Nutrient limitations of clay soils for *Desmanthus virgatus*. II. A glasshouse study of 7 soils

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Abstract

The potential for the nutrients P, S, Zn and Mo to limit growth of *Desmanthus virgatus* in clay soils from Queensland was investigated using nutrient-omission experiments in glasshouses located at Gatton (5 soils) and St Lucia (2 soils). Six of the soils were black earths and were neutral to alkaline (pH 7–8). The seventh soil was a euchrozem and was slightly acid (pH 6). The effects of nutrient deficiencies were determined by measuring top growth 47 and 103 days after planting in the Gatton trial and 73 days after planting in the St Lucia trial, as well as by the observation of deficiency symptoms.

In the first harvest of the Gatton trial, omission of P or Zn significantly ($P < 0.05$) reduced top dry weight of plants in 3 of the soils by 32–66% relative to plants fertilised with all nutrients. Responses to these nutrients decreased, and were generally not significant, at the second harvest. This was in contrast to the effect of S omission, which increased with time and was significant in 4 of the 5 soils at the second harvest, reducing top growth by 24–75% and drastically reducing pod production in 3 of the soils. A small reduction in growth in one soil also occurred due to the omission of Mo.

In the St Lucia trial, omission of Mo and Zn, as well as S and P, caused significant reductions in plant growth in a slightly acid euchrozem soil . Low pH appeared to reduce the availability of some nutrients in this soil and the application of lime increased growth in both the presence and the absence of added nutrients by 20% and 120%, respectively.

Soil and tissue S concentrations were useful indicators of S deficiency. The greatest effect of S deficiency occurred in soils with KCl-extractable S < 4 mg/kg. Concentrations of P in soil and tissue and of Mo in tissue were less useful in predicting deficiencies of these nutrients. The extent of these nutrient deficiencies needs to be verified by field experimentation.

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Introduction

Desmanthus virgatus is a summer-growing perennial legume introduced from central America for use in clay soils in tropical and subtropical areas of Queensland, Australia. This species is best suited to medium-heavy textured clay soils with slightly acid to alkaline pH (pH > 6.5), and is currently the only tropical herbaceous legume available for use in long-term pastures in these soils.

Chlorosis of desmanthus has been observed in plants grown for seed production at the Walkamin Research Station, north Queensland (J. Hopkinson, personal communication). Symptoms included yellowing of young and old leaves, stunting and, in severest cases, plant death. Application of lime, S and Mo overcame the problem in subsequent plantings. This soil is slightly acid (pH 6.0) and is atypical of many soils in which desmanthus would normally be grown. However, similar symptoms had also been observed in desmanthus growing in a neutral clay soil at Brian Pastures Research Station near Gayndah, Queensland and were related to deficiencies of S and Mo (Brandon and Date 1998). The aim of the research reported here was to determine the importance of a range of nutrient deficiencies in a wider range of soils.

Materials and methods

Soils and treatments

The soil collected from the QDPI Walkamin Research Station was a euchrozem and slightly acid (pH 6.0). The remaining 6 soils were all black earths and were neutral to alkaline (pH 7.0–8.0). Two were collected from beneath pasture at QDPI research stations (Brian Pastures Research Station and Emerald Research Station) and 4 from private properties under either cultivation (Bauhinia and Wandoan) or pasture (Clermont and Theodore). Chemical properties for each soil are given in Table 2.

Treatments applied to each soil were: (i) a complete nutrient solution (referred to as "ALL") consisting of P, K, S, Fe, Mg, Zn, Mo, B and Cu at rates shown in Table 1; (ii) the complete nutrient solution with the omission of P, S, Mo or Zn; and (iii) a control treatment (no nutrients added). Two further treatments in the Walkamin soil were lime in the presence of all nutrients (ALL+Lime) and lime alone (+Lime).

Table 1. Rates of application and compounds used in a nutrient-omission trial with desmanthus.

