Nitrogen fixation in *Desmanthus*: strain specificity of *Rhizobium* and responses to inoculation in acidic and alkaline soil

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Abstract

Forty-eight accessions of *Desmanthus* (1 each of *D. brevipes, D. fruticosus, D. illinoensis, D. subulatus,* 2 of *D. covillei* and 42 of *D. virgatus*) were screened against 17 strains of *Rhizobium* for effectiveness of N-fixation. Six effectiveness groups were defined by pattern analysis with 23 accessions forming effective associations with 10 or more strains.

Five accessions selected previously for their agronomic potential responded to inoculation, especially in acidic soil. Over all strain treatments, *D. virgatus* CPI38351 and TQ90 out-yielded (dry weight and N content) the other accessions. Proportional differences among host accessions were greater in the acid podzolic soil from Gympie than in the alkaline heavy clay soil from Gayndah. In the Gympie soil strains CB1397 and CB3126 were significantly better on accession CPI38351 but only CB3126 was better on TQ90. These results show that there are suitable strains of *Rhizobium* for adapted lines of *D. virgatus* for use in S.E. in Queensland.

Resumen

Cuarenta y cinco accesiones de Desmanthus *(1 de cada una de las especies* D. brevipes, D. fruticosus, D. illinoesis. D. subulatus, *2 de* D. covillei *y 42 de* D. virgatus*) fueron discriminadas con 17 cepas de* Rhizobium *en su efectividad para fijar N. Se definieron por efectividad 6 grupos mediante un análisis del patrón con las 23 accesiones que formaron un asociación efectiva con 10 o más cepas.*

Cinco de las accesiones seleccionadas previamente por su potencial agronómico respondieron a la inoculación, especialmente en suelo ácido.

De todos los tratamientos con las cepas, D. virgatus *CPI38351 y TQ90 superaron en rendimiento (en peso seco y en contenido de N) a las otras accesiones. Las diferencias proporcionales entre las accesiones hospederas fueron mayores en los suelos podzólicos de Gympie que en los suelos alcalinos de arcillas pesadas de Gayndah. En los suelos de Gympie las cepas CB1397 y CB3126 fueron significativamente mejores con la accesión CPI3851 pero únicamente CB3126 fue mejor con la accesión TQ90. Estas combinaciones entre cepas y accesiones realza el potencial de selección de una linea de* D. virgatus *apropiada para ser usada como planta de ramoneo en el S.E. de Queensland.*

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Introduction

Desmanthus virgatus is a native of Central and South America in both humid and semi-arid tropical and subtropical regions (Allen and Allen 1981). It occurs naturally on alkaline clay soils (Reid 1983) and has been used in Hawaii, West Indies (Allen and Allen 1981), India and other Asian countries as a fodder plant. *D. virgatus* is acknowledged for its drought tolerance (Brewbaker 1986) and its ability to grow rapidly and withstand heavy grazing (Anon. 1979). The agronomic potential of this genus as a browse plant in Australia is being assessed in a series of experiments by a CSIRO/Queensland Department of Primary Industries (QDPI) *Desmanthus* working group. Conway *et al.* (1988) have observed it to grow and yield successfully on heavy clay soils in S.E. Queensland.

Part of the assessment of *Desmanthus*, by the CSIRO/QDPI working group, was the determination of specificity requirements of rhizobial strains for effective nodulation and the need for inoculation. Allen and Allen (1981) reported effective nodulation of *Desmanthus virgatus* with isolates of rhizobia from *Prosopis chilensis* and *Leucaena leucocephala*, ineffective nodulation with isolates from *Mimosa pudica*, and no nodulation with isolates from *Glycine max* and *Lupinus polyphyllus*. Davis (1982) observed effective nodulation with isolates from *L. leucocephala*, *P. chilensis* and *M. invisa*. This paper reports the results of tests for N-fixation effectiveness of 17 strains of rhizobia of diverse origins with 48 accessions of *Desmanthus*, and inoculation response of 5 of these in 2 soils.

Materials and methods

Tests for N fixation effectiveness

Strains of Rhizobium. Seventeen strains of *Rhizobium* of diverse origin (Table 1) were selected on the basis of previous *ad hoc* information (R.A. Date and H.V.A. Bushby, unpublished data) and that of Allen and Allen (1981) and Davis (1982). Information relating to host of isolation, characteristics of the soil, and the country of origin are given in Table 1. With the exception of strain CB81, all strains were observed to be fast-growing and acid-producing in yeast mannitol medium. Strain CB81 has an intermediate growth rate and is not acid-producing.

