NODULE NUMBER, TIME TO NODULATION AND ITS EFFECTIVENESS IN ELEVEN ACCESSIONS OF GLYCINE WIGHTII

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ABSTRACT

Eleven introductions of Glycine wightii *were tested against three strains of* Rhizobium *to determine time to initial nodulation, nodule number and efficiency in symbiosis. The optimum host x strain combination for each parameter differed, suggesting that all three characters were independently controlled. Tetraploid lines tended to nodulate earlier than diploid ones.*

The time to onset of nodulation as a mean for all introductions was 28 days. The significance of this delay is discussed.

INTRODUCTION

Glycine wightii (*G. javanica*) is a perennial pasture legume playing a significant role in pasture improvement in areas of Queensland and Northern New South Wales. The existing cultivars were released on their ability to persist in a productive state under grazing in different regional environments and to contribute to pasture quality. The cultivar Tinaroo, however, sometimes establishes poorly and is slow to nodulate even when inoculated with an effective strain of nodule bacteria.

Numerous introductions have been made into Australia as a basis for improvement programmes. Concurrent with any attempt to provide cultivars with desirable agronomic attributes, particular emphasis must also be placed on the attainment of an efficient bacterial symbiosis. The studies described in this paper examined the nodulation characteristics of a range of *Glycine wightii* introductions with three strains of nodule bacteria and the effectiveness of the symbiosis was assessed.

MATERIALS AND METHODS

The eleven host introductions of *Glycine wightii,* are listed together with details of origin and chromosome number in Table 1 and the three strains of *Rhizobium* sp. are described in Table 2.

TABLE 1

Accessions of Glycine wightii *used in the investigations*

Cultivar or Accession	Geographic Origin	Chromosome Number
Tinaroo	Kenya	22‡
Cooper	Kongwa, Tanzania	22‡
Clarence	Transvaal, Sth. Africa	44§
CPI* 12600	Madras, India	
K† 52338	Kenya	22‡
CPI 25920	Kasama, Nth. Rhodesia	44§
CPI 27020	Mbarara, Uganda	44§
CPI 27022	Kampiringisa, Uganda	44§
CPI 28279	Malawi	44§
CPI 30361	Pretoria, Sth. Africa	22§
CPI 18419	Choma, Nth. Rhodesia	22‡

* Commonwealth Plant Introduction (CPI)
† Kenya Department of Agriculture (K)
‡ Pritchard and Gould (1964)
§ Pritchard (per. com. 1965)

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TABLE 2
Strains of Rhizobium sp. *used in the investigation*

Rhizobium Strain	Host Origin	Note
CB756	*Dolichos africanus*	Current commercial strain
QA922	*Glycine wightii*	Superseded commercial strain*
QA878	*Glycine tomentosa*	A prolific nodulating strain*

*Kennedy (1962)

Experiment 1

Time to initial nodulation and nodule number at 40 days were compared for each host x strain combination. Seeds were sterilized in 0.1% mercuric chloride for 10 minutes followed by four washes with sterile water. Single pre-germinated seeds were transferred to a nitrogen free mineral salts nutrient agar medium (Norris 1964), contained in 6 in. x 1 in. test tubes plugged with cotton wool. Each tube was then inoculated immediately with approximately 100,000 nodule bacteria per tube from a 10 day old culture grown on yeast mannitol agar (YMA). The tubes were arranged in a randomised block design in two water baths held at 30°C in the glasshouse. Six replications were used. After setting up the experiment in July 1967, daily observations were made on the time of commencement of nodulation. Nodule numbers in each tube were finally recorded after 40 days.

Experiment 2

The effectiveness of the symbiosis was examined in Leonard jars under sand-water culture (Norris 1964). Seeds were sterilized in mercuric chloride prior to sowing and the plants thinned to four per jar after emergence. Seven-day-old seedlings were inoculated by applying 1 ml of bacterial suspension from 10 day old YMA cultures to the base of each seedling. The jars were arranged in randomised blocks in the glasshouse using three replications. Plants were harvested after nine weeks and examined for nodulation, dry weight and nitrogen content.

RESULTS

Time to initial nodulation

Host differences for individual strains and as an average over all strains were recorded (Table 3). CPI 25920 and CPI 28279 nodulated significantly earlier than K52338, CPI 18419, Tinaroo, Cooper, CPI 12600 and CPI 30361.

Rhizobial strain differences, as an average over all lines also occurred. QA922 nodulated earlier than QA878 and earlier still than CB756.

