Relative responsiveness of some tropical pasture legumes to molybdenum

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Abstract

A glasshouse pot trial was conducted in Brisbane, Queensland, Australia, from March–June 1989. Legumes were grown in a molybdenum (Mo)-deficient red podzolic (Dr 2.21) soil. Sodium molybdate, at 6 rates from 0–200 g/ha Mo, and basal P, K, S, Zn, and Cu were mixed through the soil. Differences in growth potential between species made dry weight an unsuitable basis for comparing responsiveness. Using N concentration in plant tips and N_2 fixed when no Mo was applied, relative responsiveness to Mo was categorised as follows:

least responsive — *Stylosanthes scabra* cv. Seca (Seca stylo); intermediate responsiveness — *Macroptilium atropurpureum* cv. Siratro (siratro), *Aeschynomene americana* cv. Glenn (Glenn jointvetch), *Aeschynomene falcata* cv. Bargoo (Bargoo jointvetch) and *Chamaecrista rotundifolia* cv. Wynn (Wynn cassia); most responsive — *Neonotonia wightii* cv. Tinaroo (Tinaroo glycine) and *Desmanthus virgatus* CPI 38351. *Arachis pintoi* cv. Amarillo (pinto peanut) grew well with no added Mo due to large Mo reserves in the seed. Further research is needed to determine its relative responsiveness after seed reserves have been depleted.

Mo-fertiliser recommendations for *D. virgatus* CPI 38351 would be expected to be similar to the highly responsive glycine (100 g/ha Mo, effective for only 2 years on a strongly adsorbing soil). Residual values of applications (100 g/ha Mo) for Glenn jointvetch, Wynn cassia and Bargoo jointvetch should be similar to those for siratro (3–5 years on a strongly adsorbing soil), while residual value for Seca stylo should be similar to that for lotononis (5 years on a strongly adsorbing soil).

Pod development of Bargoo jointvetch and flowering of Glenn jointvetch and siratro were delayed at the lower rates of Mo application. Maturation of Wynn cassia was not affected by application rate. Other species had not flowered prior to harvest. Insufficient Mo may be involved in lack of persistence of some legumes.

Introduction

Tropical legumes are commonly introduced to pasture swards in northern Australia to improve nitrogen (N) status and stability of pastures. Legumes fix atmospheric N_2 and therefore have a higher protein concentration and feed value than associated grasses where soil N is low.

Molybdenum (Mo) is required for both growth and N_2 fixation by legumes. Deficiency symptoms are identical with those of N deficiency and result in reduced yields and poor persistence of the legumes. Molybdenum deficiency in grasses is rare because of their lower requirement for Mo but is relatively common in tropical legumes on a wide range of soils and parent materials. The first recorded response to Mo in a tropical legume was by glycine (*Neonotonia wightii* cv. Tinaroo) on a latosolic soil (Luck and Douglas 1966). Many subsequent recordings of Mo responsiveness in tropical legumes have been made in field and pot experiments.

Tropical legumes differ in their susceptibility to Mo deficiency. Based on reports by Roe and Jones (1966), Luck and Douglas (1966), Crack (1971), Kerridge *et al.* (1973) and Johansen *et al.* (1977), categories of 'most responsive', 'intermediate' and 'least responsive' have been developed (Bruce 1978).

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This experiment aimed to compare relative responsiveness to Mo of 9 more recently released tropical pasture legumes with those of legumes where responsiveness has been assessed.

Materials and methods

Soil and site

The surface 15 cm of a red podzolic (Dr 2.21; Northcote 1979) soil, developed on phyllite, was taken from a relatively undisturbed forest site in the North Deep Creek area, Gympie, Queensland. Chemical properties are shown in Table 1. The soil profile was shallow, showing a distinct A and B horizon, and contained a large proportion of unweathered phyllite. Evidence to date indicates that Mo deficiencies on this soil are due to an absolute shortage of Mo (Roe and Jones 1966). Mo adsorption by this soil is moderately strong (Little and Kerridge 1978).

Table 1. Properties of the red podzolic (Dr 2.21) soil (0–15 cm) from the North Deep Creek area.

