Growth responses of *Desmanthus virgatus* to inoculation with *Rhizobium* strain CB3126. II. A field trial at 4 sites in south-east Queensland.

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Abstract

Growth of *Desmanthus virgatus* cvv. Marc, Bayamo and Uman, inoculated with *Rhizobium* strain CB3126, was compared with that of uninoculated and inoculated+nitrogen-fertilised plants at 4 sites in south-east Queensland over a 3-year period. Supplementary irrigation was used to ensure prompt establishment. The proportion of nodules due to the inoculum strain was determined using serological methods and the proportion of total plant nitrogen arising from biological fixation in the second and third years was estimated using the natural abundance method.

Top growth was increased significantly by inoculation at 3 sites in the first year and at 1 site in the second year. Growth increases relative to uninoculated plants varied from 34–313% and appeared to depend on the prevalence of indigenous strains and soil nitrogen level. The inoculum accounted for few (<3%), some (0–65%) or most (>94%) of the nodules formed in soils in which nodulation of uninoculated plants was high, medium and low, respectively. The proportion of total nitrogen due to biological nitrogen fixation in the second and third years ranged from 0% in a highly fertile soil to 38–98% in 3 soils of low–moderate fertility.

The ability of strain CB3126 to increase growth of desmanthus in some soils was confirmed in these field trials. Inoculation of desmanthus seed with an effective strain of *Rhizobium* such as CB3126 is recommended.

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Introduction

Desmanthus virgatus (desmanthus), a tropical pasture legume introduced from Latin America, is recommended for use in clay soils of southern and central Queensland (Cook *et al.* 1993). Early-, mid- and late-flowering cultivars, Marc, Bayamo and Uman, are now commercially available and are marketed as a mixture under the name, "Jaribu".

Desmanthus is not highly specific in its nodulation requirement (Date 1991), but Bahnisch *et al.* (1998) have demonstrated that some Queensland soils may contain few if any native rhizobia able to nodulate desmanthus. In these soils, it is important that an effective strain is available for use as an inoculum. Previous poor performance of desmanthus at some sites in Queensland has been attributed to nodulation failure (R.M. Jones, personal communication).

In a glasshouse trial, Bahnisch *et al.* (1998) found that CB3126 increased growth of desmanthus in soils where nodulation of uninoculated plants was slow or absent. The present experiment aimed to determine the response to inoculation with strain CB3126 in the field in 4 soils in south-east Queensland. A sandy soil was included to test the ability of strain CB3126 to nodulate desmanthus in soil outside the range to which it is well adapted.

Materials and methods

Treatments and design

The 2 factors were: cultivars of *Desmanthus virgatus* (Marc, Bayamo and Uman); and inoculation/nitrogen treatments (an uninoculated control; inoculated with CB3126; and

inoculated+nitrogen fertiliser). The 4 sites included 3 neutral-alkaline clay soils typical of those for which desmanthus is recommended. One, located at the Narayen Research Station near Mundubbera, was a highly fertile soil (Table 1), previously dominated by brigalow *(Acacia harpophylla).* This site was adjacent to the collection site for the Mundubbera soil used by Bahnisch *et al.* (1998*).* The other 2 were moderately fertile black earths, at the QDPI Brian Pastures Research Station, near Gayndah, and the QDPI Research Station, Roma. The Gayndah site was the same as that used by Bahnisch *et al.* (1998). The fourth site, at the CSIRO Narayen Research Station, was an infertile light-textured sandy soil derived from granite previously dominated by speargrass (*Heteropogon contortus*).

Factorial combinations of the 2 treatments were arranged in a randomised block design at each site. Plots (= treatment combinations) consisted of 5 rows 5m long of desmanthus, sown 0.5 m apart. Blocks were separated from each other with grassed laneways to minimise the possibility of cross contamination caused by water moving from one block to another. Plots within blocks were arranged across any prevailing slope to minimise flow of water from one plot to the next.

