

Growth responses of *Desmanthus virgatus* to inoculation with *Rhizobium* strain CB3126.

I. A pot trial with 8 clay soils from central and southern Queensland

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Abstract

The need to inoculate *Desmanthus virgatus* cv. Marc in 8 clay soils from central and southern Queensland was investigated in a glasshouse experiment, by comparing growth of uninoculated plants with that of plants inoculated with the effective *Rhizobium* strain CB3126 or inoculated and fertilised with N. The success of the inoculum in forming nodules in competition with indigenous strains was tested using a serological method. The N-fixation effectiveness of indigenous strains of *Rhizobium* compared with CB3126 was evaluated in a second glasshouse experiment.

Significant ($P < 0.05$) plant growth responses to inoculation with *Rhizobium* strain CB3126 were recorded in 4 soils, 100 days after sowing. In two of these soils, a response was recorded 56 days after planting. Leaf production was increased relative to the uninoculated controls by up to 96%, 56 days after sowing, and up to 90%, 100 days after sowing. The strain CB3126 accounted for 35–96% of nodules 56 days after planting and 23–98%, 100 days after sowing. Uninoculated plants in 6 of the 8 soils formed nodules. Indigenous strains of *Rhizobium* isolated from 5 of these soils were at least 60% as effective as CB3126.

This study demonstrates that *Desmanthus virgatus* responds to inoculation with *Rhizobium*

strain CB3126 when sown into soils with few or no native *Rhizobium*. Although no response to inoculation occurred in 4 of the 8 clay soils used in the trial, inoculation with the commercially available strain CB3126 is recommended for all soils as a normal part of the sowing procedure.

Introduction

Desmanthus virgatus (*desmanthus*) is a summer-growing perennial forage legume adapted to neutral-alkaline, medium-heavy textured clay soils of tropical and subtropical Queensland (Cook *et al.* 1993). However, observations of chlorosis, poor vigour and productivity have been attributed to inadequate nodulation at some field sites (R.M. Jones, personal communication). This may be caused by failure of previously recommended inoculum strains of *Rhizobium* to form effective N-fixing associations or where few or no suitable indigenous strains of *Rhizobium* are present.

The commercial strain of *Rhizobium* (CB3126) was effective in forming nodules and fixing nitrogen in a range of *D. virgatus* accessions in pots (Date 1991a) and in the 3 commercial cultivars in 3 of 4 soils in field plots (Brandon *et al.* 1998). However, strain CB3126 appears to survive poorly on seed stored under hot, dry conditions (Becerra Stiefel *et al.* 1998). Because *desmanthus* seed is sown shallowly, due to its small seed size, these conditions are likely to occur in the dry surface of the soil following summer sowing. In such instances, effective nodulation of *desmanthus* may be dependent on the presence of indigenous strains of *Rhizobium*. However, little is known of the N-fixation effectiveness of indigenous strains able to nodulate *desmanthus* in the clay soils for which it is recommended

The objectives of this study were to investigate the response of *D. virgatus* to inoculation with *Rhizobium* strain CB3126 in a pot experiment

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using 8 clay soils; the competitive ability of CB3126 to form nodules; and the occurrence and N-fixation effectiveness of indigenous strains of *Rhizobium* able to nodulate desmanthus in these soils.

Materials and methods

Two experiments were conducted in glass-houses at the CSIRO Tropical Agriculture Cunningham Laboratory (27°37'S, 153°19'E) between February–July 1995.

Experiment 1: Inoculation responses and the competitive ability of CB3126 to form nodules

Treatments and design. Treatments consisted of 8 soils (Table 1), 3 N treatments (uninoculated, inoculated with *Rhizobium* strain CB3126, and inoculated + the equivalent of 200 kg/ha N applied in split application). There were 2 harvests, one at 56 days after sowing and another 100 days after sowing. Treatments were replicated 4 times in a randomised design. The positions of pots in the glasshouse were continuously re-randomised in association with a 3 times/day watering regime using an automatic watering machine (Andrew and Cowper 1973).

Establishment and maintenance. Plastic-lined pots, 15 cm in diameter, were filled with sieved (1 cm²) air-dried soil collected from the 0–15 cm layer of each site in December–January (1994/95). Seeds of *D. virgatus* cv. Marc were acid-scarified in concentrated H₂SO₄ for 10 min, rinsed 5 times in sterile water and germinated on water-agar plates. Eight germinated seeds, with radicles 8–10 mm long, were sown/pot to a depth

of 2 mm. Seeds were inoculated (1 mL/pot) at sowing with a suspension of a peat culture of *Rhizobium* strain CB3126 (10 g peat/100 mL sterile water). Twenty-three days after sowing, plants were thinned to 5 uniform plants per pot.