The response of individual hosts to a particular strain was not always consistent. While cultivar Tinaroo, K52338, CPI 25920, CPI 27020, CPI 27022, and CPI 28279 each nodulated earlier with QA922 than with CB756, there were no significant differences between strains with Cooper, Clarence, CPI 12600, CPI 30361 and CPI 18419. The reaction to QA878 was fairly similar to that to QA922.

Nodulation at 40 days

All plants in each accession were nodulated by each strain by the fortieth day. CPI 25920, CPI 28279, CPI 27020 and Cooper, as an average of all strains gave significantly higher nodule numbers than did CPI 12600 and CPI 30361 (Table 4). In considering strain means, QA922 produced more nodules than QA878, while each also exceeded CB756.

TABLE 3

Time to initial nodulation in Glycine wightii *grown in nutrient agar as affected by host* × Rhizobium *strain interaction*

Cultivar or Accession	Days to initial nodulation			
	Strain QA 878	Strain CB 756	Strain QA 922	Host means
Tinaroo	29.17	34.17	25.17	29.50
Cooper	28.33	33.50	31.50	31.11
Clarence	26.00	29.17	28.67	27.94
CPI 12600	31.00	34.33	30.33	31.89
K52338	31.00	33.83	21.50	28.78
CPI 25920	24.67	27.33	17.50	23.17
CPI 27020	26.00	35.67	20.33	27.33
CPI 27022	28.33	28.50	19.50	25.44
CPI 28279	27.50	27.33	17.17	24.00
CPI 30361	28.50	33.50	34.00	32.00
CPI 18419	32.00	30.83	24.00	28.94
Strain means	28.41	31.63	24.52	

L.S.D. (5%) for host means = 4.75
for strain means = 2.48
L.S.D. (5%) for strains within hosts = 8.23

The responses of individual hosts to each of the three strains was not consistent. The nodule numbers in Clarence, K52338, CPI 25920, CPI 28279, CPI 30361 did not differ significantly between strain combinations. However, Tinaroo, CPI 27020 and CPI 27022 produced more nodules in combination with QA922 than with CB756. Cooper nodulated more profusely with QA878 than with CB756, while with CPI 18419 the reverse applied.

TABLE 4

The nodule numbers on Glycine wightii *grown in leonard jars at* 40 *days as affected by host* × Rhizobium *strain interaction*

Cultivar or Accession	Nodule number per plant			
	Strain QA 878	Strain CB 756	Strain QA 922	Host means
Tinaroo	3.24 (1.93)*	1.84 (1.53)	6.36 (2.61)	3.61 (2.02)
Cooper	7.84 (2.88)	1.49 (1.41)	5.72 (2.49)	4.63 (2.22)
Clarence	3.44 (1.98)	2.46 (1.72)	4.52 (2.24)	3.43 (1.98)
CPI 12600	1.91 (1.55)	1.66 (1.47)	4.53 (2.24)	2.58 (1.75)
K52338	3.80 (2.07)	2.42 (1.70)	4.36 (2.20)	3.48 (1.99)
CPI 25920	4.47 (2.22)	4.09 (2.14)	7.14 (2.76)	5.15 (2.37)
CPI 27020	5.33 (2.41)	2.85 (1.82)	6.48 (2.64)	4.77 (2.29)
CPI 27022	2.26 (1.66)	2.20 (1.64)	6.56 (2.65)	3.45 (1.98)
CPI 28279	4.56 (2.25)	4.40 (2.21)	6.12 (2.57)	5.00 (2.34)
CPI 30361	3.25 (1.93)	2.56 (1.75)	1.44 (1.39)	2.37 (1.69)
CPI 18419	1.78 (1.51)	5.46 (2.44)	3.47 (1.99)	3.43 (1.98)
Strain means	3.66 (2.04)	2.76 (1.80)	5.01 (2.37)	

* Sq. root $(x + \frac{1}{2})$ transformation
L.S.D. (5%) for host means = (0.45)
for strain means = (0.23)
L.S.D. (5%) for strains within hosts = (0.79)

Effectiveness of the symbiosis

Plants in all treatments in the Leonard jar trial nodulated. Due to poor seed germination no worthwhile results were obtained with the variety K52338. In all 10 introductions CB756 produced the most nitrogen (Fig. 1). It was significantly better than QA878 in nine lines but significantly better than QA922 in only four lines. The strain QA878 had a consistently low symbiotic rating on all introductions, whereas all three strains were ineffective on CPI 12600. CPI 27020 in association with an effective strain, was the most productive with regard to dry matter and nitrogen content.

