THE EFFECT OF LIME AND *RHIZOBIUM* STRAIN ON THE NODULATION OF *GLYCINE WIGHTII* AND *MACROPTILIUM ATROPURPUREUM* ON ACID SOILS

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ABSTRACT

The effects of low rates of lime and two Rhizobium *strains on nodulation and early growth of* Glycine wightii *and* Macroptilium atropurpureum *(Siratro) were measured in field trials on two soil types.*

Glycine had fewer nodulated plants and made less growth than Siratro, and the difference was more pronounced on krasnozem soil, where nodulation and early growth were poor. Lime, mixed with superphosphate and banded with the seed, increased the percentage of plants nodulated and the number of nodules per nodulated plant, for glycine, but did not influence Siratro. The two Rhizobium *strains (QA922 and CB756) did not differ in the percentage of plants nodulated but the number of nodules per nodulated glycine plant was higher with strain QA922. Plant weights were not affected by the treatments, but large differences occurred between sites. The influence of the high manganese content of the krasnozem soil is discussed.*

INTRODUCTION

Glycine wightii (glycine) is a useful perennial legume for the coast of northern New South Wales and Queensland. However on certain soil types, particularly krasnozems, the young plant often lacks vigour and is slow to nodulate (Thomas and Whiteman 1971; Diatloff and Luck 1972).

Glycine is more sensitive than most other tropical legumes to low pH, low exchangeable calcium, and high manganese (Andrew 1970; Andrew and Hegarty 1969), properties which are common to many coastal soils, particularly krasnozems. Responses by glycine to high rates of broadcast lime have been obtained (Diatloff and Luck 1972). Low rates of lime, in a mixture with superphosphate, drilled with the seed, proved successful for establishing clover on krasnozem problem soils (Vincent and Waters 1957), but lime plus ammoniated superphosphate when banded with glycine seed gave variable results (Luck, Mears and Pulsford 1971).

The *Rhizobium* strain QA922 was used in commercial inoculants for glycine until 1965 but was then replaced by the wide spectrum strain CB756 (Date 1969). Field comparisons of these two strains on glycine have not been reported and there is some evidence that CB756 is a poor soil coloniser (Cloonan and Vincent 1967; Date 1973).

An experiment was carried out to see if early nodulation and growth of glycine could be increased by the use of (1) low rates of lime banded with the seed and (2) the *Rhizobium* strain QA922. *Macroptilium atropurpureum* cv Siratro was sown for comparison.

METHODS

The experiment was sown at three sites east of Lismore, New South Wales, where the average rainfall exceeds 1600 mm (65% summer-autumn). Two sites (1 and 2) were on krasnozem soils of the Wollongbar clay loam type (Nicholls, Colwell

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and Tucker 1953), which are developed from basalt and are high in free sesquioxides. The other (site 3), called a yellow earth by McGarity (1956), is derived from phyllite and is a fine sandy loam changing gradually with depth to a compact sticky clay. Both soil types are acid throughout the profile. All sites had supported a mixed sward of *Axonopus affinis* and *Paspalum dilatatum* for many years prior to their cultivation about 8 weeks before sowing.

The number of native cowpea rhizobia in five soil samples of 6 sub-samples (cores 2.5 cm x 7 cm) was counted, using a plant infection method (Siratro seedlings), just prior to sowing.

The design was a factorial of two species, Tinaroo glycine and Siratro; three inoculum treatments, uninoculated, strain CB756, and strain QA922, and two fertilizers, Mo superphosphate at 250 kg ha^{-1}, and Mo superphosphate plus lime in a ratio of 1 : 1 (premixed at least 4 weeks) at 500 kg ha^{-1}. An additional fertilizer treatment of Mo superphosphate plus lime in a ratio of 1 : 2 at 750 kg ha^{-1} was applied to glycine with strain CB756. The 13 treatments were sown in randomised blocks with 3 replications at each site. Plots were 1.5 m by 10 m and contained two rows 30 cm apart; the fertilizer and seed were hand sown in drills and covered.

All seed was lime pelleted, using gum arabic, peat (where applicable), and lime, at rates equivalent to those recommended by Roughley, Date and Walker (1966). The number of rhizobia per seed was similar for the two inoculated treatments, being approximately 6×10^4.

Site 1 was sown on December 17, 1971, sites 2 and 3 one month later. Plants at all sites were sampled for nodulation and top weights approximately six weeks after sowing. Glycine at sites 1 and 2 was sampled again at ten weeks from sowing. At each sampling 20 plants per plot were dug and the number of nodules on each plant, and the number greater than 2 mm diameter at the first sampling, and 3 mm at the later one, were counted.

RESULTS

The native population of cowpea type rhizobia at the 3 sites prior to sowing is shown in Table 1. Rhizobia were detected in only one sample from the krasnozem soils whereas quite high numbers were found in all samples of the yellow earth. Soil pH and chemical data are also shown in Table 1.