Accessions of Desmanthus. Forty-eight accessions were selected from the CSIRO/QDPI program as representative of agronomically useful lines and the total germplasm bank. Details of soil and climate at collection sites are listed in Table 2.

Plant culture and measurements. A modification of the Leonard sand-jar technique (Norris and Date 1976) was used to screen for N-fixation effectiveness. Aseptically grown plants were inoculated with appropriate strains at 5 to 7 days of age and harvested at 8 weeks from sowing. Screening was completed in a glasshouse in Brisbane during October-April for 2 consecutive summers. When necessary, day temperature was maintained below 35 °C with evaporative cooling and night temperature above 20 °C with supplementary heating.

There were 2 sets of accessions. The first of these screened 39 accessions (IDs 1-39 in Table 2) with 17 strains of rhizobia in 4 runs. Accession CPI78382 (IDs 14, 15, 16, 17) was included as a "standard" in each run. The second set of 17 accessions (IDs 40-56) was screened against only 6 of the strains (CB81, NGR8, CB1397, CB2001, CB3059, CB3060). Five accessions (IDs 6, 40, 41, 42 & 48) were common to both sets.

Table 1. Information on origin of strains of *Rhizobium*

Strain[1]	Host of isolation	Location	Rainfall	Altitude	pH	Latitude	Soil
CB3058	*Desmanthus fruticosus*	Mulege, Mexico	140	20	7.2	26 °N	desert loam
CB3132	*Desmanthus fruticosus*	Mulege, Mexico	140	20	7.2	26 °N	desert loam
CB3133	*Desmanthus aff. virgatus*	Constituentes, Mexico	160	300	7.5	25 °N	sandy loam
CB3059	*Gliricidia* (sp.)	Honiara, Solomon Is.	(2120)[2]	n.i.[4]	6.0	9 °S	black alluvial clay
CB3060	*Leucaena diversifolia*	Lansdown, Aust.	860	90	6.2	19 °S	n.i.
CB3127	*Leucaena esculenta*	Altamirano, Mexico	(1120)	400	9.0	18 °N	alluvial clay
CB81	*Leucaena leucocephala*	Brisbane, Aust.	1100	130	[6.0][3]	27 °S	n.i.
CB3126	*Leucaena leucocephala*	Altamirano, Mexico	(1120)	400	6.5	18 °N	n.i.
CB3128	*Leucaena leucocephala*	Altamirano, Mexico	(1120)	400	9.0	18 °N	alluvial clay loam
CB3129	*Leucaena leucocephala*	Merida, Mexico	(910)	(70)	9.5	21 °N	n.i.
CB3130	*Leucaena leucocephala*	Fitches Ck, Antigua	(1150)	25	9.0	17 °N	clay
MS111	*Leucaena leucocephala*	Serdang, Malaysia	(2320)	(200)	[4.5]	3 °N	n.i.
NGR8	*Leucaena leucocephala*	Port Moresby, PNG	1015	130	[6.0]	27 °S	n.i.
CB3131	*Leucaena trichodes*	Porto Veijo, Ecuador	(560)	300	8.5	1 °S	heavy clay
CB2001	*Neptunia gracilis*	Brian Pastures, Aust.	720	n.i.	[7.5]	17 °S	heavy clay
CB1397	*Neptunia plena*	Mon Repos, B. Guyana	(2030)	n.i.	n.i.	6 °N	n.i.
TAL600	*Prosopis chilensis*	Maui, Hawaii, USA	(750)	(500)	[5.5]	[21 °N]	n.i.