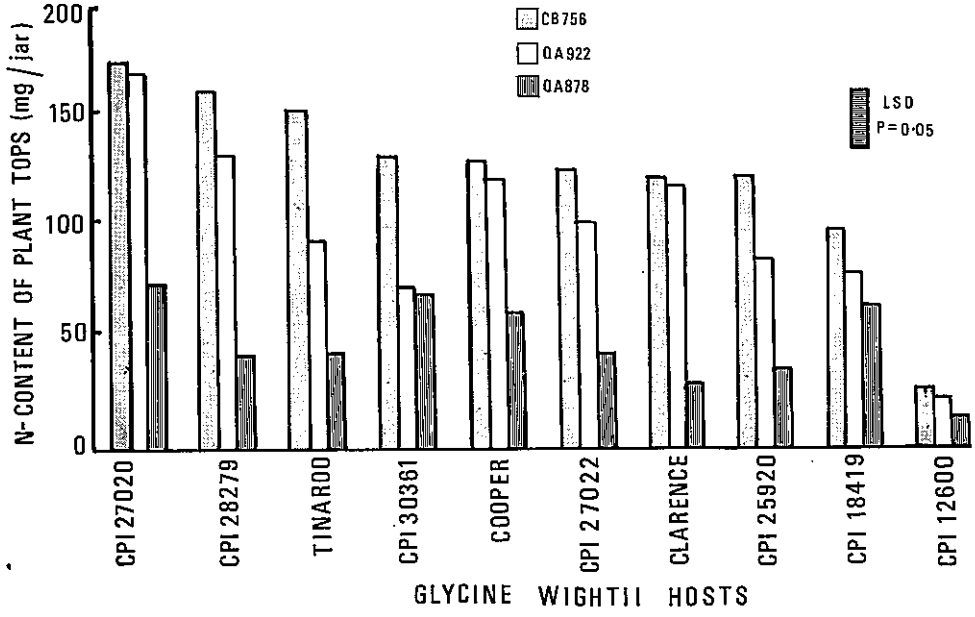

FIGURE 1
Nitrogen content of *Glycine wightii* as affected by host × Rhizobium strain interaction (showing 5% level of significance)

DISCUSSION

Under low nitrogen conditions, as might occur in mixed pastures, early effective nodulation in small seeded legumes is critical to their survival (Vincent 1954). Larger seeded legumes are initially less dependent although they too will eventually fail without nodules. In glycine the prolonged period to onset of nodulation (mean of approx. 4 weeks) weighs heavily against rapid seedling establishment. In contrast Cannon *et al.* (1967) recorded the first nodules in subterranean clover at 10-11 days after sowing under similar cultural conditions, whereas siratro (*Phaseolus atropurpureus*) in our experience can nodulate as early as 9-12 days.

The appearance of the first nodule in glycine was spread over some time within any particular host-strain combination in sharp contrast to that reported in subterranean clover by Nutman (1965), where all plants within a variety nodulated at almost the same time.

Nodule numbers and earliness to nodulate seemed to be connected possibly through the rate of nodule formation, although the existence of sparsely and abundantly nodulating lines of the host, as reported by Nutman (1958) in subterranean clover, should not be overlooked.

The ability to nodulate earlier had little effect on the final symbiotic performance of host-strain combinations, indicating the independence of the two features in that nodules that eventually form are fully effective as in the case of CB756. Although early nodulating plants theoretically had an initial growth advantage, this apparently was lost by about 8 weeks as the slower nodulating plants began to fix nitrogen at a much higher rate. In addition, the symbiotic performance and nodule number appear unrelated. Under field conditions the rate of nodule formation is much slower and more environment dependent than in sand water culture. It is not surprising, therefore, that such strain differences have not been observed in the field by the authors. The plants are often forced to overwinter with an inadequate complement of nodules, thus aggravating the problems of establishment.

Among the comparatively small number of plant introductions included in these experiments, those of the tetraploid nature tended to nodulate earlier than the diploids. This characteristic may prove useful in breeding or selecting desirable types. The earlier nodulation of strain QA922 on most varieties suggests that further improvement might be made through strain manipulation.

In view of the proven effectiveness of the existing commercial strain CB756 with the majority of introductions, it might be safely used on a wider range of introduced material while other agronomic evaluations are made. Some aberrant ineffective nodulation, as in CPI 12600, is inevitable and unless such lines show agronomic promise under fertilizer nitrogen they do not warrant any particular attention.

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