Nutrient	Nutrient source	Nutrient rate	Nutrient element
		(kg/ha)	(mg/pot)
P	$CaH_4(PO_4)_2.H_2O$	50	66.5
K[1]	K_2SO_4	60	80
	KCl	30	40
	$KHCO_3$	30	40
Mg[1]	$MgSO_4$	45	60
	$MgCl_2$	30	40
Fe	Fe EDTA	10	13.3
Cu	$CuCl_2$	5	6.65
Zn	$ZnCl_2$	10	13.3
Mo	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.5	0.665
B	H_3BO_3	2	2.66

[1]Used for all treatments except the -S treatment where the alternative sources of K ($KCl+KHCO_3$) and Mg ($MgCl_2$) were used. Thus, rate of S was equivalent to 84.6 kg/ha. Two additional treatments in the Walkamin soil were lime applied at a rate of 6.3g/pot in the presence of all nutrients (+ALL + Lime) or in the absence of all nutrients (-ALL + Lime).

Soil preparation, planting and nutrient addition

Plastic pots, 13 cm in diameter and lined with plastic bags, were filled with air-dry soil at a rate of 1.2–1.4 kg air dry soil/pot depending on soil type. Lime was mixed through the soil at a rate of 6.3g/pot (equivalent to 4.8t/ha on a pot area basis) in the 2 additional treatments in the Walkamin soil. Seed of *Desmanthus virgatus* cv. Marc was scarified in concentrated sulphuric acid for 15 minutes and washed 5 times in water before being spread on water agar in petri-dishes to germinate. Seed coats were removed following germination. Eight seeds were planted in each pot on February 16, 1996. Pots were placed in a glasshouse at the University of Queensland Gatton campus. Following planting, pots were inoculated with *Rhizobium* strain CB3126 at a rate of 0.01g commercial peat inoculum in a water slurry.

Nutrients were applied in 5–20 ml of solution to the surface of the pots, 2 weeks after planting. The time of nutrient application corresponded with a period of hot, dry weather resulting in leaf-burn of some seedlings. Most severely affected were those in the Walkamin and Clermont soils. Pots containing these soils were replanted and relocated to a glasshouse at the CSIRO Cunningham Laboratory where temperatures were 5–10°C lower. These 2 soils were then treated as a separate experiment.

Management — Gatton trial

The experiment at Gatton was arranged in a randomised complete block design with 4 replications. Using de-ionised water, pots were watered, at least once daily, to field capacity as determined using a pressure plate set at 0.3 bars. Desmanthus plants were thinned to 4 per pot, 3 weeks after planting. The treatments within replications were re-randomised at 2-week intervals.

Table 2. Chemical properties of 7 soils used in nutrient-omission experiments with desmanthus.

Soil	pH[1]	EC	Org.C[5]	CEC[5]	P (Bicarb)[2]	S (KCl)[3]	S (MCP)[4]	Zn (DTPA)[5]
		(mS/cm)	(%)	(meq%)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Gayndah	7.0	0.3	1.6	40	84	3	1	1.3
Emerald	7.3	0.1	1.2	69	8	2	1	0.5
Clermont	7.9	0.1	1.0	67	7	3	1	0.3
Theodore	7.7	0.1	1.6	65	7	3	1	0.2
Wandoan	7.1	0.2	0.9	24	6	8	2	0.1
Bauhinia	8.0	0.2	1.5	38	35	15	13	0.6
Walkamin	6.0	0.1	1.4	10	40	2	1	0.9

[1]Soil pH was measured in 1:5 H_2O.
[2]Bicarbonate extraction using 0.5 M $NaHCO_3$.
[3]KCl extraction using 3:20 0.25 M KCl at 40°C (Blair *et al.* 1991).
[4]MCP extraction using 1:5 0.01M $Ca(H_2PO_4)_2$.
[5]Standard methods used by the DPI soil testing service.

Plant tops were harvested by cutting between the second- and third-lowest nodes, 47 days after planting (Harvest 1) and allowed to regrow for a further 56 days before being reharvested in the same way (Harvest 2). Shoot material at each harvest was divided into leaf, stem and pods and dried to constant weight at 70°C. Observations for plant yellowing and leaf abscission were made 13, 25, 30, 46, 69, 88 and 103 days after planting. Plant colour was rated on a scale of 1 to 5 with 5 representing severe yellowing associated with leaf abscission.

Following the second harvest, nodules were recovered from the ALL treatment and tested to determine the proportion of nodules due to the inoculum strain using the indirect fluorescence technique with fluorscein isothiocyanate (FITC)-labelled antiserum (Somasegaran and Hoben 1985).