Property	Measured value	Test method
pH	5.3	1:5 water
Organic carbon (%)	3.9	Walkley-Black
Extractable P (mg/kg)	19	acid extractable
	11	bicarbonate
Exchangeable Ca (cmol(+)/kg)	4.6	
Exchangeable Mg (cmol(+)/kg)	5.8	
Exchangeable Na (cmol(+)/kg)	0.23	
Exchangeable K (cmol(+)/kg)	0.87	
NO_3–N (mg/kg)	4	0.2 N KCl
Clay (%)	41	dispersion test
Coarse sand (%)	23	sieving technique

Treatments and design

Treatments imposed were 9 tropical legumes and 6 rates of molybdenum fertiliser in factorial combination. The legumes were as follows:

Aeschynomene americana cv. Glenn (Glenn jointvetch) (Oram 1985)

Aeschynomene falcata cv. Bargoo (Bargoo jointvetch) (Mackay 1982)

Arachis pintoi cv. Amarillo (pinto peanut) (Cook *et al.* 1990)

Chamaechrista rotundifolia cv. Wynn (Wynn cassia) (Oram 1984)

Desmanthus virgatus CPI 38351 (Farrel, M., personal communication)

Stylosanthes scabra cv. Seca (Seca stylo) (Mackay 1982)

Vigna parkeri cv. Shaw (Shaw vigna) (Oram 1986)

Neonotonia wightii cv. Tinaroo (Tinaroo glycine) (Barnard 1972)

Macroptilium atropurpureum cv. Siratro (siratro) (Barnard 1972)

Shaw vigna appeared to suffer from iron deficiency and its roots were infested with root nematodes. Severity of deficiency symptoms was related to severity of nematode infestation. This legume will not be considered further.

Six rates of Mo as sodium molybdate (0, 15.9, 31.8, 63.5, 127.0 and 254.0 μg/pot Mo) were mixed through the soil. Rates were equivalent to 0, 12.5, 25, 50, 100 and 200 g/ha Mo and were chosen on the basis of a previous field experiment (Johansen *et al.* 1977) on the same soil type.

Basal nutrients, Rhizobia *and seed weights*

Based on previous work with this soil (Johansen *et al.* 1977), basal P, K, S, Zn and Cu were applied as follows:

130 mg/pot P as $CaH_4(PO_4)_2.H_2O$
144 mg/pot K and 59 mg/pot S as K_2SO_4
3.7 mg/pot Cu (and 1.9 mg/pot S) as $CuSO_4.5H_2O$
4.5 mg/pot Zn (and 2.2 mg/pot S) as $ZnSO_4.7H_2O$

Phosphorus was added in powder form while the remaining nutrients were added in solution.

Rhizobium strains were used as follows: CB 756 with Wynn cassia, siratro, Tinaroo glycine and Seca stylo; CB 2312 with Bargoo and Glenn jointvetch; CB 1397 with *D. virgatus*; and CIAT 3101 with pinto peanut.

Mean individual seed weights were 120 mg for pinto peanut, 13 mg for siratro, 6.6 mg for glycine, 4.2 mg for *D. virgatus* CPI 38351, 3.5 mg for Wynn cassia, 3.4 mg for Glenn jointvetch, 2.2 mg for Bargoo jointvetch and 1.4 mg for Seca stylo.

Design

The experiment was conducted as a 2-factor factorial with 3 replicates arranged in a completely randomised design. Blocking was not necessary; randomisation was achieved by placing pots on an automatic watering machine which moved pots around the glasshouse 2–3 times per day.

Preparation and management

After collection and thorough mixing of soil, 1200 g of air-dry soil, sieved to 6–8 mm was placed in plastic-lined 15 cm pots. Basal nutrients and sodium molybdate were applied to the surface, allowed to dry, and subsequently mixed thoroughly into the soil. Containers used for shaking the soil were first acid-washed and rinsed to remove any sources of Mo contamination. Prior to planting, seeds were scarified (if necessary), germinated and inoculated with peat cultures of *Rhizobium.* Approximately 16 seeds were planted in each pot. Thinning to 4 plants/pot occurred 2 weeks after planting.