[1] CB = CSIRO, Brisbane; NGR = New Guinea Dept. Agric.; TAL = NifTAL, Univ. of Hawaii; MS = Malaysian Agricultural Research & Development Institute
[2] ( ) approximation from Wernstedt (1972)
[3] [ ] approximation from R.A. Date
[4] n.i. = no information

Table 2. Information[1] on origin of *Desmanthus* species used in tests for nitrogen-fixation effectiveness

ID	Species	CPI No.	Country	Latitude	Rainfall	Altitude	pH	Soil
				(° ′)	(mm)[2]	(m)		
28	*brevipes*	90362	USA	29 18 N	(1130)[2]	(7)	n.i.[4]	n.i.
51	*covillei*	90311	Mexico	28 00 N	250	130	n.i.	n.i.
34	"	90816	"	29 35 N	250	500	6.5	sandy loam
24	*fruticosus*	84960	"	26 45 N	140	90	7.0	sandy loam from granite
29	*illinoensis*	90364	USA	29 18 N	(1000)	n.i.	n.i.	n.i.
35	*subulatus*	90857	Mexico	27 40 N	350	300	8.0	clay from basalt
2,40	*virgatus*	30205	India	(11 00 N)	(1300)	n.i.	n.i.	n.i.
3	"	33201	Guadalupe	15 57 N	1500	20	n.i.	n.i.
4	"	37143	Mexico	18 46 N	(1620)	150	n.i.	n.i.
5,6	"	37538	Argentina	31 24 S	(670)	450	n.i.	n.i.
7	"	38351[3]	Venezuela	9 28 N	(1450)	250	n.i.	n.i.
8	"	40071	Brazil	7 55 S	(560)	1410	n.i.	n.i.
9,41	"	55719	Venezuela	10 30 N	600	80	[B]	loam
10,42	"	65947	Ecuador	2 15 S	500	100	n.i.	n.i.
43	"	67642	Guatemala	15 00 N	550	240	n.i.	n.i.
44	"	76052	Mexico	21 01 N	940	5	[C]	loam from limestone
45	"	76503	"	21 05 N	900	5	n.i.	n.i.
46	"	78369	Argentina	24 12 S	700	1000	7.2	clay loam
11	"	78372	"	23 20 S	680	400	6.5	alluvial sandy loam
12	"	78373	"	23 15 S	650	300	7.8	alluvial clay loam
13	"	78379	"	24 46 S	690	1325	6.3	alluvial clay loam
47	"	78381	"	24 35 S	650	1250	7.7	loam
14,15,16,17,48	"	78382[3]	"	24 12 S	700	1000	7.2	clay loam
18	"	78383	"	24 13 S	1660	1000	7.2	alluvial clay loam
19	"	78385	"	28 05 S	1100	90	6.6	alluvial sandy loam
20	"	79653[3]	Cuba	21 59 N	600	5	7.5	sandy loam on shale
21	"	81337	Mexico	25 03 N	450	200	n.i.	clay
22	"	83570	Brazil	(20 00 S)	(1440)	(1850)	n.i.	n.i.
23	"	84508	Mexico	23 10 N	750	10	7.5	clay loam
49	"	84991	"	22 50 N	230	5	6.5	sand
25	"	85177	"	27 40 N	380	250	7.0	clay loam
26	"	85178	"	27 30 N	400	200	8.5	alluvial clay
27	"	85179	"	27 20 N	400	220	8.0	n.i.
50	"	85182	"	27 25 N	440	170	7.5	alluvial clay loam
30	"	90750	"	26 35 N	230	1100	8.0	loam
31	"	90751	"	26 50 N	320	1350	[B]	loam
32	"	90754	"	26 52 N	320	1350	7.5	clay loam
33	"	90755	"	28 30 N	300	1400	6.5	loam
52	"	90914	"	24 00 N	700	30	[A]	loam from granite
36	"	91146	"	18 27 N	1000	1450	6.5	clay loam
37	"	91326	"	16 09 N	1600	20	6.5	sand from quartzite
53	"	91496	"	20 45 N	1050	15	8.0	clay loam from limestone
54	"	92800	"	18 29 N	1400	5	[C]	clay from limestone
38	"	92803[3]	"	18 09 N	1250	50	[C]	clay
55	"	92805	"	20 06 N	1150	30	[C]	gravel from limestone
56	"	92817	Belize	(17 00 N)	(2000)	(100)	n.i.	n.i.
39	"	92818	"	(17 00 N)	(2000)	(100)	n.i.	n.i.
1	"	TQ90[3]	Australia	19 41 S	860	130	n.i.	n.i.