Germination was satisfactory at all sites and moisture was ample throughout the experiment.

Percentage of plants nodulated

Table 2 shows the percentage of plants nodulated for the two samplings. As the results from the two sites on krasnozem soils were very similar only the mean for these sites is given.

Six weeks after sowing glycine had fewer nodulated plants than Siratro on both soil types. The difference was greater, however, on the krasnozem soils, where nodulation of both species was poorer, than on the yellow earth.

A large response to inoculation was obtained for glycine on the krasnozem soils but not on the yellow earth where nodulation was high ($>$ 75% at 6 weeks) without inoculation. Lime increased the number of glycine plants nodulated, when inoculum was used, on both soil types. There was no significant difference between the two rhizobial strains, but on glycine QA922 responded to the single rate of lime whereas the double rate was required for a similar increase with CB756 at the early sampling.

For Siratro, lime had no effect and there were no differences between the two inoculum strains, but on the krasnozem soil inoculation increased the number of nodulated plants. All Siratro plants on the yellow earth were well nodulated.

TABLE 1

Soil chemical and rhizobial characteristics of the three sites prior to sowing

Soil Type	Site	No. of rhizobia g^{-1} soil (log_{10})	pH (0.1M $CaCl_2$)	P (Bray No. 1) ppm	Mn ppm	Al ppm	Exchangeable Bases (me%) Ca	Mg	K	Na
Krasnozem	1	0.54	4.55	4.4	68.1	3.50	3.88	2.49	0.38	0.15
,,	2	none detected	4.40	3.5	57.8	3.75	3.30	1.73	0.46	0.14
Yellow earth	3	3.42	4.40	22.0	4.5	4.00	4.40	3.01	0.43	0.17

Extractants Mn—1N ammonium acetate
Al—0.01M calcium chloride
E.B.—0.025M barium chloride

TABLE 2

The effect of inoculum strain and lime on percentage of plants nodulated at 6 and 10 weeks

Treatments		% plants nodulated				
		Glycine (6 weeks)		Siratro (6 weeks)		Glycine (10 weeks)
Inoculum Strain	Fertilizer	Krasnozem*	Yellow earth	Krasnozem*	Yellow earth	Krasnozem*
Nil	S†	3.3 (7.4)a‡	83.5 (67.4)abc	66.7 (55.1) } a	100	22.6 (27.7)a
	SL 1 : 1	4.2 (5.0)a	75.2 (60.8)a	72.5 (59.4) } a	100	32.6 (31.5)a
QA922	S	40.0 (39.2)b	78.5 (62.4)ab	86.7 (72.3) } b	100	80.2 (63.7)b
	SL 1 : 1	57.6 (49.6)c	91.9 (76.8)c	84.2 (71.6) } b	100	95.2 (79.5)c
CB756	S	40.9 (39.6)b	78.5 (64.0)ab	81.7 (66.0) } b	100	86.0 (70.5)bc
	SL 1 : 1	45.0 (41.9)bc	83.5 (66.1)abc	89.2 (71.3) } b	100	95.2 (79.5)c
	SL 1 : 2	57.5 (49.5)c	95.2 (79.5)c	—	—	93.5 (78.1)c

Arc sin $\sqrt{\ }$ transformation shown in brackets.

*Mean of values for sites 1 and 2.

†S = superphosphate, SL = super and lime in ratio shown.

‡Within columns, values or bracketed values, followed by the same letter do not differ (L.S.D., P = 0.05).

Number of nodules per nodulated plant

The number of nodules per nodulated plant is shown in Table 3. Nodules on Siratro on the yellow earth were not counted as they were very numerous (at least ten per plant).

TABLE 3

The effect of inoculum strain and lime on number of nodules per nodulated plant at 6 *and* 10 *weeks*

Treatments		Number of nodules per nodulated plant					
		6 weeks				10 weeks	
Inoculum Strain	Fertilizer	Glycine (mean 3 sites)		Siratro (mean sites 1 & 2)		Glycine (mean sites 1 & 2)	
Nil	S*	1.61†		2.03 ab	} ‡ m	1.36 a	} m
	SL 1 : 1	2.05		1.96 a		1.71 a	
QA922	S	2.40 ab	} m	2.95 b	} n	7.51 cd	} o
	SL 1 : 1	3.16 c		2.83 ab		8.34 d	
CB756	S	1.92 a	} n	2.58 ab	} mn	4.48 b	} n
	SL 1 : 1	2.61 bc		2.47 ab		6.62 c	
	SL 1 : 2	2.62 bc		—		7.90 cd	

*See Table 2.

†Uninoculated treatments not included in the 6 weeks glycine analysis.