Plants were grown in a glasshouse from February–June 1989. Since the legume species were adapted to summer growing conditions, temperature and lighting were increased artificially using small fan heaters and incandescent light bulbs during the cooler months.

Pots were watered automatically to field capacity twice daily, using triple deionised water to avoid Mo contamination. Field capacity was determined by allowing water to drain freely through a cylinder of soil for 2 days. Benlate (active ingredient — benomyl; application rate of 1 kg/ha) and Plictram (active ingredient — cyhexatin; application rate of 20 g of active ingredient/100 litres) were used to control *Cercospora* leaf spot and red spider mite, respectively.

Harvesting

Changes in yield and N concentration, rather than Mo concentration, were used as indicators of Mo responsiveness. Mo concentrations in plant tops are often below the limits of detection and do not always increase with increasing Mo application rates (Johansen 1978). Furthermore, contamination of samples with Mo can occur readily (Kerridge 1981).

Harvesting occurred over an 8-week period, with most rapidly growing species harvested first. The aim was to obtain approximately 6–8 g DM of tops per pot for each species. In some cases, this was not possible because of slow growth. Glenn jointvetch and siratro were harvested after 8-weeks growth; *D. virgatus* CPI 38351 after 10 weeks; Bargoo jointvetch, Wynn cassia, Seca stylo and pinto peanut after 11 weeks; and Tinaroo glycine after 13 weeks. At harvesting, plant material from each pot was separated into root, shoot tip and remainder-of-shoot components. To ensure sufficient material for chemical analysis, tip sample sizes were as follows:

- last 3 expanded leaves (including stem fraction) for Glenn jointvetch, *D. virgatus* CPI 38351 and Seca stylo;
- last 2 expanded leaves (including stem fraction) for Bargoo jointvetch, Wynn cassia, Tinaroo glycine and siratro; and
- 1 expanded leaf (including stem fraction) for pinto peanut.

Roots in each pot were washed and notes made on nodulation. Dry weights of roots, tips and remainder-of-shoots were obtained for each replicate of each species, after oven drying at 60°C for 24 hours. Bulked replicates of remainder-of-shoots and tips were finely ground and analysed for N concentration by Kjeldahl digestion and colorimetric determination.

Statistical analysis

For each species, linear, quadratic and exponential regressions were fitted to dry weight, N content and N concentration using the GENSTAT 5 analysis package. Slopes and curvatures were compared between species using ANOVA.

Results

General growth description

Pinto peanut achieved highest mean dry matter yield (8.65 g/pot DM), indicating its relative vigour regardless of Mo responsiveness. Siratro and Glenn jointvetch exhibited the most rapid growth rates (harvested 2–5 weeks before other species), with both attaining high peak yields (yields achieved at the highest rate of Mo applied in this experiment) (7.0 and 6.5 g/pot DM, respectively), but Glenn jointvetch had a relatively low mean dry matter yield compared with that of siratro. The group Wynn cassia, Bargoo jointvetch, Tinaroo glycine, Seca stylo and *D. virgatus* CPI 38351 gave lower mean (2.4–3.2 g/pot DM) and peak yields (5.0–5.5 g/pot DM).

Siratro and *D. virgatus* CPI 38351 developed nutrient deficiencies towards the end of their respective growing periods. In siratro, symptoms occurred on the intermediate leaves as pale patches in the mid portion, while leaf margins were dark green. Lower leaflets of *D. virgatus* CPI 38351 developed chlorosis and necroses, and abscissed. Causes of these deficiency symptoms were not identified.

All legumes nodulated successfully. Proportion of large, red (effective) nodules increased with increasing rate of Mo application for most legumes except pinto peanut, Seca stylo and Glenn jointvetch.

Shoot yield

For all species except pinto peanut, a strong linear response in dry matter yield to applied Mo was observed, with response continuing to the highest rate (200 g/ha Mo). It was not possible to estimate the Mo application rate required to achieve 90% of maximum plant yield because the legumes did not achieve a yield asymptote. However, significant differences in slopes of linear responses to applied Mo between species could be compared (Table 2).