[1] Origin information from Divisional Plant Introduction Records, Project AP13.
[2] ( ) approximation after Wernstedt (1972); [A] = acid, [B] = neutral, [C] = alkaline
[3] Accessories chosen by CSIRO/QDPI *Desmanthus* working group.
[4] n.i. = no information

Uninoculated controls, with and without N fertilizer, were included as "strain" treatments in both sets. N controls received the equivalent of 100 kg/ha N as KNO_3 in 3 split applications.

The dry weight of whole plants was used to calculate an index of effectiveness for each strain by accession combination. The index was defined as the dry weight of the inoculated plant expressed as a percent of the N control.

Numerical methods and pattern analysis. The numerical taxonomy package available at PATN (Belbin 1989) was used to analyse the effectiveness data of the 48 accessions. The index data for each accession were range standardized ($S_i = (X_i - Min_i)/Range_i$, where S_i = standardized value, X_i = index value for $strain_i$, Min_i = smallest index value for entry, and $Range_i$ = difference between smallest and largest index values for the same entry) to prepare two data sets (39 x 19 and 17 x 8 accessions x strains). These were analysed with the Bray and Curtis (1957) association measure (ASO command of PATN) and the symmetric matrices thus obtained were classified by the hierarchical routine FUSE (UPGMA option). The routines GDEF and DEND were used to display group structure and relationship among groups and GSTA to determine which attributes (= strains) contributed most to the groups observed. In addition, routine MST (Minimal Spanning Tree) was used to display the dissimilarity of the responses between accessions, NNB (Nearest Neighbour), which uses the association measures, to determine proximity relationships, and BOND (linkage or bond strength) to indicate the strength of the relationship between nearest neighbours.

Inoculation responses — soil pot trial

Seed of accessions CPI38351, CPI78382, CPI79653, CPI92803 and TQ90 were inoculated separately with *Rhizobium* strains CB1397, CB3060, CB3126 and CB3128 and sown in a pot experiment with 2 types of soil. One soil was an alkaline clay loam (Ug 5.15, Northcote, 1979) from the QDPI 'Brian Pastures' Research Station (Gayndah) and the other an acidic red podzolic soil (Gn 3.14, Northcote, 1979) from Gympie. The pH (soil:water, 1:5) and percentage moisture at field capacity of these soils were pH 7.5 and 41% for the Gayndah soil and pH 4.9 and 34% for the Gympie soil. Basal nutrients were applied to both soils at rates (kg/ha) equivalent to 48 P, 12 S, 15 Ca, 2.4 Cu, 2.4 Zn and 0.18 Mo. An N control (150 kg/ha N in 6 split applications from day 45) and an uninoculated control were included as 'strain' treatments. There were 4 replications of each accession x strain treatment.

Soil from each site was air-dried, crushed to pass a 1 cm sieve and added to non-draining 15 cm diameter pots. Pots were continuously randomized, to avoid glasshouse effects, and watered (3 times/day) automatically (Andrew and Cowper 1973) to maintain the soil at close to field capacity.

Plant tops were dried and weighed on 3 occasions (63, 101 and 149 days after planting for the Gayndah soil; 66, 110 and 165 days for the Gympie soil). At the second and third harvests stems and leaflets were weighed separately. Replications 1 and 3, and 2 and 4 were combined for the determination of N concentration and the calculation of N content of leaflets. After the third harvest, soil was washed from the roots and nodulation assessed on a 1 to 5 ranking (Corbin *et al.* 1977) where 0 = no nodulation grading up to 5 = many nodules on both the tap and lateral roots.

Data were analysed in 2 ways: initially as a simple accession x strain (including the +N and −N controls) factorial for each soil and harvest, and later, as a soil x accession x strain factorial split for harvest time. Since soil accounted for the majority of the variance associated with dry weight of plant tops and N content of leaflets, harvest data were combined to give cumulative yields for each soil and analysed as accession x strain factorials in a completely randomized design. Strain rather than soil at harvest was the major contributor to variance of data for N concentration.

