit nodule weight).

Increased nodule effectiveness could account for most of the growth increase in *Dolichos axillaris* and *Glycine max* var. Kahala. Both the number and effectiveness of nodules increased for *Desmodium intortum, Glycine wightii, Medicago sativa,* and *Trifolium subterraneum*. Nodule size and effectiveness increased for *G. max* var. Kanrich. Only in one species, *Coronilla varia,* could increase in nodule numbers alone account for the increased growth associated with liming, although increased numbers of nodules probably accounted for much of the response by *Lotus corniculatus,* and by *Desmodium canum* and *D. intortum* up to pH 5.3.

Increased nodulation and plant N contents were consistent with the conclusion that for most species improved $N_2$-fixation was the cause of growth improvement associated with liming. However, percent N was high in leaves of Vigna and *Phaseolus vulgaris* at all lime levels. In Phaseolus, variation in nodulation and growth were unrelated. The growth improvement was associated with decline in leaf N, suggesting that something other than N nutrition was limiting.

## INTRODUCTION

Acidity, calcium deficiency, and manganese toxicity tend to occur together in soils. Each has been shown to inhibit nodulation of various legumes. The three factors interact, high calcium tending to

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mitigate adverse effects of acidity and manganese on nodule number[4 5].

There is less evidence concerning effects of these factors on the function of established nodules[4 5]. Such an inhibition has been most clearly shown with *Trifolium subterraneum*, where suboptimal calcium in solution culture reduced nitrogen fixation without reducing nodule growth[1]. However the general emphasis in relation to soil acidity has been placed on nodulation more than on nodule function. Frequently this emphasis may have been misplaced.

Furthermore there may be circumstances wherein acid-related soil factors inhibit the growth of dinitrogen-dependent legumes in ways not directly related to nitrogen fixation. That is, the nitrogen-fixing subsystem may sometimes be less sensitive than other subsystems in the symbiosis.

We took the opportunity to test these ideas during the course of a field trial which compared lime responses of legumes on a nitrogen deficient Oxisol of Hawaii. The field trial is described elsewhere[6]. This paper describes results of plant nitrogen analyses and observations of nodule number, nodule size, and nodule effectiveness as assayed by acetylene reduction in the field.

## PROCEDURES

The field trial[6] was on a severely nitrogen deficient Oxisol (Wahiawa silty clay) derived mainly from basic igneous rock, on the island of Oahu, Hawaii. The soil received treble superphosphate, potassium chloride, zinc sulfate and sodium molybdate (1340, 56, 31, 2.6 kg/ha respectively). Lime ($CaCO_3$) was applied at rates increasing up to 22 tons/ha along the plant rows to produce a pH-gradient in the surface 15 cm from 4.7 (saturation paste) at one end of the row to 7.1 at the other end. This also caused a corresponding continuous reduction in manganese availability and an increase in saturation extract calcium from 0.3 to 3 meq/l. There were 4 replicate blocks with the lime gradient laid in different directions to counteract possible systematic site variations.

The legumes were seeded immediately after inoculation with slurries of peat inoculants (Nitragin Co. Inc., Milwaukee, Wisconsin), applied at three times the manufacturer's recommended rate. The site had carried indigenous nodulated Indigofera, Leucaena, and Crotalaria.

Nodulation and acetylene reduction were observed soon after nodules became established. Individual plants of large-seeded legumes, or 5 cm row sections of small-seeded legumes, were taken at intervals of 2–3 meters along the row, some attempt being made to sample plants that appeared representative of the adjacent meter of row. The plants were lifted with trowel or tiling spade, and separated from the loose, finely aggregated soil with as little breakage or detachment of nodules as possible. As well as possible, nodules that became detached were recovered and included in the sample. In most cases few nodules detached, but *Leucaena leucocephala* lost almost all its nodules. Because of this we present no data on Leucaena; but it did nodulate at all lime levels and its mean nodule size did not vary obviously with treatment. Most samples were not washed. Soil without nodules had insignificant acetylene-reducing activity.

