THE EFFECTS OF INOCULATION AND NITROGEN APPLICATION ON SEEDLING GROWTH AND NODULATION OF *GLYCINE WIGHTII* AND *PHASEOLUS ATROPURPUREUS* IN THE FIELD

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ABSTRACT

Seedling growth and nodulation were compared between G. wightii *and* P. atropurpureus *in the field under 4 levels of* Rhizobium *inonculation with two times of application of 28 Kg nitrogen per ha (25 lb per ac). Nodulation in* Glycine *was slow, and a mean of only 42% of plants were nodulated at 70 days over all levels of inoculum. Siratro nodulated fully and grew more rapidly than* Glycine. *Level of inoculation or application of nitrogen had no significant effects on seedling growth or nodulation in either species. Slow establishment of* Glycine *was not improved by nitrogen application. Other physiological factors require investigation to elucidate reasons for slow and poor nodulation and low seedling growth rates.*

INTRODUCTION

Slow rates of seedling growth and poor seedling establishment of *Glycine wightii* (*Glycine*) have been found on a range of soil types (Tow 1960; Murtagh 1970; Souto and Dobereiner 1970). One factor considered important is the slow rate of nodulation (Diatloff and Ferguson 1970; Thomas and Whiteman 1971), and often poor nodulation in the field (Luck, Mears and Pulsford 1971). Since poor or slow nodulation may affect nitrogen nutrition, this may in turn be reflected in low rates of seedling growth.

In order to examine the role of both inoculation and nitrogen nutrition, a field experiment was established in which interactions between levels of inoculation and time of application of nitrogen were investigated. The performance of *Glycine wightii,* cv. Tinaroo was compared with *Phaseolus atropurpureus* cv. Siratro, which is known to nodulate and establish rapidly in the soils of the experimental area.

MATERIALS AND METHODS

The trial was conducted at the C.S.I.R.O. Pasture Research Station, Samford, near Brisbane. The soil is a solodized solonetz, with a sandy loam A horizon 20-30 cm deep (pH 6.1) overlying a mottled clay B horizon (Beckman 1957, Soil G2). Irrigation equivalent to 2.5 cm of rainfall was applied at intervals of from 7 to 10 days. A basal application of 100 kg superphosphate was applied.

A randomised block design with split plots was used with four replications. Each plot was a single row which contained one species at one level as a main treatment. Each row was split for the three nitrogen treatments. Rows were 1 m apart.

Inoculation levels

The experimental treatments were; no inoculation but seed surface sterilized with "Hibitane", or an inoculation at either normal level (0.75 g or 1.5 g peat culture per 150 g of seed for siratro and *Glycine* respectively), or ten times or 100 times normal level; 15 ml of "Cellofas" sticker was applied before the peat. Excess peat culture was planted with the seed.

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The experiment was sown on January 8, 1969, and seedlings were thinned after 8 days to 10 cm within the row. The first nitrogen application was 9 days after sowing (5 days after seedling emergence). Plots were weeded regularly throughout the experimental period.

Nitrogen treatments

Nitrogen treatments were; no nitrogen application (No), 28 Kg nitrogen per ha applied 9 days after sowing (N_1), and 28 Kg nitrogen per ha (25 lb per ac) applied 18 days after sowing (N_2). This was applied as urea dissolved in water, and applied evenly to each side of the row. Nitrogen application was followed on each occasion by 2.5 cm of irrigation.

Sampling

Plants were sampled on 6 occasions, at 13, 20, 27, 34, 42 and 70 days from sowing. Blocks of soil were dug out with a spade 20 cm deep, 20 cm wide and 40 cm long to remove a sample unit of 4 plants per treatment replicate. A buffer length of 1 m was left at each end of a sub-row. Root systems were washed out in a large sieving box, and number of plants nodulated and nodule number per plant were recorded. Tops and roots were weighed separately after oven drying at 90°C.

RESULTS

Nodulation

Neither the level of inoculation, including no inoculation, nor the application of nitrogen had any significant effects at any sample occasion on nodule number per plant. Nor was there any interaction between these treatments. Thus only the main effects of these treatments on nodule number are shown in Table 1.