Sowing and maintenance of plots

Minimum tillage was used to prepare each of the sites. Grass cover was killed using the herbicide, Glyphosate (2 L/ha), and shallow furrows 0.5 m apart were made using a chisel plough. Seed of desmanthus was sown into the furrows on 11 January at Mundubbera (both sites), 12 January at Gayndah and 13 January, 1994 at Roma, using a walk-behind garden seeder that placed the seed in the ground at a depth of 0.5–1 cm and a rate of 3 kg/ha (approximately 50 seeds/m of row). Uninoculated seed was sown first at each site. Seed that had been inoculated with peat inoculum (*Rhizobium* strain CB3126) at a rate of 1 g/100 g seed using 1.5% methyl cellulose as an adhesive was then sown in the remaining 2 treatments. The planter was washed in alcohol between sites. *Chloris gayana* seed was hand-broadcast over the entire area of each site at a rate of 4 kg/ha.

Plots were irrigated within 18 h of sowing. Subsequently, irrigation was used as necessary to supplement rainfall and ensure moderate first- and second-year growth of desmanthus. Low water availability prevented use of irrigation at Mundubbera in the second year and no irrigation was used at any of the sites in the third year.

Urea was applied to the surface of the soil at a rate of 50 kg/ha N in the nitrogen treatment after sowing and again approximately 4 and 10 weeks after sowing in the first year and early (January–February) and mid-season (March–April) in the second year. Molybdenum-fortified superphosphate was applied to the granite soil at sowing and at the beginning of the second year (20 kg/ha P, 20 kg/ha S and 0.4 kg/ha Mo) to overcome possible deficiencies of P, S and Mo (Johansen *et al.* 1978). Gypsum (50 kg/ha S) was applied to the Gayndah site shortly after sowing and molybdenised superphosphate (30 kg/ha S, 6 kg/ha Mo) at the beginning of the second year following results of a pot trial which identified S and Mo as possible nutrient limitations in this soil (Brandon and Date 1998).

Grass cover was established quickly between rows of desmanthus by the naturalised Red Natal grass (*Melinis repens*) at Gayndah, and by the sown species, *Chloris gayana,* at the granite soil site at Mundubbera. Reinvasion of plots by the native species, *Eriochloa pseudacrotricha,* occurred in the second year at Roma. No grass colonisation occurred at the clay soil site at

Table 1. Soil classification and chemical characteristics of the 0–10 cm layer of 4 sites in south-east Queensland.

Site	Type[1]	pH	Organic C	Total N	NO_3	Bicarb. P
			(%)	(%)	(mg/kg)	(mg/kg)
Gayndah	Ug5.13	7.9	1.6	0.12	51	84
Mundubbera granite	Dy3.14	6.6	0.6	0.04	5	7
Mundubbera clay	Gn3.13	7.1	3.2	0.24	12	47
Roma	Ug5.22	8.2	0.07	0.07	2	5

[1]Northcote *et al.* (1975).

Mundubbera despite repeated hand sowing of *Chloris gayana* seed between the rows. Excessive grass growth was prevented in the granite soil site at Mundubbera and at Gayndah by periodic application of the selective herbicide, Fluazifop, applied at the sub-lethal rate of 1–2 L/ha. Plots at all sites were mown to 5–8 cm at the beginning of the second and third seasons.

Sampling and measurements

Nodulation. In the first year, nodules from the central 3 rows of the inoculated and uninoculated plots were sampled 7, 10 and 16 weeks after sowing at Gayndah; 7, 10 and 18 weeks after sowing at the granite site at Mundubbera; 10 and 18 weeks after sowing at Roma; and 10 and 17 weeks after sowing at the clay soil site at Mundubbera. In the second year, nodules at Gayndah and Mundubbera were sampled in April and at Roma in May.

Four desmanthus plants/plot were removed using a soil-coring tube driven into the soil to a depth of 20–25 cm. A 13 cm diameter tube was used for all sampling except for the 7- and 10-week harvests in the first year when a smaller diameter tube (9 cm) or excavation with a spade was used. Additional lateral cores (9 cm diameter) were taken, immediately either side of the main core, 16–18 weeks after sowing in the first year at all sites to evaluate the lateral spread of the inoculum.

Soil was washed from plant roots and nodules and collected in a fine sieve. All nodules collected in the first year were counted and serotyped using the indirect fluorescence technique with fluorscein isothiocyanate-labelled antiserum (Somasegaran and Hoben 1985). Up to 70 nodules/treatment were serotyped in the second year. Rating of fluorescence relative to known positive (CB3126) and negative controls (non-CB3126 strains) provided an estimate of the proportion of nodules due to CB3126.