‡Within columns, values or bracketed values, followed by the same letter do not differ (L.S.D., P = 0.05).

For glycine a response to lime was obtained with inoculum at both samplings. Also strain QA922 formed more nodules than CB756 at both times.

For Siratro there was no response to lime nor any difference between the two *Rhizobium* strains, but plants inoculated with QA922 formed more nodules than uninoculated plants.

For both species there were differences between sites. Fewer nodules per nodulated plant were formed in krasnozem soil than in yellow earth.

The number of large nodules per nodulated plant was not affected by lime at the early sampling for either species but at the second sampling of glycine, lime increased the number of large nodules on plants inoculated with CB756 from 1.7 to 3.3 per nodulated plant. For both species there were significantly more large nodules with QA922 than with CB756 at the early sampling, and the numbers were respectively, 1.13 and 0.91 for glycine, and 1.21 and 0.87 for Siratro (sites 1 and 2 only).

Plant weights

The dry weight of plant tops was similar for all treatments within species and sites at the early sampling, but there was a large difference between species (glycine 142 mg, Siratro 345 mg per plant) and the relative difference was greater on the krasnozem soil, where growth was poor, than on the yellow earth. Growth of both species was better on the yellow earth (glycine 221, Siratro 424 mg) than at site 2 (76 and 198 mg respectively). These sites are comparable as they were sown at the same time and experienced similar weather conditions.

After 10 weeks, plant growth was markedly better for both species on the yellow earth than on the krasnozem soils, but it was not measured at the former site as runners from different plants and rows were intertwined. Glycine plant weights were not affected by treatments on the krasnozem soils.

DISCUSSION

The soil properties at the three sites (low phosphorus, calcium and pH) are those which, without amendment, are often associated with nodulation problems. The application of superphosphate and lime, together with an efficient *Rhizobium* strain should allow satisfactory establishment. This was achieved for both species on the yellow earth even without lime, although for glycine nodulation was improved where lime was used.

On the krasnozem soils the problem of poor nodulation and growth of glycine was not overcome by using strain QA922, or lime although some improvement in nodulation was caused by them. Although nodulation of Siratro was satisfactory, growth was restricted on this soil type.

As plant growth was not related to nodulation some factor other than nitrogen was apparently responsible for the poor growth of both species on the krasnozem soils. The difference in manganese content of the two soils is noteworthy and could account for the differences observed. The levels in the krasnozem soils were high enough for adverse effects to be expected, as 10 ppm were found by Andrew and Hegarty (1969) to reduce the growth of Siratro and glycine by 50 per cent. Also as glycine is more sensitive to a low calcium to manganese ratio than Siratro (Andrew and White 1963) this could account for the relatively poorer growth of glycine on the krasnozems.

Soil manganese could also have caused the poorer nodulation on the krasnozem soils as nodule formation on glycine, particularly cultivar Tinaroo, is severely restricted by high manganese levels (Souto and Dobereiner 1970). In the present experiment reddish purple spots were observed on leaves of some glycine plants at site 2, and this has been noted as a symptom of manganese toxicity in glycine (Andrew and Pieters 1970). The low rates of lime used, and premixing it with superphosphate, would limit its effect on manganese availability.

As a nodulation response to lime occurred on both soil types and was confined to glycine, it was probably due to increased calcium and perhaps soil pH. These were similar for all soils, and such that nodulation responses by glycine could be expected (Andrew 1970). The improvement in nodulation was not as great as that obtained for glycine when 2.5 tonne ha^{-1} of lime was applied to similar soils by Diatloff and Luck (1972). In their case this rate was effective in halving the manganese content of glycine leaves, and such rates are probably required to significantly reduce soil manganese levels.

The poorer nodulation with strain CB756 than QA922 supports the conclusion of Nicholas and Haydock (1971) that CB756 is not a particularly good strain for glycine. From the results reported here, it appears to have a higher calcium and/or pH requirement than QA922. The difference between the *Rhizobium* strains, however, was not great, and seeking a more efficient strain for glycine on unfavourable soils may be justified.

The high percentage of nodulated Siratro plants in the uninoculated treatments on the krasnozem soils is in marked contrast to that of glycine. In view of the very low native population found prior to sowing, Siratro apparently exerted a strong stimulating effect on the multiplication of the 'native' rhizobia. Glycine either had a much weaker effect or only a small proportion of the rhizobia formed nodules. The latter would appear more likely in view of Whiteman's (1972) results. Alternatively the roots of Siratro may have explored more soil. However, this is unlikely to be the major cause, as the roots of glycine at 10 weeks were more extensive than Siratro at 6 weeks, and yet the percentages of nodulated plants in the uninoculated treatments were 28 and 70% respectively.