Management — St Lucia trial

Following replanting on March 17, the pots containing the euchrozem soil from Walkamin and the black earth soil from Clermont were transferred to St Lucia where they were placed on an automatic watering machine (Andrew and Cowper 1973) which rotated the pots around the glasshouse and watered them to 90% field capacity, 3 times a day. Ratings for yellowing and leaf abscission were taken at 41, 55 and 73 days after planting. Shoot material, harvested 73 days after planting, was divided into leaf, stem and pod. Ratings for yellowing were as described for the Gatton trial.

Plant chemical analysis

Leaf tissue of selected treatments harvested 103 days after planting at Gatton (Harvest 2) and 73 days after planting at St Lucia was analysed for: N and P using Kjeldahl digestion followed by colorimetric analysis; Mo using an inductively coupled emission spectrophotometer; and Cl using titrimetry (Greenberg *et al.* 1992) Plant samples for the limed and unlimed treatments in the ALL and unfertilised treatments in the Walkamin soil were also analysed for Mn, Al, Ca, Mg, K, Cu, B and Zn using inductively coupled mass and emission spectrophotometers. To reduce costs, while still allowing valid statistical comparison, ground leaf material from Replications 1 and 3 and 2 and 4 were combined prior to analysis.

Statistical analysis

Yield and nutrient data for the Gatton and St Lucia trials were analysed separately. Yield data for Harvests 1 and 2 of the Gatton trial were analysed using analysis of variance for a randomised block design. Yield data for the St Lucia trial were analysed using analysis of variance for a completely randomised design. The effect of the 2 extra lime treatments in the Walkamin soil of the St Lucia trial was analysed using data for this soil only. In all cases, treatment means were compared using least significant differences.

Results

Dry matter responses — Gatton

At the first harvest, the omission of P or Zn significantly ($P<0.05$) reduced top dry weight of desmanthus by 48–66% relative to the ALL treatment in the Theodore and Bauhinia soils and 32–49% in the Wandoan soil (Figure 1).

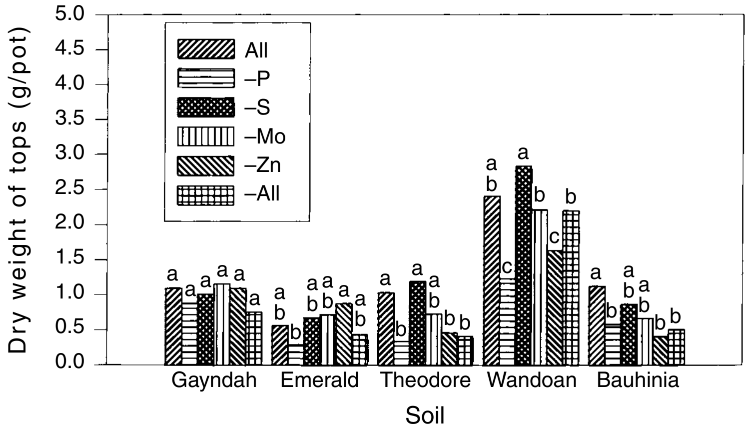
At the second harvest, growth of plant tops in the Theodore soil only was limited by P omission which resulted in a 38% reduction in top dry weight relative to the ALL treatment (Figure 2). Omission of S significantly ($P<0.05$) reduced top weight in the Gayndah, Emerald, Theodore and Wandoan soils by 24–74% relative to the ALL treatment and effectively eliminated seed set in the Gayndah, Emerald and Theodore soils (data not presented). The omission of Mo significantly reduced ($P<0.05$) dry weight of tops in the second harvest in the Emerald soil by 38%.

Dry matter responses — St Lucia

The omission of P, S, or Mo significantly ($P<0.05$) reduced dry weight of tops in the Clermont soil by 60, 30 and 24%, respectively (Figure 3). Omission of P, S, Mo and Zn significantly reduced growth of desmanthus in the Walkamin soil by 27, 47, 51 and 26%, respectively (Figure 3). There was a significant ($P<0.05$) response to lime in total dry weights in both the presence and the absence of other nutrients (Figure 3).