Basal nutrients were applied to all soils at rates (kg/ha) equivalent to 100 P, 150 K, 25 Zn, 10 Cu, 2 Mo and 10 Mn, 9 days after planting. Nitrogen was applied to the appropriate pots in the form of NH₄NO₃, 34, 50, 78 and 92 days after sowing. Pots were watered with demineralised water to 90% of field capacity, determined using a pressure plate set at 0.3 bars.

Pots were maintained free of weeds throughout the experiment and supplementary heating was used to maintain the ambient temperature above 18°C. The diurnal soil temperature at a depth of 5 cm during the experiment ranged between 17 and 36°C (min./max.).

Measurements. All plants were cut 1 cm above the third node, 56 days after sowing. Plants for the second harvest were allowed to regrow for a further 44 days, when they were recut at the same height. For both harvests, plant tops were oven-dried for 48 h at 60°C for determination of dry matter yields of whole tops and leaves. At the second harvest, leaflets of Replications 1 and 3, and 2 and 4 of the inoculated and uninoculated treatments were combined for the determination of N concentration and the calculation of total N content of the leaflets.

At both harvests, the soil was washed from the roots of the inoculated and uninoculated treatments and all nodules collected. The nodules were stored at –15°C prior to serological typing using the indirect FITC-labelled antibody procedure (Somasegaran and Hoben 1985). After

Table 1. Characteristics of 8 soils from south-east Queensland used in Experiment 1.

Location	Property/site name	Soil type ¹	pH (1:5 soil:H ₂ O)	Total N (%)
Gayndah	Brian Pastures Research Station	Ug5.32	7.3	0.12
Roma	"Bindaroo"	Db1.43	7.2	0.08
Mundubbera	Narayan Research Station	Gn3.13	6.6	0.30
Emerald	Emerald Research Station	Ug5.12	7.4	0.05
Biloela	"Kapalee"	Ug5.16	8.6	0.06
Theodore	"Rangeview"	Ug5.12	7.4	0.10
Bauhinia	"Mungabunda"	Ug5.15	7.6	0.07
Wandoan	"Kookaburra"	Ug5.16	7.4	0.04

¹Northcote *et al.* (1975).

nodule strains were identified, the nodule residues were oven-dried for 48 h at 60°C and weighed. Nodules not sampled for serotyping were also dried and weighed and total nodule weight calculated.

Experiment 2: Effectiveness in N fixation of indigenous strains of Rhizobium

A modification of the Leonard sand-jar technique (Norris and Date 1976) was used to evaluate the effectiveness in N fixation of indigenous strains of *Rhizobium* that nodulated *D. virgatus* in the uninoculated control treatments of the initial pot experiment.

Methods and measurements. Random subsamples of nodules from the uninoculated Gayndah, Emerald, Biloela, Theodore, Bauhinia and Wandoan soils collected at Harvest 1 were surface-sterilised and crushed to form the inoculum source for aseptically-grown plants. The same procedure was undertaken for nodules collected from the inoculated Roma treatments (no nodules formed on uninoculated plants) for use as the CB3126 control. The treatments were replicated 8 times in a completely randomised design. Four sand-jars which received no inoculum were used as a control.

Plants were cut at the second node 75 days after planting and the roots washed from the sand. Serological typing on a random sub-sample of 30 nodules taken from the Roma inoculum was done to check that all nodules resulted from infection by CB3126. Plant tops and roots were oven-dried at 60°C for 48 h for the determination of dry matter yields.

Statistical analysis

All data were analysed using an analysis of variance model for a completely randomised design. A square-root transformation was performed before analysis of the leaflet N-concentration data. Significant differences between treatments were detected by LSD analysis ($P < 0.05$).

Results

Experiment 1: Inoculation response and competitive ability of CB3126

Dry matter yield. Treatment responses, as measured by leaf dry weight (Figures 1 and 2), were identical with those recorded for top dry weight and total nitrogen content (data not presented). A significant ($P < 0.05$) soil \times N treatment interaction was recorded at both harvest times.