For acetylene reduction assay, shoots were cut off just above the uppermost nodule, excess root was trimmed, and the nodules and attached roots were put into jars of capacity 50, 100, or 250 ml depending on the sample size, with a few drops of water to maintain high humidity. Each jar was sealed with a screw cap into which had been installed a rubber serum stopper. Acetylene from a portable carbide/water generator was then injected through the serum stopper to bring the atmosphere in the jar to 0.1 atmosphere with respect to acetylene, and the jars were incubated in the field for 1.0 hours. A small gas sample was then withdrawn for subsequent determination of ethylene by gas chromatography. Lapsed time from digging to gas sample withdrawal never exceeded 1.5 hours. Incubation jars were at all times shaded and all tests were done between 10:00 a.m. and 3:00 p.m. These precautions, with the aid of the local climate, kept incubation temperatures between 27 and 29°C.

The plant tops, trimmed at ground level, were refrigerated in plastic bags until counted and weighed fresh.

Nodules from the acetylene reduction tests were counted and weighed after being detached, washed, and blotted. Nodule dry weights were not obtained because complete removal of soil was difficult, and though soil caused little error in fresh weight it could have caused large error in dry weight.

The data presented in Fig. 3 are mean weights per nodule and mean rates of acetylene reduction per unit nodule fresh weight. These two quantities were more precise than the corresponding quantities measured on a per plant basis, because of variable numbers of nodules per plant. Nodule count per plant not only was imprecise but probably had significant systematic error, since more of the nodules seemed to get lost if the plants were large or distally nodulated than if they were small or only proximally nodulated. Quantitative nodule recovery was constrained by the need to minimize damage to the experiment.

Kjeldahl N analyses were done on leaf samples taken at flowering or at the final harvest[6].

## RESULTS AND DISCUSSION

Of the several pH-related soil factors which may influence fixation, aluminum toxicity and molybdenum deficiency are unlikely on this soil. A decrease in extractable manganese may have enhanced growth and nodule function in the pH range 4.7 to 5.5.

Increased calcium availability associated with liming probably was beneficial throughout the entire pH range but especially above pH 5.8[6]. Nevertheless, we have plotted plant data against soil pH, because pH provides a familiar, stable and convenient scale to summarize changes induced by lime.

Fig. 1 compares the yield responses of the various species at final harvest. These data have been discussed elsewhere[6] along with