Higher nodule numbers were not achieved at the expense of nodule size as those treatments with more nodules also had as many or more large nodules. Hence QA922 and the lime treatments would have had more nodular tissue and so should have fixed more nitrogen. The lack of response in plant growth indicates that nitrogen was not limiting and for the yellow earth, where growth was good, available soil nitrogen was apparently adequate, at least up to 6 weeks.

The slower nodulation and early growth of glycine than Siratro, even on the more favourable yellow earth, agrees with other findings (Wilson 1972; Whiteman 1972) and seems characteristic of the species.

In conclusion neither low rates of lime nor an alternate *Rhizobium* strain overcame tardy nodulation or poor early growth of glycine on krasnozem soil. As manganese toxicity is incriminated, the use of less sensitive legumes, such as Siratro and *Desmodium* species, would be wise on suspect soils. Although the early maturing glycine cultivar Clarence is better suited to such soils (Mears 1967; Diatloff and Luck 1972), seed is not readily available. Also, as more tolerant strains of desired maturity may be available, these should be sought.

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REFERENCES

ANDREW, C. S. (1970)—Mineral Characterisation of Species. Division of Tropical Pastures, C.S.I.R.O. Annual Report 1969-70. 78-9.

ANDREW, C. S., and HEGARTY, M. P. (1969)—Comparative responses to manganese excess of eight tropical and four temperate pasture legume species. *Australian Journal of Agricultural Research* **20**: 687-96.

ANDREW, C. S., and PIETERS, W. H. (1970)—Manganese toxicity symptoms of one temperate and seven tropical pasture legumes. Division of Tropical Pastures Technical Paper No. 4, C.S.I.R.O.

ANDREW, C. S., and WHITE, R. E. (1963)—Mineral characterisation of pasture species. Division of Tropical Pastures, C.S.I.R.O. Annual Report 1962-63. 36.

CLOONAN, M. J., and VINCENT, J. M. (1967)—The nodulation of annual summer legumes sown on the far north coast of New South Wales. *Australian Journal of Experimental Agriculture and Animal Husbandry* **7**: 181-189.

DATE, R. A. (1969)—A decade of legume inoculant quality control in Australia. *Journal of the Australian Institute of Agricultural Science* **35**: 27-37.

DATE, R. A. (1973)—Rhizobium ecology. Division of Tropical Pastures, C.S.I.R.O. Annual Report 1972-73. 57-58.

DIATLOFF, A., and LUCK, P. E. (1972)—The effects of the interactions between seed inoculation, pelleting and fertilizer on growth and nodulation of desmodium and glycine on two soils in S.E. Queensland. *Tropical Grasslands* **6**: 33-38.

LUCK, P. E., MEARS, P. T., and PULSFORD, J. S. (1971)—Pelleting and fertilizer effects on nodulation of *Glycine wightii*. *Tropical Grasslands* **5**: 81-87.

MCGARITY, J. W. (1956)—Soils of the Richmond Tweed Region. M.Sc. Thesis University of Sydney.

MEARS, P. T. (1967)—Tropical pastures in the Richmond-Tweed region of New South Wales. *Tropical Grasslands* **1**: 98-104.

NICHOLAS, D. B., and HAYDOCK, K. P. (1971)—Variation in growth and nodulation of *Glycine wightii* under controlled environment. *Australian Journal of Agricultural Research* **22**: 223-30.

NICHOLLS, K. D., COLWELL, J. D., and TUCKER, B. M. (1953)—A survey of soils and some aspects of soil fertility in the Lismore district, New South Wales. C.S.I.R.O., Division of Soils: Soils and Land Use Series No. 11.

ROUGHLEY, R. J., DATE, R. A., and WALKER, M. H. (1966)—Inoculating and lime pelleting legume seed. *Agricultural Gazette of New South Wales* **77**: 142-6.

SOUTO, S. M., and DOBEREINER, J. (1970)—Problems in the establishment of *Glycine javanica* in a tropical region. Proceedings of XI International Grasslands Congress, Surfers Paradise, Australia, 1970. 127-131.

THOMAS, D., and WHITEMAN, P. C. (1971)—The effect of soil type on the establishment, early growth and nodulation of *Glycine wightii*. *Australian Journal of Experimental Agriculture and Animal Husbandry* **11**: 513-20.

VINCENT, J. M., and WATERS, L. M. (1957)—Seven years pasture research on the far north coast of New South Wales. University of Sydney, School of Agriculture, Report No. 2. 25-35.

WILSON, J. R. (1972)—Comparative nodulation, nitrogen fixation and growth of *Glycine wightii* and *Phaseolus atropurpureus* seedlings. *Australian Journal of Agricultural Research* **23**: 1-8.

WHITEMAN, P. C. (1972)—The effects of inoculation and nitrogen application on seedling growth and nodulation of *Glycine wightii* and *Phaseolus atropurpureus* in the field. *Tropical Grasslands* **6**: 11-16.

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