Plant colour

Most severe symptoms of yellowing (*i.e.* rating of >3) were observed when S was omitted from the Gayndah soil and in the control treatment at Emerald. Yellowing also was severe in the



Walkamin soil when Mo was omitted (data not presented).

Figure 1. Dry weight of desmanthus tops in Harvest 1 of the Gatton trial. Treatments associated with the same letter within soils are not significantly different (P>0.05).

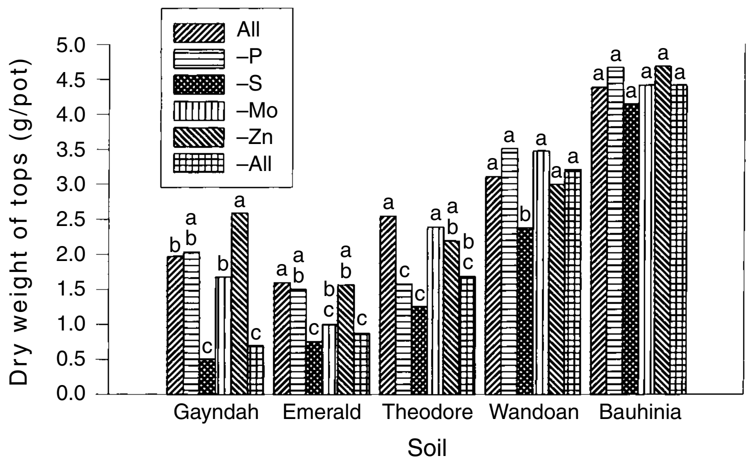


Figure 2. Dry weight of desmanthus tops in Harvest 2 of the Gatton trial. Treatments associated with the same letter within soils are not significantly different (P>0.05).

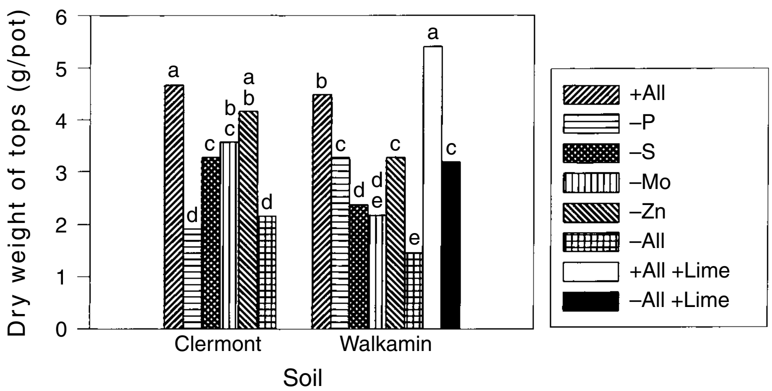


Figure 3. Dry weight of desmanthus tops for Clermont and Walkamin soils for the St Lucia trial. Treatments associated with the same letter within soils are not significantly different (P>0.05).

Nutrient analysis

Omission of S reduced the concentration of S in leaves of plants growing in all soils (Table 3). Phosphorus concentrations were reduced by omission of P in all but the Bauhinia and Gayndah soils, which were high in available P (Table 2). Nitrogen concentrations were reduced by omission of S in all but the Walkamin soil and by omission of Mo in the Walkamin and Emerald soils (Table 3). Application of lime in the Walkamin soil increased S and P concentrations in unfertilised plants and Mo concentration in the fertilised treatment. Chloride concentrations were less than 0.7% in all soils (data not presented).

Nodulation

Plant roots of desmanthus grown in the ALL treatment in each of the 7 soils were well nodulated. The proportion of nodules due to the inoculum strain CB3126 in 6 of the soils ranged from 75–98%. The proportion of nodules due to the inoculum in the remaining soil (Theodore) was 15%.