TABLE 1

Main effects of species, inoculum level and nitrogen application over all sample occasions on mean nodule number per plant for Siratro, *and (a) mean nodule number per plant and (b) mean number of nodules per nodulated plant for* Glycine

Species	Inoculum Level				Nitrogen Level		
	0	1	10	100	N_0	N_1	N_2
Siratro	3.78	4.70	3.10	3.85	3.29	4.34	3.83
Glycine (a)	0.54	0.49	0.55	0.35	0.42	0.41	0.66
(b)	1.69	1.38	1.66	1.30	1.40	1.33	1.83

Siratro had significantly higher ($P < .001$) nodule numbers than *Glycine* under all treatments and at all sample occasions (Fig. 1). It also nodulated earlier than *Glycine* (Fig. 1), and a much higher percentage of the total plant population was nodulated (Table 2). Except at the first sample date, all Siratro plants in all treatments were nodulated, which contrasts strongly with the situation in *Glycine*. However the level of inoculum appears to have little effect on percentage nodulation in *Glycine* (Table 2). The apparent reduction in nodulation at the highest inoculum level results from the anomalous value at harvest day 34.

Even though 44% of plants were nodulated up to day 42, the nodules on *Glycine* were small and white and only about 10% appeared to be effective at this stage, whereas Siratro nodules were pink and functional. By harvest day 70 although nodule number per *Glycine* plant was still low, most of the nodules appeared to be effective.

Plant Dry Weight

Again treatment effects, except species, were not significant at any harvest. Both shoot and root weights and leaf area per plant were higher in Siratro at all harvests (Fig. 1).

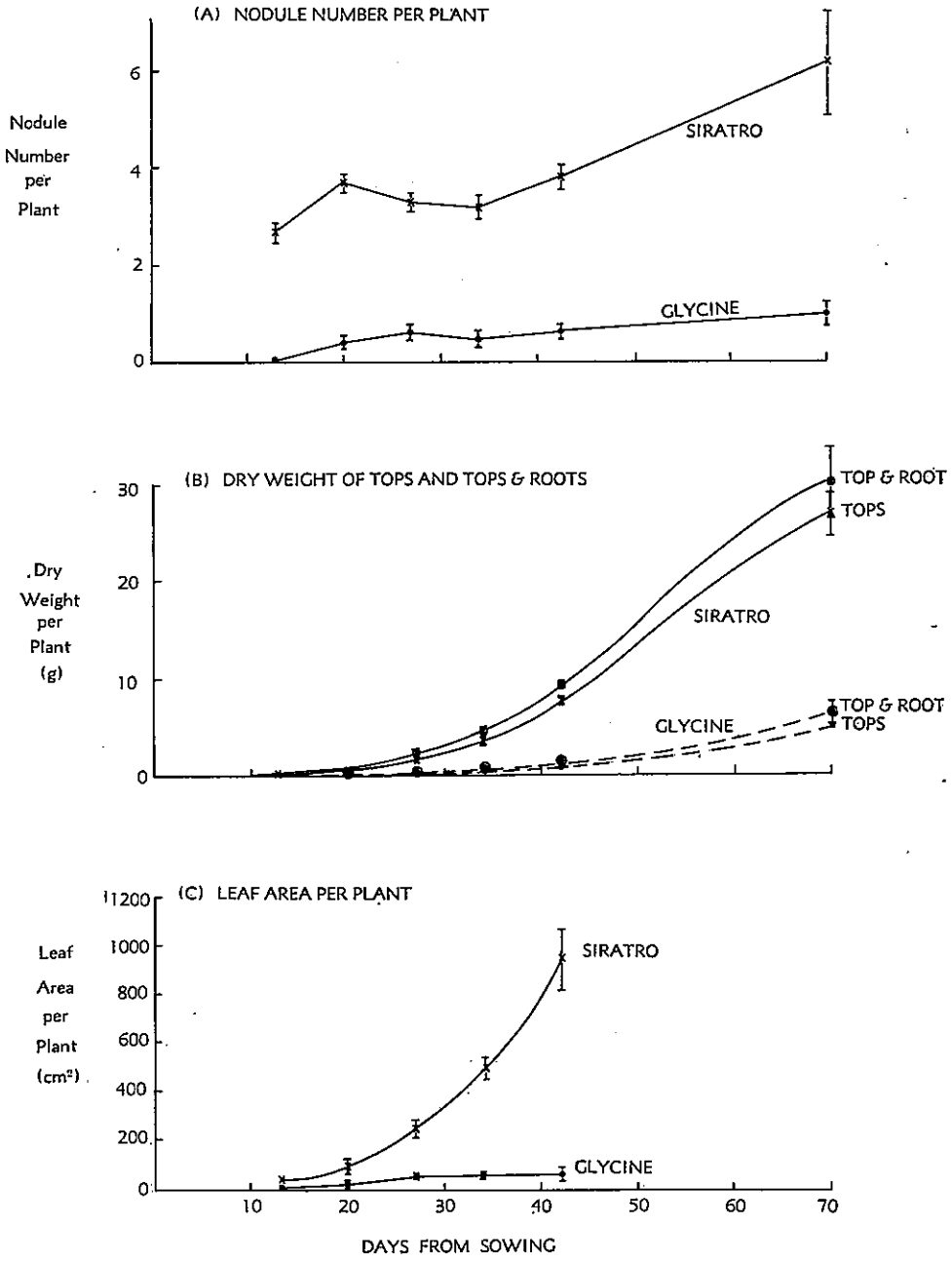

Figure 1. Changes with time in (A) mean nodule number per plant (B) dry weight of tops and roots, (C) mean leaf area per plant. Standard errors of means are indicated by vertical bars.