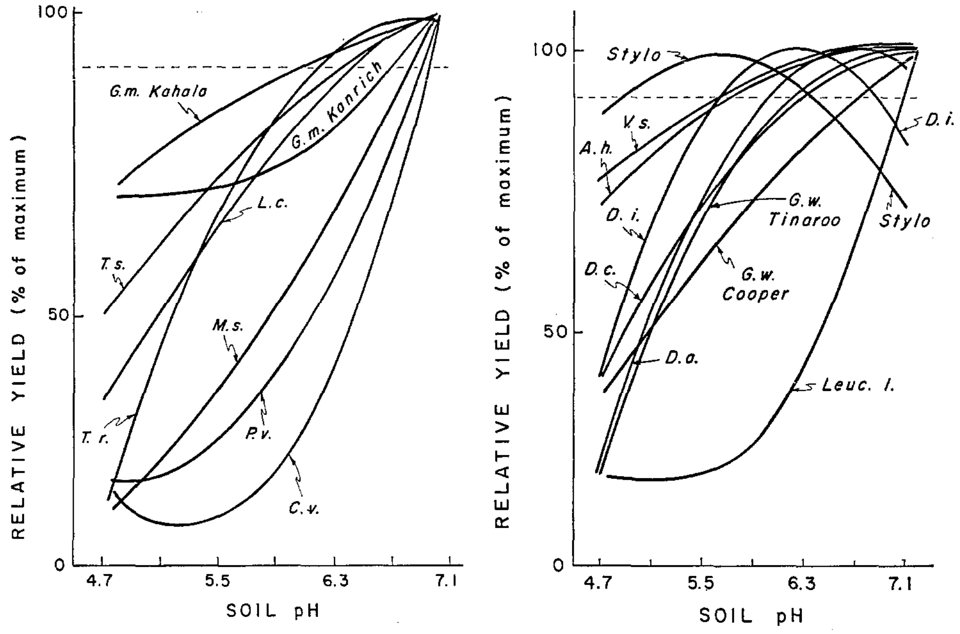
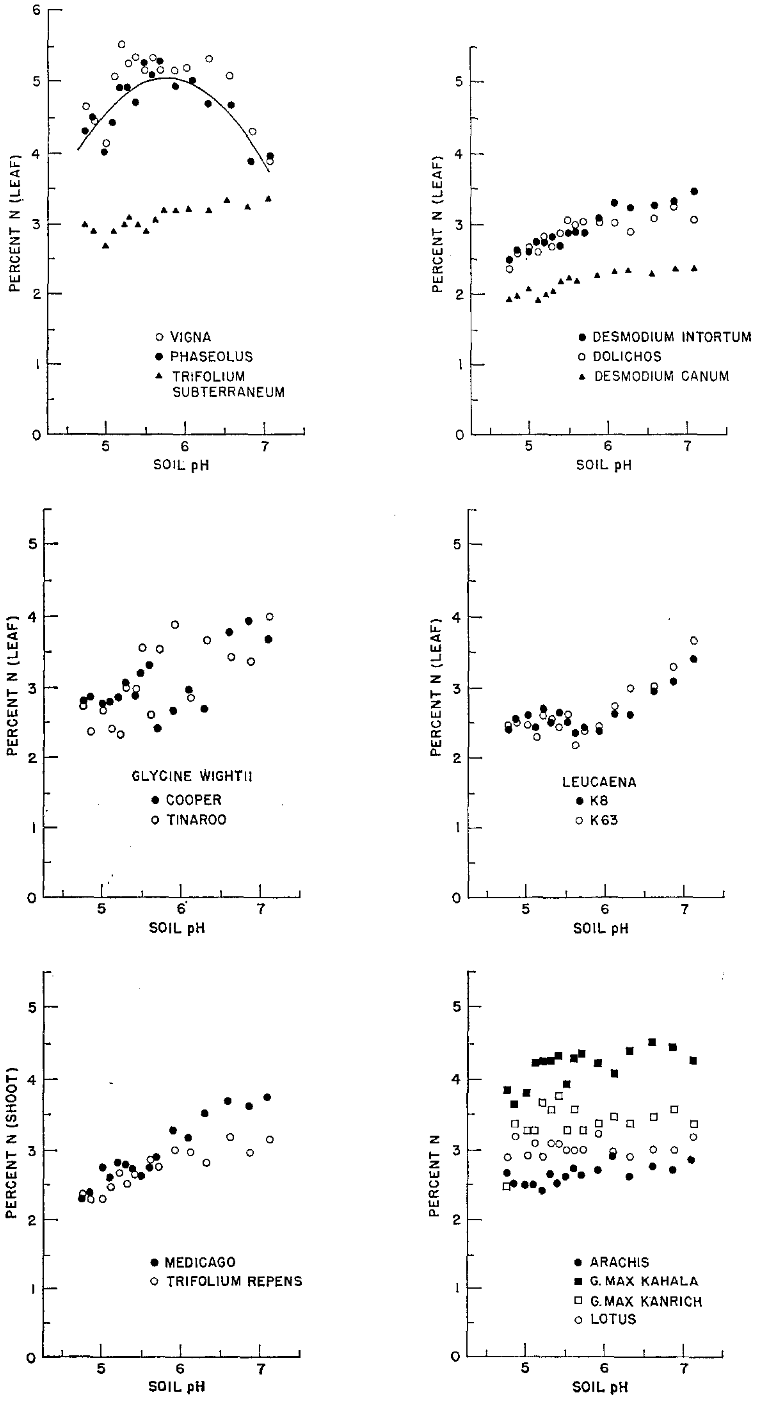