Results

Effectiveness responses

The effectiveness response patterns of the 17 strains of *Rhizobium* varied over the range of *Desmanthus* accessions tested. The number of strains forming effective (≥50% N control) associations varied from 0 to 16. Six response groups were recognized within the main data matrix of 39 accessions x 17 strains. The MST representation of the effectiveness responses showed a downward trend of decreasing number of strains forming effective associations, and an increasing dissimilarity between responses (Figure 1). Distances between accessions represent the degree of dissimilarity of the effectiveness responses. For example, the response of accession 20 is more closely related to that of 7 than it is to 9. The strength of the relationship between accessions is indicated in Figure 1 by superimposing the results of the NNB/BOND analyses on the MST (see caption Figure 1). Thus, accession 5 is more strongly bonded to 6 than it is to 12, even though the dissimilarity distance between the pairs is similar. The relationship among the 6 groups (Figure 2) indicated that the majority of accessions was closely related (Groups II, III, V). Group I had the greatest dissimilarity and formed the most effective associations.

The diagnostic strains (i.e. those contributing most to the grouping of accessions in Figures 1 and 2) were CB3128, CB3129, CB3130 (Cramer

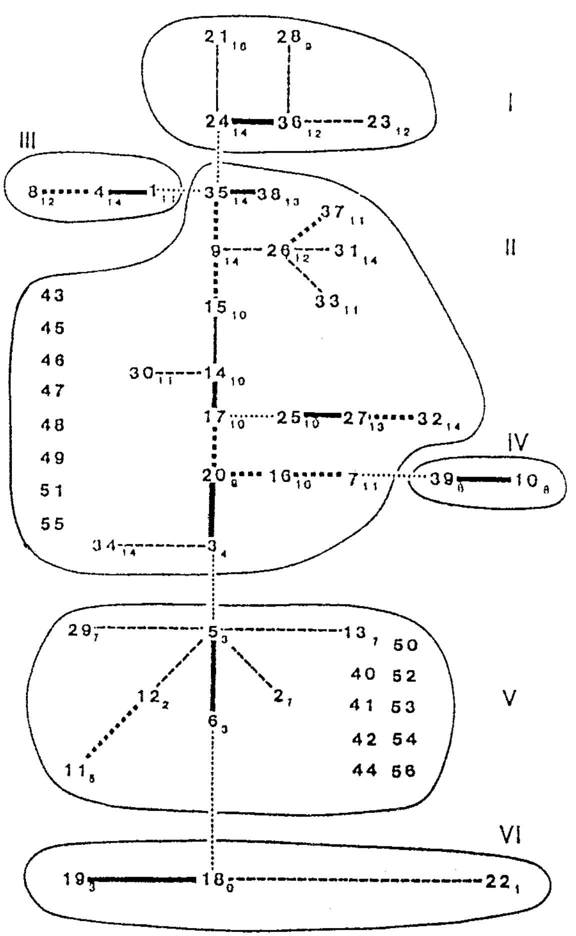

Figure 1. MST representation of effectiveness response patterns of 48 accessions of *Desmanthus*. Distances between accessions represent unrelatedness (= dissimilarity) between accessions. Numbers in bold type are accession IDs as listed in Table 1. The number of strains effective with each accession is indicated as a subscript to the accession ID. Groups I to VI are the response groups defined by NNB and BOND. Nearest neighbour BOND strengths between accessions are coded ▬▬▬, ——— ('strong'), ▬ ▬ ▬, – – – – ('intermediate') and ...... ('weak') in decreasing order.

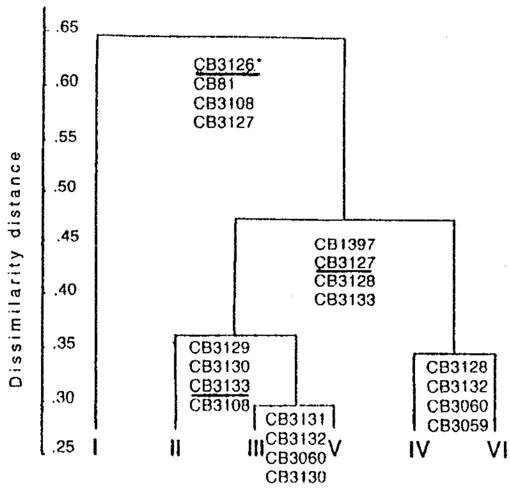

Figure 2. Dendogram of groups of *Desmanthus* accessions obtained when classed by DEND on basis of effectiveness response indices. * = diagnostic strains of *Rhizobium* in decreasing order of contribution to each dichotomy. Horizongal line indicates a significant break in contribution. Composition of groups is defined by Figure 1.

values 0.80–0.84) and CB1397, CB3131 and CB3132 (Cramer values 0.75–0.79). Strains contributing most to individual dichotomies are included on the dendrogram of Figure 2.