Plant growth. The 4 plant tops of desmanthus removed prior to soil coring in the central rows of each plot were dried to constant weight in an oven at 60°C. An additional 4–6 plants were harvested from the border rows of each plot in the first year and 2–4 plants in the second year. These were added to tops sampled from the middle rows and an individual plant dry weight calculated from the total dry weight of plant tops.

N fixation. Following drying, leaves were separated from plants tops, ground and analysed for nitrogen concentration following kjeldahl digestion. Leaf material from inoculated and uninoculated plots of the Bayamo cultivar at the Roma and Mundubbera sites was also analysed for nitrogen isotopes using a mass spectrophotometer to allow estimation of the proportion of nitrogen fixed using the natural abundance method (Peoples and Craswell 1992). Whole plant tops of Bayamo (3–5 plants/pot) were sampled in the third year. The amount of nitrogen fixed by the nodulated legume was determined using the ratio of natural isotopes of N ($\delta^{15}N$) in non-N-fixing control plants compared with that for a legume relying entirely on biological nitrogen fixation for its N supply (β value). The β value was obtained by analysing leaves of whole plant tops of Bayamo inoculated with strain CB3126 and grown in sand-jars. The β value for leaf tissue was used in calculating the amount of N fixed in the second year (2.0). The β value for whole tops was used in calculating the amount of nitrogen fixed in the third year (2.7).

Non-nitrogen-fixing plants used as controls in the second year were: *Melinis repens* at Gayndah; *Chloris gayana* at the granite site at Mundubbera; *Urochloa panicoides* at the clay site at Mundubbera; and *Chloris virgatus* at Roma. Whole tops of Bayamo (3–5 plants/plot) were sampled from the same plots in the third year for analysis, with controls being *Melinis repens* at Gayndah, *Eriochloa pseudoacratricha* at Roma, *Chloris gayana* at the granite soil site at Mundubbera and *Salsola kali* at the clay soil site at Mundubbera. The samples of *Melinis repens* collected in the second year at Gayndah were discarded accidentally and estimation of nitrogen fixation at this site was therefore not possible.

Results

Proportion of nodules identified as strain CB3126

Data for nodules collected at the various harvests in the first year were summed (Table 2) due to the low number of nodules collected at any one harvest. These data do not include nodule number from the side cores taken at the end of Year 1.

Plants in the uninoculated treatment nodulated only at Gayndah and Roma, whereas those in the

inoculated treatment were well nodulated at all sites except the fertile clay soil at Mundubbera (Table 2).

Strain CB3126 accounted for essentially all the nodules in the inoculated treatment at the granite site at Mundubbera in both years (Table 2) and for the few nodules formed in the fertile clay soil in the first year. This was in contrast to the well nodulated plants at Roma, where all nodules formed on inoculated plants were from native rhizobia. At Gayndah, 7–44% of nodules in the first year and 0–65% in the second year were due to CB3126 (Table 2). In the second year at Gayndah, the proportion of CB3126 nodules on Marc was lower than on Bayamo, resulting in a significant ($P<0.05$) cultivar × treatment interaction. There was no significant ($P>0.05$) interaction between cultivars and inoculation at the other sites.

There was little evidence of lateral spread of inoculum at the Gayndah and Roma sites. None of the nodules (>500) from the lateral cores at Gayndah was due to CB3126. At Roma, only 3 of 25 nodules from lateral cores sampled beside Marc, Bayamo and Uman plants were due to the inoculum strain. However, at the granite site at Mundubbera, over 90% of the 40 nodules sampled from the lateral cores of the 3 cultivars were due to CB3126.

Plant growth

In the first year, inoculation significantly ($P<0.05$) increased yield of desmanthus at Gayndah and at both sites at Mundubbera. Greatest response occurred in the infertile granite soil at Mundubbera (313% increase in growth relative to uninoculated controls), but growth increases of 34–76% occurred in inoculated treatments in the clay soils (Table 3). In the second year, inoculation increased yield by 246% in the granite soil ($P<0.05$) but had no significant effect ($P>0.05$) at the other sites (Table 4).

