Nutrient limitations of clay soils for *Desmanthus virgatus*. I. What is the cause of chlorosis in field-grown desmanthus on a black earth soil?

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Abstract

Nutrient-omission and addition trials, in pots, were used to determine whether nutrient deficiencies were responsible for stunting and chlorosis in stands of *Desmanthus virgatus* growing in a black earth soil at Gayndah in south-east Queensland. After 11-weeks growth, omission of S or Mo, relative to a complete treatment, reduced yield by 55% and 42%, respectively, in inoculated plants and 92% and 14%, respectively, in N-fertilised plants. Omission of P, Cu or Mn reduced growth of inoculated plants by 8–24% but had no significant effect on yield of N-fertilised plants.

The combined addition of P, S, Mo, K, Ca, Mg, Zn, Cu, Mn and B to inoculated plants almost doubled yield of plant tops 11 weeks after planting, but there was no significant yield response when P, S or Mo was applied alone.

The results suggest that S, Mo and possibly P, Cu and Mn may contribute to the development of chlorosis and low yield of inoculated desmanthus at Gayndah. A critical S concentration in leaf tissue of 0.20% was determined in an S-rate trial.

Introduction

Desmanthus virgatus is a shrubby tropical pasture legume introduced from central America for use in clay soils in southern and central Queensland as a component of permanent pasture. Although it is well adapted and persistent in a number of clay soils (Cook *et al.* 1993), symptoms of chlorosis have been observed in old stands of desmanthus planted at the Brian Pastures Research Station of the Queensland Department of Primary Industries (R.L. Clem, personal communication).

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Inoculation with an improved *Rhizobium* strain CB3126 reduced chlorosis and increased growth of desmanthus in this soil (Bahnisch *et al.* 1998). Therefore, poor nodulation may have contributed to the low productivity of desmanthus in a grazing trial at this site which was planted prior to the commercialisation of this inoculum strain (Burrows and Porter 1993). However, chlorosis was observed in plants well nodulated by strain CB3126 in more recent work by Brandon *et al.* (1998) and Bahnisch *et al.* (1998), suggesting nutrient deficiencies other than N.

The 3 glasshouse experiments reported here examined the nutritional responses of desmanthus growing in soil from Brian Pastures.

Materials and methods

The soil used for all experiments was collected from the top 10–15 cm of a black earth derived from basalt at the Brian Pastures Research Station (25°39′S, 157°42′E) near Gayndah in Queensland. The surface soil was moderate to high in most nutrients except sulphur (Baker and Eldershaw 1993) (Table 1).

Table 1. Soil analysis results for the Brian Pastures soil used in Experiments 1–3.

Measurement	Method[1]	Result
pH	1:5 H_2O	7.0
EC (mS/cm)	1:5 H_2O	0.3
Cl (mg/kg)	1:5 H_2O	69.0
Organic carbon (%)		1.6
P-Bicarb (mg/kg)	1:100 0.5M $NaHCO_3$	84.0
N- Total (%)	Kjeldahl digest	0.12
K (meq/100g)	1M NH_4Cl pH 7	1.5
Ca (meq/100g)	1M NH_4Cl pH 7	29.0
Mg (meq/100g)	1M NH_4Cl pH 7	8.9
S-KCl (mg/kg)	3:20 0.25M KCl[2]	3.0
S-MCP (mg/kg)	1:5 0.01M $Ca(H_2PO_4)_2$	1.0
Cu (mg/kg)	0.005M DTPA	2.8
Zn (mg/kg)	0.005M DTPA	1.3
Mn (mg/kg)	0.005M DTPA	13.0
B (mg/kg)	0.01M $CaCl_2$	<0.10

[1]All methods except the KCl extraction of S were standard soil analysis procedures used by the Department of Primary Industries Agricultural Chemistry Branch.
[2]The KCl method is described by Blair *et al.* (1991).

Experiment 1 — Nutrient-addition experiment

There were 8 nutrient combinations (see Table 2 for rates and forms of nutrients applied) including a complete nutrient treatment (referred to as "ALL") and 2 control (Nil) treatments, 1 with and 1 without applied N. The experiment was replicated twice and arranged in a randomised block design in a glasshouse located at the CSIRO Cunningham Laboratory in Brisbane.

Table 2. Nutrient treatments and top dry weight of *Rhizobium*-inoculated desmanthus after 11-weeks growth relative to the All treatment (5.7 g) in a soil from Brian Pastures (Experiment 1).