A similar distribution and grouping of accessions was obtained when all 48 accessions, plus standards and repeats (= total of 56) were analysed on the basis of their responses to only 6 of the strains. Effectiveness responses of the additional 17 accessions were most closely allied to those of groups II and V of the original analysis (see Figure 1).

Strains CB1397, CB3126, CB3128, CB3130 and CB3132 formed effective associations with most of the accessions, while CB81 and CB2001 formed only a limited number, but strain CB3058, even though forming nodules, failed to form any effective associations. Results for a selected accession from each effectiveness group plus the 5 accessions chosen by the CSIRO/QDPI working group as those with most agronomic potential, are recorded in Table 3.

In the analysis of variance of dry weight there were significant effects of host accession and strain of *Rhizobium* but no interaction between them. Mean dry weight indices for strains are included in Table 3.

An arbitrary grouping of accessions, based on the number of strains forming effective associations (effectiveness index ⩾50%), is provided in Table 4.

Table 3. Examples of *Rhizobium* screening of selected accessions of *Desmanthus*

Strain of *Rhizobium*	CPI Accession Number and (ID) of *Desmanthus* spp. 78385	78372	65947	92803[1]	78382[1]	38351[1]	79653[1]	81137	TQ90[1]	Mean top dry weight[2]	Number accessions effective[3]
	(Dry weights of whole plants as % of +N control)										
CB3058	15	3	4	13	9	7	4	11	4	7	0
CB3132	30	50	55	142	65	63	55	207	70	76	30
CB3133	53	8	17	144	90	74	71	80	83	53	19
CB3059	15	5	35	80	45	68	59	69	47	44	17
CB3060	12	10	66	144	74	78	40	110	72	57	19
CB3127	13	59	69	126	86	90	62	140	78	70	31
CB81	57	4	16	45	14	21	7	131	35	26	4
CB3126	10	32	28	100	54	39	21	143	41	82	30
CB3128	17	54	58	79	97	77	49	158	86	75	29
CB3129	11	18	104	144	68	134	62	100	83	63	24
CB3130	12	28	114	123	37	103	58	107	78	75	30
MS111	71	34	36	99	46	33	18	136	82	56	18
NGR8	11	40	46	23	61	79	61	120	71	61	25
CB3131	14	34	59	108	91	77	46	114	86	61	26
CB2001	7	53	44	45	57	37	9	84	43	37	9
CB1397	14	66	62	126	84	70	83	120	86	86	35
TAL600	12	70	35	117	73	33	53	98	9	66	23
−N	11	3	7	11	20	19	5	12	7	8	0
No. strains effective	3	6	8	13	12	11	9	16	11		
Response group	VI	V	IV	II	II	II	II	I	III		

[1] Accession chosen by QDPI/CSIRO *Desmanthus* working group.
[2] LSD between strain means = 10
[3] from 39 accession x 17 strain set

Table 4. An arbitrary grouping of 48 accessions (CPI Nos.) of *Desmanthus* based on an effectiveness index ⩾50%

Number of strains effective 0–3	4–6	7–9	10–12	13–17
37538	33201	30205	TQ90	37143
78373	78372	65949	38351	55719
78383		78379	40071	81337
78385		79653	78382	84960(3)
83570		90362(1)[1]	84508	85179
		90364(4)	85177	90751
			85178	90754
			90750	90816(2)
			90755	90857(5)
			91146	92803
			91326	
	67642	76052	78369	based on
	76053	78381	84991	only 6
	90914	85182		strains &
	92800	90311(2)		allocated
		91496		to group
		92805		according
		92817		to MST
				analysis

[1] (1) *D. brevipes*, (2) *D. covillei*, (3) *D. fruticosus*, (4) *D. illinoensis*, (5) *D. subulatus*, remainder *D. virgatus*

Inoculation responses — soil pot trial

There were significant effects of soil, harvest time, host accession, strain of *Rhizobium* and their interactions on plant dry weight. Within soils, dry weights of plant tops were similar for the 3 harvest periods, i.e. means of 6.9, 5.7 and 4.4 g/pot and 1.2, 1.6 and 1.3 g/pot, respectively, for harvests 1, 2 and 3 in the Gayndah and Gympie soils. Since soils accounted for 89% and the soil x harvest interaction only 3% of the total variance for dry weight the data were separated on the basis of soils and summed over harvests to give cumulative yields (harvest 1 + 2 + 3 for plant dry weight; harvest 2 + 3 for dry weight of leaflets and their N concentration and calculation of N content.