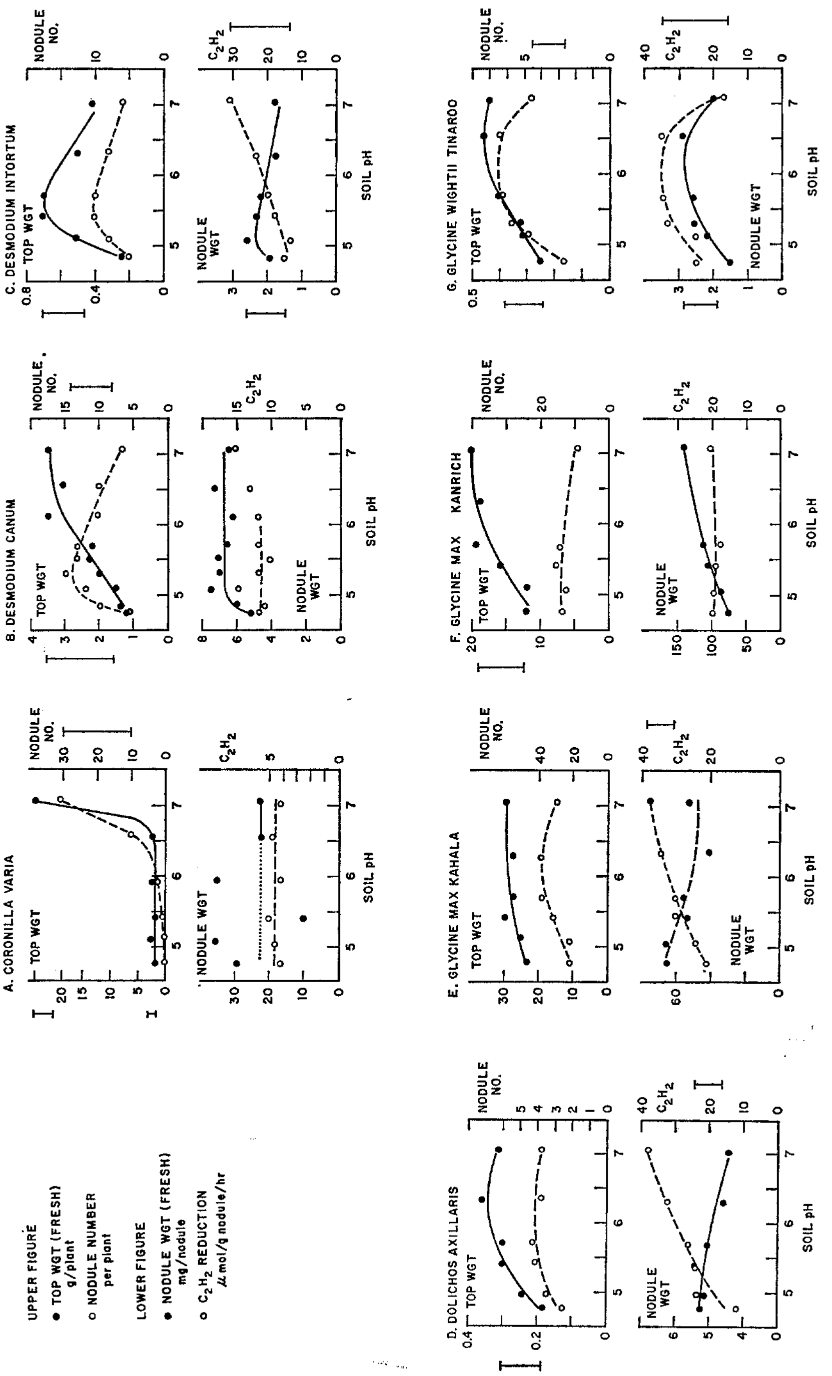
Fig. 1. Yield responses to liming at final harvest: relative yield as a percentage of maximum attained yield for each species is plotted against saturation paste soil pH.