Treatment	Nutrients	Rate[1]	Form	Dry weight	Colour rating[2]
		(kg/ha)		(%)	
1	P	100	Na_2HPO_4	67	4
2	S	60	Na_2SO_4	58	4
3	Mo	0.2	$(NH_4)_6Mo_7O_{24}$	54	3
4	K	60	KCl		
	Ca	100	$CaCl_2$	19	1
	Mg	25	$MgCl_2$		
5	Zn	2.5	$ZnSO_4$		
	Cu	2	$CuSO_4$	79	4
	Mn	2	$MnSO_4$		
	B	0.6	H_3BO_3		
6	ALL[3]			100	5
7	Control			53	2
8	Control (+N)	50	NH_4NO_3	53	1
LSD (P=0.05)				14	

[1]1 kg/ha was equivalent to 1.3 mg/pot.

[2]Colour was rated subjectively on a scale from 1 (yellow) to 5 (green).

[3]The ALL treatment included all nutrients in Treatments 1–5, applied at rates listed for Treatments 1–5.

Seed of *Desmanthus virgatus* cv. Marc (CPI 78373) was acid-scarified, pre-germinated on 1% water agar and planted into plastic-lined pots containing 1.2 kg air-dried soil at a rate of 8 seedlings/pot on April 6, 1994. Seedlings were inoculated with *Rhizobium* strain CB3126 in a peat-water suspension at a rate of 0.1 g peat/pot at the time of planting. Pots were watered daily to 90% field capacity (determined gravimetrically from a free-draining column of soil). Nutrients were applied to the surface of the soil in 10 ml of solution, 6 days after planting.

Plant tops were harvested 11 weeks after planting, and chlorosis rated subjectively on a 1–5 scale (1=yellow, 5=green). Plant material was dried and weighed and leaflets removed from Treatments 4, 6 and 7. These were analysed for: nitrogen using colorimetry following Kjeldahl digestion; sulphur using an inductively coupled plasma emission spectrophotometer; and chloride using titrimetry (Greenberg *et al.* 1992).

Experiment 2 — Nutrient-omission experiment

Nutrient combinations examining the effects of omission of P, S, Mo, Zn, Cu, Mn and B were applied to desmanthus in either the presence or the absence of inorganic N. Plants not fertilised with inorganic N were inoculated with *Rhizobium* strain CB3126. The N-fertilised and inoculated treatments were placed on separate benches in a glasshouse and were analysed as separate experiments. Each experiment was arranged in a completely randomised design. There were 4 replications.

Planting details were the same as for Experiment 1. Nutrients were applied in solution, 1 week after planting. Nutrient application rates and forms for the complete (ALL) treatment are given in Table 3. The other treatments were the same except for the omission of the treatment nutrient. Plants in the N-fertilised treatments received N (as NH_4NO_3 at 50 kg/ha N) 1, 5 and 9 weeks after planting.

Table 3. Top dry weight of inoculated and N-fertilised desmanthus relative to the ALL treatment (Experiment 2). Absolute weights of the ALL treatment were 5.9 g and 3.7 g for Harvests 1 and 2, respectively, in the inoculated treatment and 8.0 g and 4.7 g in the N-fertilised treatment.

	Inoculated		Nitrogen-fertilised	
Treatment	Harvest 1	Harvest 2	Harvest 1	Harvest 2
		(%)		
ALL[1]	100	100	100	100
-P	88	92	99	100
-S	53	30	24	6
-Mo	59	62	85	87
-Zn	97	97	98	96
-Cu	95	76	103	104
-Mn	86	78	103	102
-B	95	89	98	98
LSD (P=0.05)	10	16	11	6

[1]Nutrients in the ALL treatment were applied at the following rates (kg/ha) using the corresponding forms: 100 P [$CaH_4(PO_4)$], 60 K (KCl), 60 S ($MgSO_4$), 10 Zn ($ZnCl_2$), 5 Cu ($CuCl_2$), 5 Mn ($MnCl_2$), 2 B (H_3BO_3) and 0.5 Mo [$(NH_4)_6Mo_7O_{24}$]. (1 kg/ha was equivalent to 1.3 mg/pot).

Plant tops were harvested 7 weeks after planting, by clipping plants between the second- and third-lowest nodes and then allowing them to

regrow for a further 4 weeks before harvesting them again in the same way. Plant tops were dried at 60°C and weighed. Leaves of plants in the ALL and -S treatments were analysed for N and S as described previously.