For both soils there were significant effects of host accession ($P<0.01$), strain of *Rhizobium* ($P<0.01$) and their interaction ($P<0.05$ Gayndah; $P<0.01$ Gympie) on dry weight of tops (Table 5) and N content of leaflets (Table 6). Dry weight yields of accessions CPI38351 and TQ90 generally were higher than for the other 3 accessions. Strains CB1397 and CB3126 generally were better than CB3060 and CB3128 in Gaydah soil

Table 5. Cumulative dry weight yield for 3 harvests for 5 accessions of *Desmanthus virgatus* in Gayndah (149 days) and Gympie (165 days) soils

Accession (CPI)	CB1397	CB3060	Strain of *Rhizobium* CB3126	CB3128	+N	−N
			(g/plant)			
Gayndah						
38351	21.6	19.2	21.0	21.0	21.7	16.8
78382	18.6	15.1	16.2	14.8	18.4	16.5
79653	13.0	11.4	14.5	12.7	15.0	9.4
92803	17.1	13.1	13.6	15.7	18.9	12.1
TQ90	22.2	18.9	19.5	21.1	21.4	20.4
LSD (P = 0.05)	Accession x strain = 2.7					
	Strain within accession = 1.2					
Gympie						
38351	7.5	5.6	7.9	7.0	13.1	2.4
78382	2.2	1.6	2.5	2.9	7.3	1.4
79653	2.0	2.1	1.4	2.5	8.1	1.5
92803	2.3	1.3	2.2	1.1	8.8	1.1
TQ90	2.8	3.6	4.8	2.5	11.5	1.6
LSD (P = 0.05)	Accession x strain = 1.9					
	Strain within accession = 0.9					

Table 6. Cumulative nitrogen content in leaflets for 5 accessions of *Desmanthus virgatus* in Gayndah (63 to 149 days) and Gympie (66 to 165 days) soils

Accession (CPI)	CB1397	CB3060	Strain of *Rhizobium* CB3126	CB3128	+N	−N
			(mg/pot)			
Gayndah						
38351	451	324	374	422	447	340
78382	395	269	320	242	349	320
79653	342	259	317	330	352	197
92803	394	260	314	342	490	183
TQ90	483	471	395	495	449	451
LSD (P = 0.05)	Accession x strain = 95					
	Strain within accession = 43					
Gympie						
38351	271	217	296	236	230	26
78382	74	47	75	96	220	25
79653	70	55	47	74	217	28
92803	70	26	50	20	315	22
TQ90	69	117	140	106	233	25
LSD (P = 0.05)	Accession x strain = 78					
	Strain within accession = 35					

but there were significant interactions with accession in Gympie soil. All strains were significantly ($P<0.05$) worse than the N control in the Gympie soil.

Similarly, the cumulative N content of leaflets of accessions CPI38351 and TQ90 was higher than that for the other accessions, but strain CB1397 was significantly poorer with TQ90 than with the other 3 strains (Table 6). The interaction effect between host and strain was more evident in the acid Gympie soil than in the alkaline Gayndah soil. The N concentration data were not analysed separately since soil and soil x harvest contributed <8% of the variance. Strain contributed 36% and there was a significant ($P<0.01$) soil x host x strain interaction (Table 7). There was less variation in N concentration of leaflets in Gayndah soil than in Gympie soil with most effect due to strains CB1397 and CB3126 and accessions CPI92803 and TQ90 in Gympie soil. Levels for uninoculated control were significantly less than those for strains in most cases and differences were much greater in Gympie soil.