Yield achieved with the application of nitrogen was significantly ($P<0.05$) higher than that achieved with inoculation at Gayndah and on the granite soil at Mundubbera, but there was no significant ($P>0.05$) increase at the other sites. At Gayndah, first-year yield increased by 220% and second-year yield by 130% relative to inoculated plants. Corresponding increases in the granite soil at Mundubbera were 60 % ($P<0.05$) and 30% ($P<0.05$). There was a significant ($P<0.05$) cultivar × inoculation/nitrogen treatment interaction at Gayndah and at the granite sites where the later-flowering Uman and Bayamo cultivars respond better to inoculation or nitrogen application than the less robust, early flowering cultivar, Marc.

Table 2. The percentage of nodules due to strain CB3126 in desmanthus inoculated with CB3126 at sowing or uninoculated at 4 sites in south-east Queensland in the first and second years. Values in brackets are total nodule number. Within years, percentages followed by different letters are significantly different ($P<0.05$) following √arcsin transformation (upper case letters for interactions; lower case letters for comparison of means).

Site	Cultivar	Year 1		Year 2	
		Uninoculated	Inoculated	Uninoculated	Inoculated
Gayndah	Marc	0 (244)	7 (272)	5A (41)	0A (27)
	Bayamo	4 (289)	44 (310)	0A (108)	44B (134)
	Uman	0 (153)	36 (230)	0A (151)	65B (154)
	Mean	1a	29b	2a	36b
Mundubbera granite	Marc	0 (1)	98 (118)	0 (0)	99 (92)
	Bayamo	0 (1)	95 (196)	0 (0)	100 (124)
	Uman	0 (1)	97 (195)	0 (0)	94 (127)
	Mean	0a	96b	0a	98a
Mundubbera clay	Marc	0 (0)	0 (0)	0 (0)	0 (0)
	Bayamo	0 (0)	86 (7)	0 (0)	0 (0)
	Uman	100 (4)	100 (9)	0 (0)	0 (0)
	Mean	—	—	0	0
Roma	Marc	0 (57)	1 (54)	0 (103)	0 (53)
	Bayamo	0 (24)	2 (35)	0 (192)	0 (155)
	Uman	1 (68)	2 (162)	2 (154)	1 (216)
	Mean	0	2	1	0

Table 3. Mean plant weight (g/plant) of desmanthus plants inoculated with *Rhizobium* strain CB3126, uninoculated or fertilised with nitrogen (+N) harvested in the first year at 4 sites in south-east Queensland. Within soils, values followed by different letters are significantly different (P<0.05) (upper case letters for interactions; lower case letters for comparison of means).

Site (Weeks from sowing)	Cultivar	Treatment			**Mean**
		Uninoculated	Inoculated	Inoculated +N	
Gayndah (16 weeks)	Marc	3.8AB	4.9AB	14.2D	7.6a
	Bayamo	1.8A	3.7AB	19.4E	8.3b
	Uman	2.3AB	5.1B	10.1C	5.8a
	Mean	2.6a	4.6b	14.6c	
Mundubbera granite (17 weeks)	Marc	0.9	1.4	2.5	1.6a
	Bayamo	1.0	5.8	10.7	5.8b
	Uman	0.5	2.8	2.7	1.8a
	Mean	0.8a	3.3b	5.3c	
Mundubbera clay (18 weeks)	Marc	28.5	29.5	28.6	28.9a
	Bayamo	46.6	64.5	69.4	60.1b
	Uman	52.4	77.0	98.1	75.9c
	Mean	42.5a	57.0b	65.4b	
Roma (18 weeks)	Marc	13.6	20.0	18.7	17.4a
	Bayamo	11.4	11.3	15.0	12.5a
	Uman	15.9	28.7	56.4	33.6b
	Mean	13.6a	20.0ab	30.0b	

Table 4. Mean weight (g/plant) of desmanthus plants inoculated with *Rhizobium* strain CB3126, uninoculated or inoculated and fertilised with nitrogen harvested in the first year at 4 sites in south-east Queensland. Within soils, values followed by different letters are significantly different (P<0.05) (upper case letters for interactions; lower case letters for comparison of means).