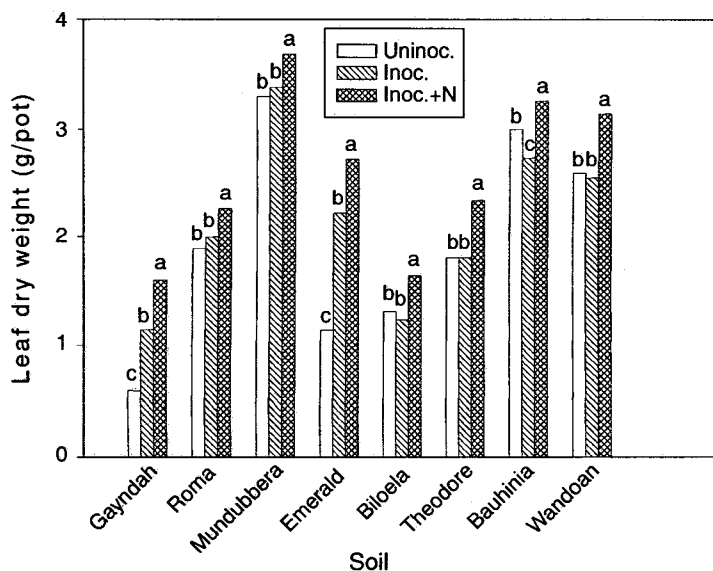


Figure 1. Leaf dry weight of *Desmanthus virgatus* in uninoculated, inoculated and inoculated+N treatments, 56 days after sowing. Letters on histogram bars denote significant differences ($P < 0.05$) within soils.

At Harvest 1, inoculation significantly increased leaf yield relative to uninoculated controls in the Gayndah and Emerald soils by 93 and 96%, respectively. Inoculation significantly reduced leaf production in the Bauhinia soil by 9%. All soils responded to applied N when compared with either the inoculated or uninoculated treatments.

At Harvest 2, inoculation significantly increased leaf yield, relative to the uninoculated controls in the Gayndah, Roma, Mundubbera and Emerald soils, by 53, 90, 84 and 30%, respectively (Figure 2). A leaf dry weight response to applied N in comparison with the inoculated and uninoculated treatments was recorded in all soils.

Nitrogen status. Concentration of nitrogen in leaflets from Harvest 2 was increased by inoculation in the Gayndah, Roma, Mundubbera and Bauhinia soils (Table 2).

Nodulation. No nodules were found on roots of uninoculated plants in the Roma and Mundubbera soils at Harvest 1 (Table 3) or Harvest 2 (data not presented), indicating that few or no indigenous strains occurred in these soils. Inoculation with CB3126 increased nodule mass in Roma and Mundubbera soils to levels similar to those of other soils. Inoculation with CB3126 also increased nodule mass in plants grown in the

Table 2. Nitrogen concentration (%) in leaflets of *Desmanthus* inoculated with CB3126 and uninoculated, 100 days after sowing. Figures in brackets are square root-transformed data.

Soil	Nitrogen concentration			
	Uninoculated		Inoculated	
Gayndah	2.91	(1.71b) ¹	3.09	(1.76a) ¹
Roma	2.02	(1.42b)	3.68	(1.92a)
Mundubbera	1.93	(1.39b)	3.45	(1.86a)
Emerald	3.25	(1.80a)	3.25	(1.80a)
Biloela	3.49	(1.87a)	3.41	(1.85a)
Theodore	3.44	(1.85a)	3.43	(1.85a)
Bauhinia	3.10	(1.76b)	3.61	(1.90a)
Wandoan	3.35	(1.83a)	3.33	(1.82a)

¹ Means followed by different letters within rows are significantly different ($P < 0.05$).

Emerald soil in Harvest 1 (Table 3) but not in Harvest 2 (data not presented). No significant effects of inoculation occurred in the other soils, in which uninoculated plants were all well nodulated.

In those soils where uninoculated plants form nodules, nodules formed from strain CB3126 in the inoculated treatment were 35–96% of the total at Harvest 1 and 23–98% at Harvest 2 (Table 3). Between 56 and 100 days after sowing, the proportion of nodules due to CB3126 appeared to decrease in the Biloela, Theodore