Largest accumulation of dry matter per unit of applied Mo was by Glenn jointvetch which had a linear response slope of 0.026 g DM/g Mo (Table 2). Pinto peanut had the smallest response slope of only 0.004 g DM/g Mo, with yields above 8 g/pot and similar at all rates of Mo. The other legumes (Tinaroo glycine, *D. virgatus* CPI 38351, Bargoo jointvetch, Wynn cassia, siratro and Seca stylo) exhibited slopes of 0.019–0.014 g DM/g Mo applied.

A similar ranking was obtained when comparing percentages of peak yield achieved when no Mo was applied. Glenn jointvetch was the most responsive on this basis and achieved only 18% of peak yield without Mo. The least responsive was pinto peanut which achieved 87% of peak dry matter yield without Mo fertiliser (Table 2).

Root dry weight response was similar to that of shoot response and is not shown.

Nitrogen in plant tops

N uptake in response to increasing Mo application showed relative responsiveness trends very similar to those obtained for dry matter yield and is not shown. As for yield, Mo application required for 90% maximum N uptake could not be estimated because an asymptote was not reached.

Amount of N_2 fixed without added Mo was calculated by subtracting the amount of N in Tinaroo glycine in the absence of Mo fertiliser. Tinaroo glycine was obviously N-deficient at all but the highest rate of Mo and was assumed unable to fix N_2 on this soil without Mo application. Its N content when no Mo was applied was assumed to represent available soil N. Legumes with higher N content when no Mo was applied were assumed to have had sufficient Mo from soil or seed to have fixed N_2. When no Mo was applied, pinto peanut fixed 15 times more N_2 than was in Tinaroo glycine (Table 3), with smaller amounts fixed by siratro (5 times), Seca stylo (2.6 times) and Glenn and Bargoo jointvetch and Wynn cassia (1.4–1.5 times). N content of *D. virgatus* CPI 38351 was as low as that of Tinaroo glycine, indicating that it was unable to fix any N_2 without Mo application.

Table 2. Rate of increase in shoot yield to applied Mo, and percentage of peak yield achieved when no Mo was applied, for 8 tropical pasture legumes grown in pots in Mo-deficient, strongly adsorbing soil.

Species	Slope of linear response of shoot dry matter yield (g/pot) to applied Mo			% of peak yield achieved without applied Mo[1]	
	Slope[2]	±s.e.	Rank[3]		Rank
A. americana cv. Glenn	0.026a	0.0023	1	18	1
A. falcata cv. Bargoo	0.018b	0.0024	2	24	2
A. pintoi cv. Amarillo	0.004d	0.0038	4	87	5
C. rotundifolia cv. Wynn	0.018b	0.0015	2	25	2
D. virgatus CPI 38351	0.019b	0.0011	2	23	2
M. atropurpureum cv. Siratro	0.014c	0.0025	3	62	4
N. wightii cv. Tinaroo	0.019b	0.0017	2	24	2
S. scabra cv. Seca	0.014c	0.0018	3	42	3

[1] Peak yield is that achieved at the highest rate of Mo used in this experiment (200 g/ha Mo).
[2] Values followed by the same letter are not significantly different.
[3] 1 = most responsive to Mo or highest Mo requirement; 5 = least responsive.

Table 3. Estimated N_2 fixation when no molybdenum was applied, relative to that fixed by Tinaroo glycine, for 8 tropical pasture legumes grown in pots of Mo-deficient, strongly adsorbing soil.

Species	N content in plant tops (no Mo applied)		
	Actual	Relative to *N. wightii*	Rank[1]
	(mg/pot)	(%)	
A. americana cv. Glenn	21	140	2
A. falcata cv. Bargoo	23	150	2
A. pintoi cv. Amarillo	222	1500	5
C. rotundifolia cv. Wynn	22	150	2
D. virgatus CPI 38351	15	100	1
M. atropurpureum cv. Siratro	75	500	4
N. wightii cv. Tinaroo	15	100	1
S. scabra cv. Seca	39	260	3

[1] 1 = most responsive to applied Mo, or highest Mo requirement; 5 = least responsive.