Discussion

Sulphur

Sulphur was the most widespread deficiency identified in the trial. KCl-extractable S was lower than the critical concentration of 6.5 mg/kg determined by Blair *et al.* (1991) in 5 of the soils, and MCP-extractable S was lower than the critical concentration of 4 mg/kg determined by Probert and Jones (1977) in 6 of the soils. Tissue concentrations were also lower than the 0.2% S suggested as critical for desmanthus by Brandon and Date (1998). However, concentrations measured in the 2 non-responsive soils (0.17–0.19%) were also low, perhaps as a result of nutrient depletion in the pots. This is consistent with the finding that concentrations in plants grown in the Gatton trial (14 weeks total

Table 3. Concentration of nutrients in leaflets of desmanthus grown in various nutrient-omission treatments in 7 soils. Values on the same line followed by letters that are the same are not significantly different (P>0.05).

Experiment location	Soil	Sulphur		Phosphorus		Nitrogen			
		All	-S	All	-P	All	-S	-Mo	Nil
		(%)		(%)		(%)			
Gatton	Gayndah	0.32a	0.13b	0.22a	0.21a	2.6a	2.0b	2.4a	2.0b
	Emerald	0.28a	0.13b	0.18a	0.13b	2.2a	1.7b	1.7b	1.8b
	Theodore	0.35a	0.15b	0.16a	0.12b	2.6a	2.1b	2.4a	1.9b
	Wandoan	0.40a	0.17b	0.23a	0.18b	2.5a	2.1b	2.4a	2.0b
	Bauhinia	0.27a	0.19b	0.18a	0.15a	2.1a	1.8b	2.3a	1.7b
St Lucia	Clermont	0.40a	0.17b	0.20a	0.16b	2.8a	2.4b	2.6a	2.4b
	Walkamin	0.46a	0.19b	0.19a	0.17b	2.8a	2.7a	2.0b	2.2b

duration) were generally lower than those found in the St Lucia trial (10 weeks total duration).

Although available S may be increased by the mineralisation associated with the preparation of soil for pot trials, S responses by legumes in glasshouse experiments are quite common (Standley *et al.* 1990). Jones (1970) found that responses to S by lucerne, grown in surface soil in pots, did not always correlate with responses in the field, because high concentrations of S in subsoil layers of the black earth profile often compensated for low concentrations in the surface of the profile. However, in soils such as that at Gayndah which have low subsoil S (Prinsen *et al*. 1992), a correlation might be expected. Application of S at this field site reduced chlorosis and increased tissue S levels in leaves from 0.1–0.4% in established desmanthus (authors, unpublished data).

Application of S has also been found necessary to prevent chlorosis of desmanthus at the QDPI Walkamin Research Station (J. Hopkinson, personal communication). This soil is low in S and desmanthus responded significantly to application of basal nutrients, including S, in a pot trial (M. Gilbert, personal communication). This has been confirmed in the field, where concentrations of S measured in the cultivar Uman at Walkamin of 0.07% (J.Hopkinson, personal communication), in the absence of fertiliser, were lower than critical concentrations suggested by Brandon and Date (1998) for desmanthus or by Andrew (1977) for a range of tropical and temperate legumes.

Since desmanthus was the only species used in the current trials, it is not known whether the S requirement of desmanthus is higher than that of other legumes. Application of S at the Walkamin site appeared to stimulate growth of many other legumes growing as weeds in desmanthus seed production areas (J.Hopkinson, personal communication), suggesting a general deficiency of this nutrient. Similarly, at the Gayndah site, a response to S has been observed not only in desmanthus, but also in the tree legume *Leucaena leucocephala* (Prinsen *et al*. 1992).

However, the pot trial data, along with field observations, suggest that S deficiency is a factor affecting the growth of desmanthus and can result in symptoms of chlorosis. Sulphur deficiency may also limit the plant's ability to persist in pastures through seedling recruitment as omission of S effectively prevented production of pods in the Emerald, Gayndah and Theodore soils. S-deficient plants at Gayndah also had lower concentrations of N, probably as a result of reduced nodule number (Brandon and Date 1998) and synthesis of proteins (Marschner 1986).

Least affected by S deficiency were the plants in the 2 soils used previously for cropping (Bauhinia and Wandoan). Both soils had KCl-extractable S concentrations in surface layers higher than those considered critical by Blair *et al*. (1991). The Wandoan soil, however, had an MCP-extractable S level of 2 mg/kg, which was less than that considered critical by Probert and Jones (1977). Blair *et al.* (1991) suggested that KCl extraction removes more S at intermediate S ranges than MCP extraction and that this can sometimes result in better correlations with plant-available S than results using the MCP method of extraction.