Experiment 3 —Sulphur-rate experiment

Five rates of S (0, 5, 15, 30 and 60 kg/ha S) were applied as K_2SO_4 to 2 soils, the Brian Pastures soil (3.0 mg/kg KCl-extractable S) as used in Experiments 1 and 2 and a soil collected from Emerald (2.0 mg/kg KCl-extractable S). Sodium molybdate and KH_2PO_4 were applied to all pots (32 kg/ha P, 40 kg/ha K and 0.5 kg/ha Mo) to ensure that these nutrients were not limiting in either soil. Soil preparation, planting method, inoculation with *Rhizobium* and post-planting management were all similar to those described previously. There were 4 replications arranged in a completely randomised design.

Plant tops from each pot were harvested by clipping between the second- and third-lowest nodes 7 weeks after planting and again at 13 weeks. Nodule numbers and weights were estimated from the roots of 2 replications of the highest and lowest rates of S in the Brian Pastures soil. Plant tops were dried and weighed. Leaves were then removed from the dried plant tops and combined across replications. Sulphur and N concentrations were determined as previously described.

Results

Experiment 1 —Nutrient-addition experiment

Dry weight. Best growth occurred for the ALL treatment with plant growth almost double that of the control treatments (Table 2). Using the ALL treatment as the standard (100%), the next highest treatments were +(Zn, Cu, Mn and B) (79%) and +P (67%). Poorest growth occurred in the +(K, Ca and Mg) treatment (19%) which was less than the control treatment (53%).

Chlorosis. Severity of chlorosis was reduced by application of P, S and Mo and the combined application of +(Zn, Cu, Mn and B) (which had also supplied 2.4 kg/ha S). Chlorosis was increased by application of +(K, Ca and Mg) as chlorides and by application of N.

Tissue nutrient concentration. The leaves of the control treatment (unfertilised with N) contained 2.4% N and 0.13% S, compared with 3.0% N and 0.36% S in the ALL treatment. Chloride concentration in leaf tissue of plants in the +(K, Ca and Mg) treatment was 0.85% compared with 0.52% in the ALL treatment and 0.54% in the control treatment (unfertilised with N).

Experiment 2 —Nutrient-omission experiment

Dry weight. At Harvest 1, omission of S significantly ($P<0.05$) reduced top growth of inoculated plants by 47% and N-fertilised plants by 76% (Table 3). Omission of Mo significantly ($P<0.05$) reduced plant growth by 41% in inoculated plants and by 15% in N-fertilised plants. Omission of P or Mn caused small but significant reduction in growth of inoculated plants (12% and 14%, respectively) but had no effects on N-fertilised plants (Table 3).

Similar results were found for Harvest 2, although effects of S deficiency tended to increase for both inoculated and N-fertilised plants and omission of Cu significantly ($P<0.05$) reduced growth of inoculated desmanthus by 24%.

Plant tissue analysis. Leaves of inoculated desmanthus in the ALL treatment contained 3.3% N and 0.48% S compared with 2.6% N and 0.17% S for the -S treatment.

Experiment 3 —Sulphur-rate experiment

Tissue nutrient analysis and plant growth. A curvi-linear relationship was found between leaf tissue S concentration and relative yield of plant tops harvested 13 weeks after planting (Figure 1). Near-maximum yield (90%) was achieved in both soils at a tissue S concentration in leaves of approximately 0.20% and an N:S ratio of 14. This occurred at an application rate of between 15 and 30 kg/ha S. Nitrogen concentration in leaf tissue increased from 1.8–1.9% in plants not fertilised with S to 3.3–3.4% in plants fertilised with S at 60 kg/ha S.

Nodulation. Nodule number and weight/pot increased from 270 and 58 mg, respectively, without applied S to 490 and 187 mg at 60 kg/ha S.

Discussion

Several nutrients limited growth of desmanthus in the black earth soil from Brian Pastures, the largest responses being obtained to S and Mo.

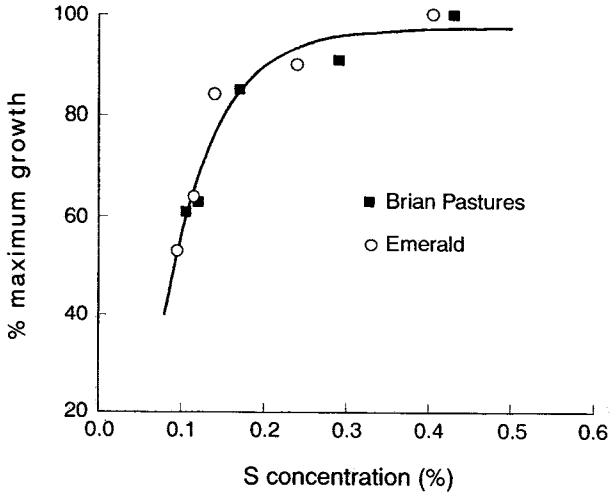

Figure 1. Relationship between plant growth expressed as a percentage of that achieved at 60 kg/ha S and S concentration measured in leaves of desmanthus grown at sulphur rates of 0–60 kg/ha S in soils from Brian Pastures ■ and Emerald ○.