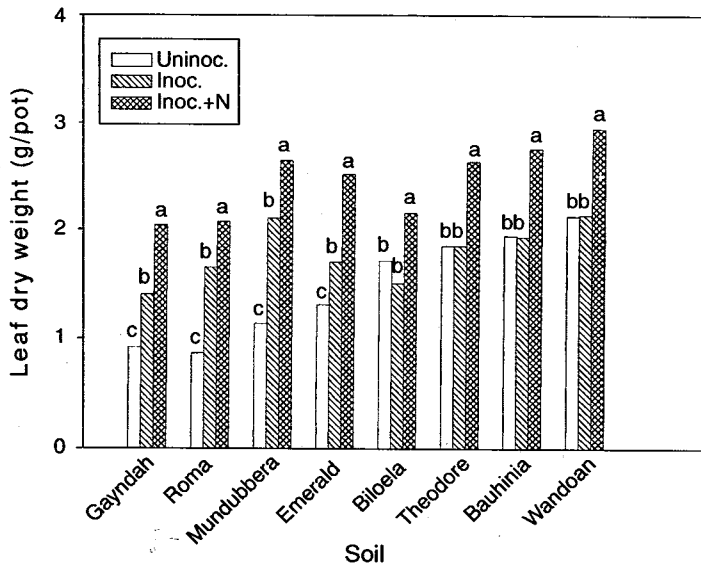


Figure 2. Leaf dry weight of *Desmanthus virgatus* in uninoculated, inoculated and inoculated+N treatments, 100 days after sowing, i.e. 44 days of regrowth after first harvest. Letters on histogram bars denote significant differences ($P < 0.05$) within soils.

and Wandoan soils, to increase in the Bauhinia soil and to remain relatively constant in the Gayndah, Roma, Mundubbera and Emerald soils. A small percentage (<10%) of nodules from uninoculated controls in soils from Bauhinia, Theodore and Wandoan were identified as containing rhizobia that serologically cross-reacted with antiserum prepared against strain CB3126. The fluorescent reaction in these cases was of much lower intensity compared with that of nodules formed by CB3126.

Table 3. Nodule dry weight of *desmanthus* uninoculated or inoculated with *Rhizobium* strain CB3126, 100 days after sowing. The proportions of nodules in the inoculated treatment due to CB3126, 56 and 100 days after sowing, are also presented.

Soil	Nodule dry weight		CB3126 nodules	
	Uninoculated	Inoculated	56 d	100 d
	(mg/pot)		(%)	
Gayndah	33.9a ¹	60.8a	86	82
Roma	0.0b	110.8a	100	100
Mundubbera	0.0b	121.5a	100	100
Emerald	44.3b	124.7a	96	98
Biloela	86.0a	115.3a	47	32
Theodore	110.0a	116.8a	72	43
Bauhinia	193.4a	162.9a	88	96
Wandoan	176.3a	155.8a	35	23

¹Means within rows and items followed by different letters are significantly different ($P < 0.05$).

Experiment 2: Effectiveness in N fixation of indigenous strains of *Rhizobium*

Indigenous strains of *Rhizobium* from the Emerald, Biloela and Theodore soils were as effective ($P < 0.05$) as CB3126 and resulted in total yields 96–113% of those from plants inoculated with CB3126. Strains from Gayndah, Bauhinia and Wandoan were inferior and resulted in yields of 62, 27 and 67% of CB3126, respectively. Low yields in the uninoculated control confirmed that the system was N-free and serological typing confirmed that nodules originating from the Roma inoculum source were due to CB3126 (Figure 3).

Discussion

The response of *Desmanthus virgatus* cv. Marc to inoculation with the *Rhizobium* strain CB3126 appeared to depend on a number of factors including the soil N supply, the competitive ability of CB3126 to form nodules, and the presence and N-fixation effectiveness of indigenous rhizobia.

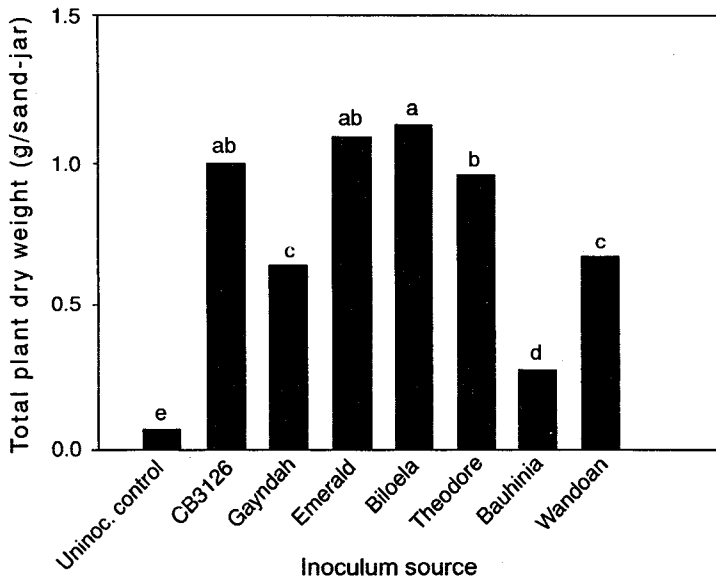


Figure 3. Total dry weight of *Desmanthus virgatus* inoculated with indigenous rhizobia from different clay soils in comparison with the commercial inoculum (CB3126) grown in a nitrogen-free system. Treatments marked with different letters are significantly different ($P < 0.05$).