Mo content of the legume seeds could not be measured due to instrument problems.

Nitrogen in plant tips

A significant increase in N concentration in plant tips (to 3.5–5%) in response to Mo application occurred in all species except Tinaroo glycine and pinto peanut (Figure 1). In glycine it increased only slightly, to 2.4% at the highest rate of Mo, despite a strong visual growth response. Pinto peanut showed no response, but N concentration was high (approximately 4%) even without added Mo.

N concentrations in tips of Tinaroo glycine and *D. virgatus* CPI 38351 increased linearly to the highest rate of Mo. More than 90% of the variation was accounted for by the linear function, with no improvement by fitting an exponential curve. For the remaining species (other than pinto peanut), maximum N% had almost been reached at 200 g/ha Mo. More of the variance was accounted for by fitting an exponential (88–98% variance accounted for) than a linear (66–87% variance accounted for) function. Neither a linear nor an exponential function could be fitted to N response by pinto peanut to applied Mo.

It was possible to estimate the rate of Mo required to achieve 90% of maximum (extrapolated from regressions in Figure 1) N concentration in plant tips for all legumes except pinto peanut, and legumes can be ranked on this basis (Table 4).

Table 4. Estimated Mo application required for maximum N% in tips of 7 tropical pasture legumes grown in pots on an Mo-deficient, strongly adsorbing soil.

Species	Estimated Mo application required for 90% of estimated maximum N% in plant tips		
		±s.e.	Rank[1]
	(g/ha Mo)		
A. americana cv. Glenn	74	19	3
A. falcata cv. Bargoo	123	29	2
A. pintoi cv. Amarillo	—[2]	—	—
C. rotundifolia cv. Wynn	87	20	3
D. virgatus CPI 38351	430	223	1
M. atropurpureum cv. Siratro	84	60	3
N. wightii cv. Tinaroo	318	307	1
S. scabra cv. Seca	42	17	4

[1] 1 = most responsive to Mo or highest Mo requirement; 4 = least responsive.

[2] It was not possible to obtain an estimate for *A. pintoi* because an exponential function could not be fitted to the data.

Discussion

Species differences in relative responsiveness to molybdenum

This study has added significantly to the knowledge of relative responsiveness to Mo application of tropical legumes, as proposed by Bruce (1978) (Table 5).

Table 5. Relative molybdenum responsiveness of some tropical pasture legumes.

Source	Most responsive	Intermediate	Least responsive	Not responsive
Bruce (1978)	Tinaroo glycine Greenleaf desmodium	siratro phasey bean	Townsville stylo Schofield stylo Cook stylo Lotononis	
This study	Tinaroo glycine *D. virgatus* CPI 38351	siratro[1] Wynn roundleaf cassia Glenn jointvetch Bargoo jointvetch	Seca stylo	pinto peanut

[1] Results for siratro were associated with a high standard error, possibly related to its nutrient deficiency.