Site (Month of harvest)	Cultivar	Treatment			**Mean**
		Uninoculated	Inoculated	Inoculated +N	
Gayndah (April)	Marc	2.6A	2.2A	7.3AB	4.0a
	Bayamo	8.6AB	11.8AB	27.9B	6.1a
	Uman	12.9AB	26.0B	58.2C	32.4b
	Mean	8.1a	13.4a	31.2c	
Mundubbera granite (April)	Marc	0.6	2.0	1.6	1.4a
	Bayamo	2.0	6.8	11.3	6.7b
	Uman	1.4	4.8	5.1	3.8c
	Mean	1.3a	4.5b	5.9b	
Mundubbera clay (April)	Marc	7.5	5.9	6.9	6.7a
	Bayamo	13.1	16.0	10.2	13.1b
	Uman	12.9	11.1	12.9	12.3b
	Mean	11.2a	11.0a	10.0a	
Roma (June)	Marc	4.2	6.0	7.9	6.0a
	Bayamo	41.4	48.3	38.1	42.6b
	Uman	60.7	73.8	105.4	80.0c
	Mean	35.4a	42.7a	50.4a	

Proportion of nitrogen fixed by Rhizobium

The proportion of nitrogen fixed biologically in the second and third years at Roma was similar (40–70%) in both inoculated and uninoculated plots (Table 5). Nitrogen fixed in the fertile clay soil at Mundubbera in the second year also was similar in both treatments (20–30%) but higher than that in the third year (0%). Inoculated plants fixed a high proportion of nitrogen at the granite site at Mundubbera in both years (69–78%). The proportion of nitrogen in plant tops in uninoculated plants was less than 0% (–7% and –25% — see discussion). Nitrogen fixation in the third year at Gayndah was high in both inoculated and uninoculated treatments (80–98%).

Table 5. Proportion of nitrogen attributed to N fixation for leaves (second year) and whole plant tops (third year) estimated using the natural abundance method.

Site	Year 2		Year 3	
	Uninoc.	Inoc.	Uninoc.	Inoc.
		(%)		
Gayndah	—	—	98	82
Mundubbera granite	–7	78	–25	69
Mundubbera clay	22	26	0	0
Roma	40	48	68	47

Discussion

Growth responses of *Desmanthus virgatus* to inoculation with *Rhizobium* strain CB3126 have been demonstrated at 3 of 4 field sites in south-east Queensland. The largest response was observed in the sandy granitic soil at Mundubbera in which no suitable native rhizobia were present. The current cultivars of desmanthus are not recommended for sandy soil. Similar responses to inoculation were found in a pot trial by Bahnisch *et al.* (1998) in 4 of 8 clay soils from south-east Queensland, in which *D. virgatus* is a recommended pasture species. No native rhizobia were observed in 2 of these 4 clay soils.

In the field experiments, and pot experiments by Bahnisch *et al.* (1998), serological typing confirmed that, where inoculation responses were obtained, *Rhizobium* strain CB3126 formed most of the nodules. Inoculation with an effective strain of *Rhizobium,* therefore, is essential in these soils. It is worth noting that significant improvement in early establishment and growth can be obtained even where native rhizobia are either few in number or less effective as fixers of nitrogen (Bahnisch *et al.* 1998).

The lack of a companion grass and the high N fertility of the clay soil at Mundubbera may have been responsible for the low nodule numbers observed in inoculated plants. When desmanthus was grown in a similar soil in pots, roots of inoculated plants formed nodules (Bahnisch *et al.* 1998). Nodules were observed on roots of desmanthus growing in a vigorous grass pasture in a nearby grazing trial (authors, unpublished data). The lack of nodulation in desmanthus grown in pure swards in the current trial suggests the need to grow it with a companion grass in soils that have high levels of nitrogen, if significant levels of fixation are to be achieved.

Although there were significant differences in plant dry matter production and the proportion of nodules formed by CB3126 in the 3 cultivars at Gayndah and in plant dry matter in both years in the granitic soil at Mundubbera, this was attributed to longer vegetative growing periods rather than to direct differences in effectiveness of the inoculum on the cultivars. For example, inoculation or nitrogen fertilisation of the later maturing Bayamo and Uman cultivars allowed them to express their genetic potential better. There was more rapid replacement of the inoculum with indigenous strains on the early maturing Marc at Gayndah, perhaps due to longer periods of inactive growth.