Sulphur

Omission of S resulted in reductions in plant yield in both harvests of inoculated and N-fertilised plants. Sulphur concentrations in the surface soil of 1 mg/kg (MCP extraction) and 3 mg/kg (KCl extraction) were lower than the levels found critical for growth of pasture legumes by Probert and Jones (1977) (4.0 mg/kg MCP extraction) and Blair *et al.* (1991) (6.5 mg/kg KCl extraction).

A positive response to S has been reported in old leucaena stands growing in a similar soil at the Brian Pastures Research Station with phosphate-extractable S of 3.3 mg/kg (0–120 cm) (Prinsen *et al.* 1992). Application of 20–40 kg/ha S in these trials doubled growth of unirrigated leucaena (Prinsen *et al.* 1992).

A tentative critical S concentration of 0.20% and an N:S ratio of 14 in leaves of desmanthus are within the range of 0.14–0.20% and N:S ratio of 14–26 in whole plant tops of a variety of tropical pasture legumes reported by Andrew (1977). Concentration of S in leaves of plants growing in unamended soil in pots was below 0.20%. Concentrations in leaves of desmanthus growing in the field were also low (0.12%) but were increased to 0.40% following application of S at a rate of approximately 20 kg/ha S (R.L. Clem, personal communication). In addition to reducing plant growth, deficiency of S reduced N concentrations in leaf tissue, and decreased the number and weight of nodules.

Molybdenum

Omission of Mo also resulted in substantial reductions in top yield, particularly where plants were relying on *Rhizobium* for N fixation (44% reduction). This finding is consistent with the role of Mo in the N-fixation process (Bergersen 1971). Although more commonly associated with acid soils, Mo deficiency has been observed in 2 clay soils (Standley *et al.* 1990). This may reflect low absolute amounts of Mo in the soil rather than low availability. Gross soil deficiency was responsible for responses in *Desmodium intortum* grown in limed soils derived from metamorphic rock collected from the Atherton Tableland (Kerridge *et al.* 1972). Although not measured in this trial, soil Mo concentration is often a poor predictor of the need to fertilise with Mo (Baker and Eldershaw 1993). Similarly, tissue concentrations of Mo measured in desmanthus grown in a range of clay soils were not useful in predicting response to Mo in experiments by Spies *et al.* (1998).

Phosphorus, manganese and copper

Responses to P, Cu and Mn were unexpected given that soil analytical data were higher than suggested critical levels (Baker and Eldershaw 1993). Responses, however, were small and significant only in plants relying on *Rhizobium* for N fixation perhaps due to their involvement in N fixation (Bergersen 1971). Large responses to these nutrients are not expected in the field.

Chloride

Growth was reduced following the multiple addition of K, Ca and Mg as chlorides in Experiment 1. Chloride, rather than sulphate, forms of these nutrients were used to avoid confounding response to these nutrients with a S response. However, this resulted in a total application rate in excess of 300 kg /ha Cl. Jones (1973) reported that application of KCl at 100–200 kg/ha Cl to a field trial killed seedlings of *Desmodium intortum*, with surviving seedlings having a concentration of Cl in whole tops of 1.1–3.5%. Although the concentration of 0.85% measured in the current trial was lower, reduced growth in this treatment was attributed to Cl toxicity rather than negative effects of the nutrients K, Ca and Mg.

Conclusion

The nutrients S and Mo were found to be limiting to growth of desmanthus in pots, suggesting that deficiencies of these nutrients may be involved in the observation of chlorosis of stands in the field. A critical S concentration in leaves of 0.20% S was determined in pots, and was higher than S concentrations measured in the field. However, the effect of the application of these nutrients needs to be verified in the field. A wider range of soils needs to be screened to determine if these deficiencies occur in other clay soils.

Acknowledgements

Thanks are extended to Hans Liebech for setting up and maintaining Experiment 2 and to David Eagles and Reddy Thuma for assistance with Experiment 3. Thanks also to INCITEC for determination of S concentration in soils. The financial support of the Meat Research Corporation is gratefully acknowledged.

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(Received for publication August 14, 1997; accepted January 28, 1998)