Table 7. Nitrogen concentration in leaflets for 5 accessions of *Desmanthus virgatus* growth in Gayndah and Gympie soils

Accession (CPI)	Strain of *Rhizobium* CB1397	CB3060	CB3126	CB3128	+N	−N
			(%)			
Gayndah						
38351	3.31	3.02	3.01	3.30	3.00	3.00
78382	3.14	2.65	2.82	2.33	3.01	2.87
79653	3.49	3.01	3.08	3.27	3.11	2.55
92803	3.77	3.22	3.64	3.72	3.11	2.86
TQ90	3.13	3.15	2.87	3.18	2.79	2.89
Gympie						
38351	3.66	3.02	3.66	3.37	2.30	1.78
78382	3.21	3.11	2.91	2.60	3.28	2.19
79653	3.15	3.29	3.03	3.05	3.22	2.31
92803	3.58	3.09	3.18	2.07	3.78	2.28
TQ90	2.94	3.72	3.06	3.28	2.26	2.07

LSD ($P = 0.05$) Accession x strain = 0.45

Nodulation (nodule score) of all treatments in Gayndah soil was rated at either 4 or 5 (modified version of Corbin *et al.* 1977) except for the plus N controls in accessions CPI38351, CPI78382 and CPI79653 which were scored at 0 (= not nodulated). In Gympie soil, both the plus N and uninoculated controls were scored at 0 and the inoculated treatments at 2.5 to 4.

Discussion

Although a distinct grouping of accessions was observed, the relative similarity of effectiveness responses of the 48 accessions suggests that *Desmanthus* does not have a highly specific requirement for strain of *Rhizobium* for effective N-fixation. There was a tendency for accessions with more strains effective to have similar effectiveness response patterns. In general, these accessions originate from areas of < 1000 mm rainfall whereas those accessions effectively nodulated by only a few strains were from wetter areas. There were no relationships among effectiveness group characteristics and other provenance information (soil type, altitude, tropical or sub-tropical) as suggested for *Stylosanthes* (Date and Norris 1979). Four of the 5 most effective strains were also part of the group of 5 strains forming effective associations with 30 or more accessions. Only one (CB3132) of the 6 most broadly effective strains (CB1397, CB3126, CB3127, CB3128, CB3130, CB3132) originated from *Desmanthus*. However, it is perhaps significant that all 6 of the most effective/broadspectrum strains have provenances in central and northern South American countries which constitute the centre of diversity of *Desmanthus*. The effectiveness of these strains on a range of accessions of their host of origin (*Leucaena* and *Neptunia*) is not known although CB3126, CB3127, CB3128, CB3129 and CB3130 are effective on *L. leucocephala* cv. Cunningham (R.A. Date, unpublished data).

The inoculation response experiment clearly demonstrated the need for inoculation of *Desmanthus* in the acidic podzolic soil from Gympie and it is reasonable to assume that it would respond similarly in other acidic soils. The lack of nodules on the uninoculated control plants in the Gympie soil suggests that suitable rhizobia were either absent or very sparse. In contrast, plants in all treatments, in the clay soil from Gayndah, including the uninoculated control, were well nodulated. Cumulative yield was improved by inoculation but the proportional improvement was much less than in Gympie soil. The effect was more marked for N content of leaflets than it was for dry weight of tops.

Although there were significant differences among the N concentrations of the leaflets of the inoculated and +N treatments in the pot trial, the values were of a similar order in both soils. This suggests that, although growth was less in Gympie soil than in Gayndah soil (means of 1.4 and 5.7 g/pot), N supply was not the main factor limiting growth. Nodulation and growth of *Leucaena* in acid soils is improved by increasing P and Ca application (S. Ruaysoongnern, N. Brandon; personal communications). Since both *Leucaena* and *Desmanthus* have alkali soil origins and both are members of the Tribe Mimoseae, *Desmanthus* also may respond to P and Ca application when grown in acid soils. It is recommended that *Desmanthus* be inoculated in any new sowing. *Rhizobium* strain CB1397 is recommended as the best overall strain for the range of accessions but CB3126 is preferred for CPI38351 and TQ90 when sown in acid soil. Both strains have been made available to AIRCS (Australian Inoculants Research and Control Service) for distribution to Australian legume inoculant manufacturers. The fact that *D. virgatus* CPI38351 and TQ90 out-yielded the other accessions in both soil types suggests that these accessions may have more potential than CPI78382, CPI79653 and CPI92803 as browse plants especially in acid soils but this needs to be assessed together with other aspects in the series of trials being done by the CSIRO/QDPI *Desmanthus* working group.

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