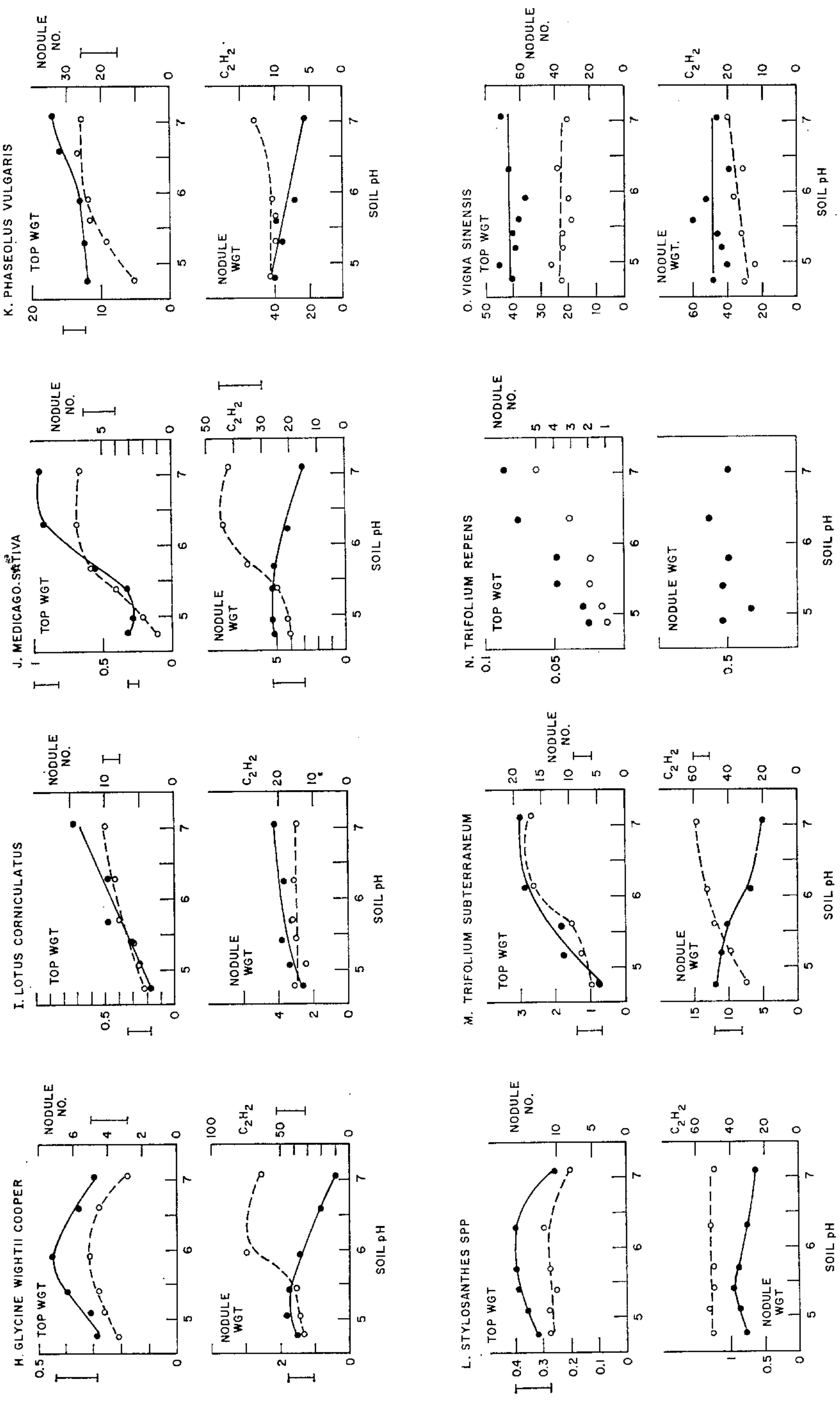
Identifying symbols refer to plant types as follows (alphabetically): A.h. *Arachis hypogaea;* C.v. *Coronilla varia;* D.c. *Desmodium canum;* D.i. *Desmodium intortum;* D.a. *Dolichos axillaris;* G.m. *Glycine max* vars. Kahala and Kanrich; G.w. *Glycine wightii* vars Tinaroo and Cooper; L.l. *Leucaena leucocephala;* L.c. *Lotus corniculatus;* M.s. *Medicago sativa;* P.v. *Phaseolus vulgaris;* Stylo *Stylosanthes gracilis* and *S. fruticosa;* T.r. *Trifolium repens;* T.s. *Trifolium subterraneum;* V.s. *Vigna sinensis.*

Fig. 2. *(Page* 595*)* Plant N (tops) or leaf N in relation to soil pH.

Fig. 3. *(Pages* 596 *and* 597*)* Nodulation and acetylene reduction data, with shoot fresh weight at time of nodule observations. Species arranged alphabetically. *Vertical bar* alongside an ordinate represents LSD ($p = 0.05$) between any two individual points.