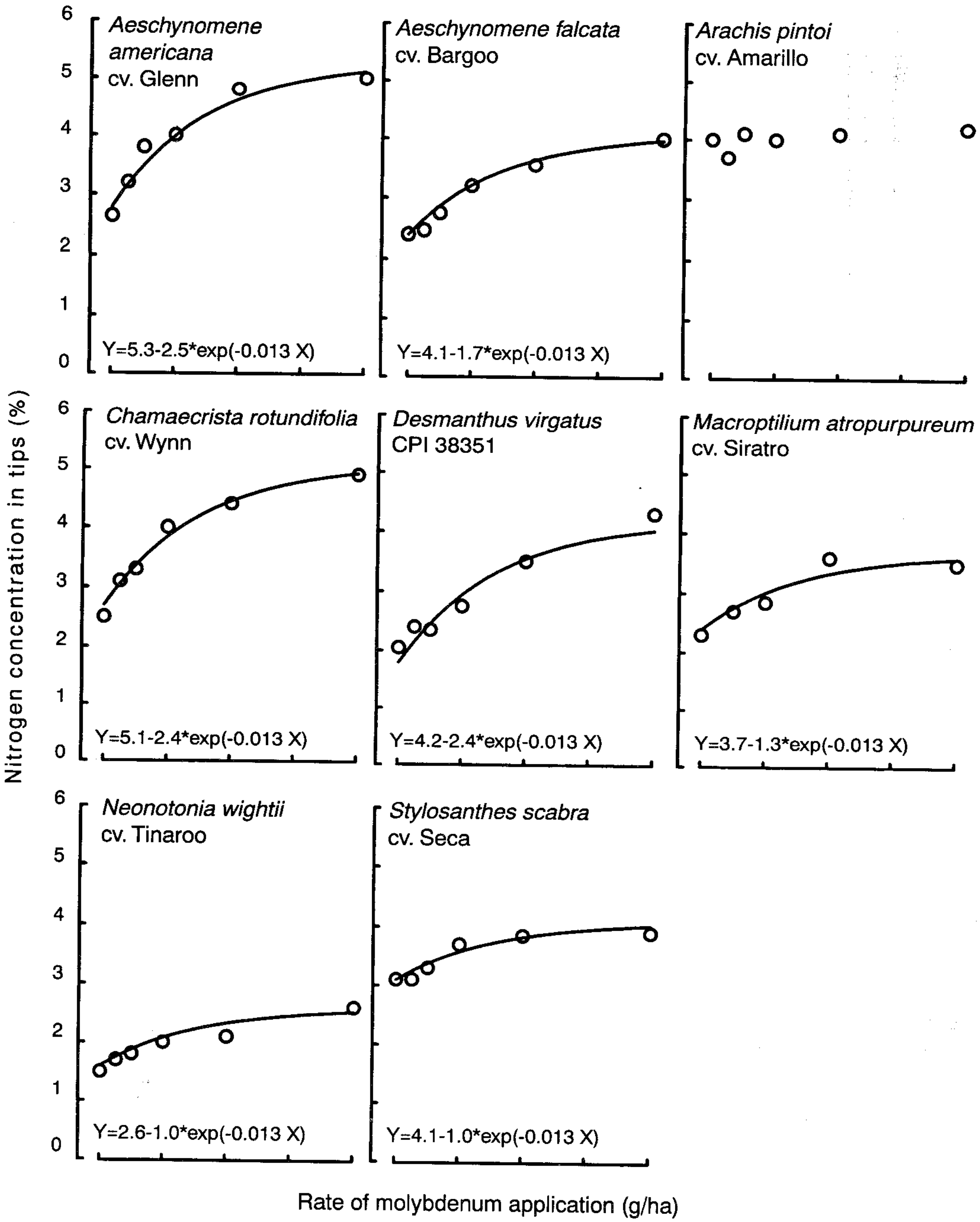

Figure 1. Effect of molybdenum application on nitrogen concentration in plant tips of 8 tropical pasture legumes grown in pots of a molybdenum-deficient, strongly adsorbing, red podzolic (Dr 2.21) soil.

Changes in N% in tips, rather than shoot weight, in response to applied Mo was used to compare relative Mo responsiveness, because of differences in growth potential between species. Two species could show similar rates of increase in yield (slope) in response to applied Mo but have different critical values for Mo. Conversely, 2 species with different slopes could have the same Mo requirement. The large increase in shoot yield per unit of applied Mo of Glenn jointvetch was attributable to more vigorous growth relative to other species (it was the first legume harvested). Its relatively low Mo requirement was indicated by high N% in tips without added Mo, and the fact that, at 200 g/ha Mo, Glenn jointvetch obtained almost sufficient Mo to achieve maximum N_2 fixation.

Standard errors associated with Tinaroo glycine and *D. virgatus* CPI 38351 were large because N% in shoot tips had not reached an asymptote at the highest rate of Mo application but this accentuated rather than cast doubt on their responsiveness to Mo. The strong visual symptoms of N deficiency in Tinaroo glycine at all but the highest rate of Mo were evidence of its high Mo requirement, confirming the assessment from previous field experiments (Johansen *et al.* 1977; 1978). Thus, grouping these 2 legumes in the 'most responsive' category was easily justified.