There appeared to be cycling of nodules within seasons in all cultivars, with nodule death occurring following periods of drought, and nodule formation occurring in response to improved soil moisture. For example, no nodules were observed in a preliminary harvest at the end of the second year (data not presented), but small nodules were present in large numbers 3 weeks later, following irrigation and increased plant growth. The ephemeral nature of nodule formation means that the strain must persist in the soil during periods of inactive plant growth.

Although strain CB3126 was replaced by native strains on Marc at Gayndah, it was able to over-winter and initiate nodules on lateral roots in the granite soil at Mundubbera where there were no effective native strains. It also survived in the clay soil at Mundubbera until nodulation was observed in the middle of the first year. This ability to survive in the soil may be necessary

where desmanthus is sown into fertile soil, delaying the onset of nodulation. However, survival in the current trial may have been aided by irrigation which was applied shortly after sowing. More recent work has shown that high soil temperatures similar to that in dry soils can quickly kill inoculum placed on desmanthus seed (Becerra Stiefel *et al.* 1998).

The proportion of plant nitrogen fixed biologically in the Roma and Gayndah soils was in the order of 40–98%. This is similar to the range of nitrogen fixed by other pasture legumes (Peoples and Craswell 1992). Variation between sites in the proportion of nitrogen fixed probably related to factors such as pre-existing soil-N level. Very little nitrogen was fixed in plants growing in the fertile clay soil at Mundubbera (0–25%). Similarly low fixation rates were found by Sanford *et al.* (1993) in field-grown temperate legumes where soil-nitrogen level was high.

The negative fixation rates estimated for uninoculated desmanthus in the granite soil at Mundubbera might be due to differences in nitrogen-uptake patterns between legume and the non-fixing grass control (Pate *et al.* 1994). Dicotyledonous non-fixing plants are recommended, but were not present in sufficient numbers at most sites in the trial. N fixation associated with the *Chloris gayana* may have introduced some error in the estimation of nitrogen fixed by the legume.

The poorer growth of inoculated desmanthus relative to nitrogen-fertilised plots, particularly in the granite soil at Mundubbera and at Gayndah, suggests that there may be limitations to growth other than N. *Rhizobium* strain CB3126 was effective on a range of desmanthus accessions in early screening work (Date 1991), and was as effective as indigenous strains isolated from Queensland soil in work by Bahnisch *et al.* (1998). We suggest that nutritional or other soil limitations may have limited the amount of N fixed and therefore growth at these sites. Concurrent pot-trial work on nutrition of desmanthus indicated that both S and Mo may be limiting in the Gayndah soil (Spies *et al.* 1998). Although S was applied in the first year, Mo was not applied until the beginning of the second year. Therefore, Mo may have been a limitation in the first year. Results of pot-trial work showed that Mo deficiency in this soil reduced growth of 11-week-old plants inoculated with CB3126 by 40%, but by only 13% in nitrogen-fertilised plants (Brandon and Date 1998).

Bolger *et al.* (1995) reported an increase in nitrogen fixation (kg/ha) in subterranean clover-grass pastures in infertile sandy soils following addition of P, S and K. However, increased N was due primarily to increased total dry matter production not increased proportion of N fixed by the legume component. Therefore, despite a high proportion of nitrogen fixed by desmanthus at Gayndah, low yields in association with grass pastures (Burrows and Porter 1993; R.L. Clem, personal communication) may limit input of nitrogen on a kg/ha basis. More work is needed to define better the nutritional and other soil factors needed to ensure vigorous desmanthus growth.

Although desmanthus has been collected from soil types ranging from clays to sands (Burt 1993), the current cultivars were collected from clays and did not appear well adapted to the lighter-textured soil at Mundubbera despite application of nutrients S, P and Mo at sowing. Only the largest-growing cultivar, Bayamo, inoculated with CB3126 or fertilised with N, survived the dry conditions in the second year. The death of uninoculated plants in this soil, however, highlights the importance of ensuring that compatible rhizobia are present when evaluating accessions on this soil.

Conclusion

Evidence from this trial and the trial of Bahnisch *et al.* (1998) shows that strain CB3126 can increase growth of desmanthus in soils where indigenous strains are absent or sparse in number. It is, therefore, recommended that desmanthus seed be inoculated with this commercially available strain prior to sowing in all soils.

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