PERCENT N (LEAF)
SOIL pH
○ VIGNA
● PHASEOLUS
▲ TRIFOLIUM SUBTERRANEUM
PERCENT N (LEAF)
SOIL pH
● DESMODIUM INTORTUM
○ DOLICHOS
▲ DESMODIUM CANUM
PERCENT N (LEAF)
SOIL pH
GLYCINE WIGHTII
● COOPER
○ TINAROO
PERCENT N (LEAF)
SOIL pH
LEUCAENA
● K8
○ K63
PERCENT N (SHOOT)
SOIL pH
● MEDICAGO
○ TRIFOLIUM REPENS
PERCENT N
SOIL pH
● ARACHIS
■ G. MAX KAHALA
□ G. MAX KANRICH
○ LOTUS
0
1
2
3
4
5
6
5
6
7


UPPER FIGURE
● TOP WGT (FRESH) g/plant
○ NODULE NUMBER per plant
LOWER FIGURE
● NODULE WGT (FRESH) mg/nodule
○ $C_2H_2$ REDUCTION μ mol/g nodule/hr
A. CORONILLA VARIA
B. DESMODIUM CANUM
C. DESMODIUM INTORTUM
D. DOLICHOS AXILLARIS
E. GLYCINE MAX KAHALA
F. GLYCINE MAX KANRICH
G. GLYCINE WIGHTII TINAROO
TOP WGT
NODULE NO.
NODULE WGT
$C_2H_2$
SOIL pH


H. GLYCINE WIGHTII COOPER
TOP WGT
NODULE NO.
NODULE WGT
$C_2H_2$
I. LOTUS CORNICULATUS
TOP WGT
NODULE NO.
NODULE WGT
$C_2H_2$
J. MEDICAGO SATIVA
TOP WGT
NODULE NO.
NODULE WGT
$C_2H_2$
K. PHASEOLUS VULGARIS
TOP WGT
NODULE NO.
NODULE WGT
$C_2H_2$
L. STYLOSANTHES SPP
TOP WGT
NODULE NO.
NODULE WGT
$C_2H_2$
M. TRIFOLIUM SUBTERRANEUM
TOP WGT
NODULE NO.
NODULE WGT
$C_2H_2$
N. TRIFOLIUM REPENS
TOP WGT
NODULE NO.
NODULE WGT
O. VIGNA SINENSIS
TOP WGT
NODULE NO.
NODULE WGT.
$C_2H_2$
SOIL pH
5
6
7

evidence that the responses did not result from the influence of lime on the mineralization of organic soil nitrogen, which was too slow to support significant growth.

At the highest rates of lime, yield declined in Stylosanthes (Fig. 1), and percentage plant N declined in *P. vulgaris* and *V. sinensis* (Fig. 2). In *V. sinensis*, fixation would seem to have supplied N at luxury levels: the plants could afford a drop in leaf N without growth reduction at high pH. In *P. vulgaris* the decline in foliar N coincided with the principal increase in growth and yield, with implications which will be discussed below.

Plant N in the other species increased or stayed constant with increasing lime (Fig. 2), consistent with the idea that the main beneficial influence of lime on growth was due to increased nitrogen fixation.

Nodule number and weight tended to correlate with plant size (Fig. 3). This, of course, need not imply that increased growth results from increased nodulation. The reverse is possible, and indeed probable in those cases where very high lime levels depressed growth, because such depression was not accompanied by nitrogen deficiency symptoms. However, at lower lime levels, where increasing lime improved both growth and nodulation together, it also alleviated symptoms of nitrogen deficiency, making it reasonable to suppose that the growth improvement resulted at least in part from improved nodulation.

Suboptimal calcium concentrations have been shown to reduce nodule effectiveness (nitrogen fixation per unit nodule weight)[1], in solution-grown Trifolium[1]. Some experiments utilizing soils as growth media strongly suggest a similar effect of excessive manganese[2 5 8]. But effects of soil acidity on nodule effectiveness have received less attention than effects of acidity on nodule initiation and formation[4 5]. Fig. 3 is evidence that lime had important influence on nodule effectiveness. The relative contribution of number, size, and effectiveness to the total response varied between species.

Increased nodule number could account for the growth response in *Coronilla varia* (Fig. 3a), for much of the response in *Lotus corniculatus* (Fig. 3i) and in Desmodium up to pH 5.5 (Fig. 3b, 3c). Also nodule number in *Glycine wightii* (Fig. 3a, 3h), *Medicago sativa* (Fig. 3j), and Trifolium (Fig. 3m, 3n), increased markedly over some portion of the pH range.