Phosphorus and zinc

Although P and Zn limited early growth of desmanthus in some of the soils, there was generally no effect of the omission of these nutrients at final harvest. Although not measured in this trial, increased infection of roots by VAM fungi may have contributed to the reduced response to P in the second harvest of the Gatton trial. Extraction of P and Zn by plant roots is known to be increased by infection with vesicular arbuscular mycorrhizal (VAM) fungi (Jehne 1980). Hyphae of these fungi become intimately associated with root cells and also permeate the surrounding soil, increasing the absorption of some nutrients, particularly P and Zn. Brandon *et al.* (1997) found that low infection by VAM fungi reduced growth of the legume *Leucaena leucocephala* even in soils with moderate levels of available phosphorus. This may explain the early (Harvest 1) response to P in the Bauhinia soil (35 mg/kg bicarbonate-extractable P), and the response to P in the Walkamin soil (40 mg/kg bicarbonate-extractable P).

The effect of P omission remained significant in the Theodore soil in the second harvest. This soil was low in available P (7 mg/kg P), and had the lowest tissue P levels at the final harvest (0.12%). However, final tissue concentrations in a number of soils were low (<0.15%), perhaps reflecting low available P levels (4 soils with <9 mg/kg P) and nutrient depletion caused by limited soil volume in pots. The large response to P in the euchrozem soil, in which measured levels of available P were high (40 mg/kg bicarbonate-extractable P), can be explained by its ability to fix phosphorus in forms unavailable to plants due to high levels of hydrous oxides of Fe and Al. Similar results were found by Kerridge *et al.* (1972) using the legume *Desmodium intortum* grown in pots in 20 soils collected from the Atherton Tableland (1–36 mg/kg P measured following extraction with H_2SO_4).

It is difficult to predict the importance of P limitations in the field from results of this trial, because of the likely role of VAM infection on P nutrition of desmanthus. Significant responses, however, appear most likely in soils low in P (<9 mg/kg bicarbonate-extractable P), or very low in VAM fungi as can occur following long periods of fallow (Thompson 1987).

Molybdenum

Molybdenum was a significant limitation to plant growth in the Walkamin and Emerald soils. However, concentrations of Mo in plant tissue were extremely variable and not useful in predicting responsive soils.

Mo availability is decreased by low pH and by specific absorption in highly weathered soils. The response to Mo in the Walkamin soil, therefore, is consistent with results of Kerridge *et al.* (1972) for other soils from the Atherton Tableland. Less expected was the response to Mo in the Emerald soil, which was slightly alkaline in pH and probably indicates low absolute amounts in the soil rather than low availability. Similar limitations of Mo to legume growth in clay soils have been found by Standley *et al.* (1990) and Brandon and Date (1998).

Liming

Application of lime increased growth in the Walkamin soil in both the presence and the absence of other applied nutrients. Analysis of leaf tissue of plants growing in these treatments showed that S and P were increased by application of lime to unfertilised plants. The only nutrient increased by lime application in plants fertilised with ALL nutrients was Mo. Similar effects of liming on Mo concentration in euchrozem soils were reported by Kerridge (1978). The positive response to lime, therefore, may relate to improved nutrient availability. It did not appear to be related to Ca as concentration of Ca was similar in both limed and unlimed plants, in both the presence and the absence of other nutrients. It also did not appear to be due to Al or Mn toxicity as concentrations of both these nutrients were similarly low in both treatments.

Conclusion

Results of this pot trial, and associated field observations, suggest that nutrient availability may be a significant limitation to growth of desmanthus in some clay soils. Deficiencies of S are most likely in soils with extractable S levels of less than 6.5 mg/kg as determined using the KCl extraction method. High levels of subsoil S, however, may compensate for low surface soil levels in some soils in field-grown plants.

Poor growth and chlorosis of desmanthus growing in the Walkamin soil appeared to be due to S, Mo and P deficiency. Application of these nutrients in the field has been shown to prevent chlorosis of desmanthus in seed crops.

More work needs to be done in the field to evaluate responses of desmanthus to nutrients such as S, Mo and P in neutral to alkaline clay soils.

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