N supply

An apparent high level of soil nitrogen masked growth responses to inoculum at the first harvest in the fertile Mundubbera soil. Levels of soil nitrogen early in the trial may have been higher than normal due to increased mineralisation caused by storage and disturbance of the soil during the potting procedure. However, the removal of tops at the first harvest appeared to deplete the supply of available soil nitrogen allowing the expression of an inoculation response at the second harvest. This suggests that plants growing in highly fertile soils may still need to nodulate in the longer term. This may be especially true in the field where desmanthus plants usually will grow in competition with grasses (R.L. Clem, personal communication).

Presence and N-fixation effectiveness of indigenous strains

The absence of nodules on the uninoculated plants at both harvests in the Mundubbera red eucrazem and Roma red-brown earth indicated that compatible rhizobia were absent. Other soils found lacking in indigenous strains able to nodulate desmanthus include a sandy soil from Mundubbera (Brandon *et al.* 1998), an acidic red podzolic soil from Gympie (Date 1991a) and a clay soil from Tara (authors, unpublished data). Uninoculated plants in most clay soils in the current trial, however, nodulated. The presence of indigenous strains of *Rhizobium* able to nodulate *D. virgatus* is thought to be due to cross-infection with rhizobia associated with indigenous Mimosaceae legumes. For example, *Neptunia gracilis* is present in some clay soils of central and southern Queensland and its *Rhizobium* is known to form effective associations with some accessions of *D. virgatus* (Date 1991a). No *N. gracilis* was observed at either Mundubbera or Roma.

Although most soils contained strains of *Rhizobium* compatible with desmanthus, positive responses to inoculation may still occur in soils where population levels are low. This appears to have been the case in the Emerald soil where native strains were equally effective as *Rhizobium* strain CB3126, but a positive response was still observed to inoculation. The decline in the proportional response to inoculation between 56 and

100 days after planting, low nodule mass in the uninoculated relative to the inoculated and the extremely high proportion of nodules due to the inoculum strain at both harvests support this hypothesis.

A positive response to inoculation can be expected where effectiveness of native strains is lower than that of the inoculum, such as in the Gayndah, Bauhinia and Wandoan soils. Despite strains in these soils being only 27–67% as effective as CB3126, leaf dry weights of well-nodulated uninoculated plants in these soils were similar to those of plants inoculated with CB3126. However, N concentration in leaf tissue of plants growing in the Bauhinia soil was significantly lower than in inoculated plants at the end of the trial, which may reflect the much lower effectiveness (27%) of native strains in this soil.

The response of plants to applied N, whether inoculated with CB3126 or in uninoculated plants nodulated effectively by native strain, suggests that neither CB3126 nor native rhizobia is meeting the plant's demand for N. This observation needs confirmation in field-grown plants. Plant dry weight responses varied with soil and cultivar (Brandon *et al.* 1998).

Competitive ability of CB3126 to form nodules

The inoculum strain CB3126 effectively nodulated plants growing in soils with no indigenous *Rhizobium*. It also produced a majority of nodules in inoculated plants in 2 of the 4 soils in which uninoculated plants were well nodulated, showing that it is moderately competitive against native strains. The proportion of nodules due to CB3126 remained high in most soils at the second harvest, but appeared to increase in the Bauhinia soil, in which native strains were less effective, and may decrease slightly in other soils such as Theodore, in which native strains were as effective as CB3126. Similar replacement of inoculum with effective strains of indigenous rhizobia was reported by Date (1991b) in field experiments, with *Desmodium intortum* and *Neonotonia wightii*, in which the proportion of nodules formed by the inoculum declined from 30–90% in the establishment year to <10% within 2–5 years, without a corresponding reduction in yield.

Conclusion

Six of 8 soils used in the current trial had indigenous strains of *Rhizobium* able to nodulate desmanthus, and most of these were effective relative to CB3126. However, inoculation with CB3126 increased plant growth in 4 soils in which nodulation by indigenous strains was low. It is, therefore, recommended that desmanthus be inoculated with the commercially available strain CB3126 as a normal part of the sowing procedure.

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