TABLE 2

The effect of inoculation level at each sample occasion on percentage of Glycine *plants nodulated, (means over nitrogen levels). Mean values for Siratro are included for comparison*

% of Plants Nodulated

Sample Occasion (Days from Sowing)	(Glycine) Inoculum Level 0	1	10	100	Mean	Siratro Mean
13	6	2	0	0	2	98
20	40	29	23	51	36	100
27	41	43	38	25	37	100
34	41	46	40	10	34	100
42	46	46	54	29	44	100
70	33	40	54	42	42	100
Mean	34.5	34.3	34.8	29.2	32.5	99.7

DISCUSSION

The trial site had until late 1967 carried mixed swards including *Glycine* and Siratro which had been ploughed, followed by grass swards. The level of seed inoculation appeared to have little effect on nodulation but this may have been due to the presence of a high level of inoculum already in the soil. The percentage of plants nodulated in the uninoculated *Glycine* treatments was similar to that recorded by Luck, Mears and Pulsford (1971) at Lismore but much higher than they recorded at Maleny. There was little improvement in the percentage of plants nodulated after day 20, suggesting either that a proportion of plants in the population were incompatible with the *Rhizobium* strains present, or that conditions after day 20 were not suitable for further infection or nodulation, or that the existing *Rhizobium* population had declined. Wilson (1971) also noted a reduction in new nodule development after 33 days in a pot trial. In contrast, Siratro, which is nodulated by the same *Rhizobium* strains as is *Glycine*, rapidly established full nodulation under the same conditions.

It is interesting to note that initial nodulation of *Glycine* was detected earlier in the field, between days 13 and 20 days from sowing, than in previous pot trials (27 days, Thomas and Whiteman 1971) or in nutrient agar (23 days, Nicholas and Haydock 1971) and (29 days, Diatloff and Ferguson 1970). These workers have shown a variety by *Rhizobium* strain interaction on earliness of nodulation. The extreme earliness to nodulate in the present trial would indicate that some nodulation in the field resulted from infection by a range of indigenous strains already present. However, Wilson (1971) detected nodulation in some *Glycine* (cv. Cooper) plants as early as 9 days from sowing in sand culture.

Although soil nitrogen levels after three months fallow and cultivation may have been sufficiently high to mask responses to applied nitrogen, the lack of response in plant growth suggests that nitrogen was not a major limiting factor to seedling growth in *Glycine* even though nodulation and seedling growth were poor. In the case of Siratro, nodulation and plant growth were not affected by the levels of nitrogen applied, or by the timing of application.

Previous studies (Diatloff and Ferguson 1970; Thomas and Whiteman 1971) have shown *G. wightii* to be slow to nodulate, particularly the cultivar Tinaroo. Other field studies have also shown that the percentage of plants which nodulate may be low even when inoculated (Luck, Mears and Pulsford 1971). Although further research into the reasons for slow and often limited nodulation is required, limitations of nitrogen nutrition may not be the major factor in poor seedling

growth. Other aspects of seedling physiology, including uptake of other elements, and photosynthetic relationships also require investigation. Improvement in nodulating ability in this species also appears possible through breeding and selection (Nicholas 1971; Nicholas and Haydock 1971). Once the problems of establishment are overcome Glycine may form the basis of productive and persistent pastures even on soils which are not normally considered ideal for this species (Whiteman 1969). Wilson (1971) has also shown that once the initial nodule development phase is completed, there was little difference between Siratro and *Glycine* in rate of growth and nitrogen accumulation, proportion of nodule weight to whole plant weight or nitrogen content in the whole plant. However the proportion of plants nodulated in field studies appears to be much lower than in pot and culture experiments.

ACKNOWLEDGEMENTS

I am indebted to Mr. P. H. Smith for technical assistance throughout this trial and to Mr. R. Panitz for inoculating the seed to the required levels. I also thank Mr. K. P. Haydock, and Mrs. A. Peake for their assistance in the statistical analysis of the data.

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[Accepted for publication November 12, 1971]