Equally important was nodule effectiveness as judged by the data on acetylene reduction. Increases in effectiveness could account for most of the observed growth response in *Dolichos axillaris* (Fig. 3d) and *Glycine max* var. Kahala (Fig. 3e). Effectiveness increased together with nodule numbers in the pH range up to 5.5 or 6 in four species: *Desmodium intortum* (Fig. 3c), *Glycine wightii* (Fig. 3g, 3h), *Medicago sativa* (Fig. 3j), and *Trifolium subterraneum* (Fig. 3n). In three of these, nodule size tended to decrease as lime increased nodule number and effectiveness.

Nodule size increase could account for most of the yield increase in *Glycine max* var. Kanrich (Fig. 3f), and was substantial along with other effects in *G. wightii* var. Tinaroo (Fig. 3h).

There were no notable changes in nodule number, size, or effectiveness in those species whose growth increased least with lime *viz.* *Stylosanthes* spp (Fig. 31), *Vigna sinensis* (Fig. 30), and *Arachis hypogaea* (only nodule count available, data not shown).

*Phaseolus vulgaris* (Fig. 3k) increased its nodule number with increasing lime up to pH 6, with a corresponding increase in leaf N but no corresponding growth improvement. The growth improvement which did occur in Phaseolus, above pH 6, was accompanied by no improvement in nodule characteristics and by a decrease in foliar % N from a very high 5% to 4% (Fig. 2). This implies that growth increase was not due to relief of a limitation on nitrogen fixation but rather to relief of some other limitation. The only clue to the nature of the limitation is that *P. vulgaris* leaves had an unidentified interveinal chlorosis and contained approximately 230 ppm zinc and 1100 ppm manganese at all lime levels (Okazaki, Fox, and Munns, unpublished). Conceivably this means that toxicity of zinc or another heavy metal was mitigated as the pH or calcium supply increased.

For some species, the nodulation and acetylene reduction data of Figure 3 give a different impression of the effect of lime on nitrogen fixation than do the yield data of Fig. 1. For example, Fig. 3 implies that high lime rates should have reduced nitrogen fixation in *D. canum* and *G. wightii* var. Tinaroo, and should have caused no improvement in *G. max* and *V. sinensis*. But Fig. 1 shows that all these species eventually gave positive growth increases in the upper range of lime rates. In part the discrepancies may result from difficulties in recovering nodules from the soil and the possibility that loss of nodules during sampling increased systematically with increased growth. However, the discrepancies may represent real changes in response between early nodulation observations and final harvest some weeks later. We have evidence[7] that early lime-induced depressions of growth in some of the species were transitory, giving place to positive responses as the experiment proceeded.

The preceding is an example of the recognized impossibility of assessing seasonal nitrogen fixation from single observations of nodulation and acetylene reduction[3]. Apart from any uncertainty in calibrating acetylene reduction, temporal variation in fixation rate makes it necessary to integrate multiple assessments of fixation throughout the growth period. Our observations made at a single time have little value in this regard. Nevertheless they do serve well enough to compare effects of lime at a particular time during early growth; and to show that legume/rhizobium combinations respond to soil acidity correction in a variety of ways, including increase in nodule size and effectiveness as well as increase in nodule number. This latter point is important in assessing acid-soil effects.

Thus it is not dependable to assess the acid-soil tolerance or lime response of a legume-rhizobium combination simply by measuring nodule number or weight, much less by merely observing the presence or absence of effective-looking nodules. Most of the species investigated here formed apparently healthy nodules at pH 4.7, yet growth was increased by lime. In solution culture, similarly large increases in growth associated with increased pH or calcium concentration have been noted with well nodulated plants of genera Medicago, Trifolium, Glycine, Macroptilium and Desmodium (C. S. Andrew, personal communication).

Finally the data for *Phaseolus vulgaris* serve as a reminder that a legume's response to acidity correction may be unrelated to nitrogen fixation, except insofar as improved growth permits greater fixation. When this case goes unrecognized, even the best estimates of nitrogen fixation miss the point.

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