The estimate of Mo required for 90% of maximum N concentration in tips for siratro was associated with a large s.e., making it difficult to choose between 'intermediate' and 'least responsive' categories. The 'intermediate' category was chosen because visual observations suggested siratro was more responsive than Seca stylo. 'Intermediate' is also similar to the categorisation by Bruce (1978).

Reasons for species differences

Since the same soil was used for each species, it could be assumed that all species had similar soil-available Mo. Differences could therefore be due to differences in:

- supply of Mo from the seed;
- ability to extract Mo from soil; or
- ability of the species to fix N when the supply of Mo to root nodules is low.

Ability of pinto peanut to meet its Mo requirements without external applications of Mo could be attributed to its large seed size. Its seeds were as large (120 mg) as those of crop legumes such as soybean. Gurley and Giddens (1969) found that soybean seeds, if taken from plants grown under conditions of adequate Mo supply, could supply sufficient Mo for completion of the entire plant life cycle. Further research is required to determine the relative responsiveness of perennial pinto peanut plants after seed Mo reserves have been depleted, and the ability of new seedlings to self-regenerate on Mo-deficient soils.

Without actual measurements of seed Mo contents, speculation on the contribution of seed Mo for the other legumes is difficult because seed sizes were only 13 mg or less. Seca stylo was most effective in fixing N_2 when no Mo was applied, yet had the smallest seed size (1.4 g). Differences in relative responsiveness to Mo between species (excluding pinto peanut) are most probably due to varying abilities of legumes to extract Mo from the soil.

Fertiliser recommendations

Comparisons of relative Mo requirements are more widely applicable than actual Mo requirements or critical Mo levels in plants, because Mo application requirements will vary with adsorption capacity of the soil, and because any estimate of Mo requirement obtained from a pot experiment would over-estimate Mo requirement in a field situation. Legumes had not reached a yield asymptote even at 200 g/ha Mo in this experiment, as Mo was mixed into the strongly adsorbing soil rather than applied to the soil surface.

By grouping legumes into relative responsiveness categories, fertiliser recommendations can be made for more recently released legumes on any soil type where recommendations for legumes of similar known responsiveness already exist. In commercial practice, pasture seeds are often coated with Mo but follow-up applications may be required on strongly adsorbing soils. Generally, Mo is applied to tropical pastures of intermediate or low responsiveness at a rate of 100 g/ha Mo (as supported by the results of Luck and Douglas 1966; Kerridge 1972; Kerridge and White 1977; and Johansen *et al.* 1977). The number of years (usually 3–5; Teitzel *et al.* 1978; Cook 1978) before reapplication is necessary (its residual value) depends on soil type and legume species. Johansen *et al.* (1977) found that 100 g/ha Mo ensured maximum yield of lotononis, a 'least responsive' legume, for at least 5 years on a highly adsorbing soil. A similar residual value could be expected for applications to Seca stylo pastures on the same soil type. The widely accepted recommendation of 100 g/ha Mo should also be sufficient for Bargoo jointvetch, Wynn cassia, Glenn jointvetch and siratro but residual value will be lower than for Seca stylo (reapplications after 3–5 years on highly adsorbing soils).

To meet Mo requirements of 'most responsive' legumes (Tinaroo glycine and *D. virgatus* CPI 38351), 2 approaches are possible — application rate could be increased above 100 g/ha Mo, or 100 g/ha Mo could be applied more frequently. Douglas and Luck (1964), Ostrowski (1969) and Cook (1978) recommended rates of 200–300 g/ha Mo for glycine but care needs to be taken because of possible detrimental effect of excess Mo on copper and sulphate metabolism in animals (Dick 1956; Johansen 1978). Johansen *et al.* (1977) and Kerridge (1972) found that, for responsive species (glycine and greenleaf desmodium) on strongly adsorbing soils, 100 g/ha Mo was sufficient but remained effective for only 2 years.

Pastures containing pinto peanut are unlikely to respond to seed coating with Mo, because of large seed reserves of Mo. Relative Mo responsiveness of pinto peanut after seed reserves have been depleted, or when seed reserves are low, remains to be determined.

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