# Diagnosis and measurement of multiple soil deficiencies by a subtractive technique

#### Summary

The technique allows rapid diagnosis of the immediate and incipient deficiencies of various plant nutrients which might occur, singly or in combination, at a particular site. Degrees of deficiency, expressed in terms of standardised plant growth, permit between cut and between trial comparisons, and are therefore a valid comparison of one soil type with another.

# Introduction

To formulate a mixture to be tested in a field trial, reliable information is needed about all fertilisers likely to increase yield in the soil concerned. This information is most effective as quantitative degrees of deficiency in terms of plant growth. A pot trial, capable of such measurements for any number of fertilizer elements simultaneously, and originally proposed for tropical soils  $^{3}$  6, has been adapted for temperate conditions, in particular to the needs of pastoral farming in New Zealand.

#### Methods and materials

The method involves growth of indicator plants in soil with balanced nutrient solutions periodically added. 'Control' plants receive a complete solution of all essential mineral elements, and are therefore independent of supplies from the soil. 'Subtracted' plants, treated identically except for the omission of one element only from their nutrient solution, are dependent on the soil for this element, and limitation of their growth in comparison with control plants is therefore a quantitative measure of the degree of deficiency for the element concerned.

#### Preparation of pots and inoculation of leguminous test plants

About 100 kg of a representative soil sample, lightly crushed to pass a 6.5-mm sieve, is thoroughly mixed, and filled into  $10 \times 10$  cm plastic pots to 2.5 cm from the top; between soil comparisons are therefore on a volume basis. Soil in each pot is saturated with a 0.15 per cent aqueous solution of formaldehyde (excess draining through a 1.5-cm hole in the bottom of the pot) which performs the dual function of providing moisture for subsequent germinaton of test-plants, and of controlling root-rotting organisms.

Seventy two hours later a 1-cm layer of soil is removed from the top of each pot, and about 150 seeds of the test plant sprinkled uniformly over the exposed surface and covered with this layer of soil. After germination in a cool building, planted seeds are transferred to a glass-house when strong enough to withstand direct sunlight; leguminous test plants, need light spraying with an aqueous suspension of methyl 1 (butylcarbamyl)-2-benzimidazole carbamate at this stage to control damping-off organisms.

Preliminary work showed lucerne (*Medicago sativa*) to be the most suitable leguminous test plant, because of its hardiness, resistance to drought, and rapid upright growth. Inefficient nodulation of legumes produces uneven growth, high experimental error, and loss of sensitivity in diagnosing deficiencies of elements other than nitrogen; a single inoculation before germination is rarely effective, and to obtain sufficient uniformity of growth with lucerne, it is usually essential to re-inoculate several times with an aqueous suspension of *Rhizobium meliloti* cultured in yeast-mannitol agar <sup>4</sup> applied at the rate of 50 ml per pot.

#### Treatments, test plants, and experimental design

Of the 12 essential elements found deficient, singly or in combination in certain types of soil <sup>2</sup>, calcium has a role in relation to soil structure and acidity, and to cycling nitrogen, calling for amounts in the soil well above any deficiency level, while iron is deficient very rarely and only in particular soil types. In an unknown situation, therefore, optimum use of available facilities demands investigation of deficiencies in N, P, S, K, Mg, B, Cu, Mn, Mo, and Zn. In practice, certain elements may be neglected according to soil type or test plant. For example, molybdenum deficiency is best investigated through a leguminous test plant, when investigation of nitrogen deficiency must be be omitted; where, however, nitrogen deficiency is to be measured, perennial ryegrass (*Lolium perenne*) is a suitable indicator, and molybdenum may usually be eliminated.

Analysis of variance of results from randomised blocks, which are suitable designs, permits significance of the difference between a subtracted treatment and the control to be estimated by a modified t-test, for which comparisons of this kind are best designed so that  $m_c/m_t = \sqrt{n}$ , where  $m_c$  is the number of observations on the control and  $m_t$  the number of observations on each of n treatments <sup>1</sup>.

#### Nutrient solutions and moisture stress

Stock solutions of major (N, P, S, K, Mg, Ca) and of minor (B, Cu, Mn, Mo, Zn) elements are prepared separately, the composition of each solution varying according to treatment and test plant. Solutions for application to pots are prepared by diluting 200 ml of a solution from Table 1, plus 200 ml of a solution from Table 2, to 4.5 litres with pure water containing 0.15 ppm of ferric citrate. For the control treatment no elements are omitted; for a subtracted treatment one element only is omitted. Where major elements are subtracted, the complete minor nutrient solution is used; where minor elements are sub-

# TABLE 1

Composition of stock solutions of major nutrients in g or (ml) per litre, for (a) non-leguminous and (b) leguminous, test plants

Constituent		Element subtracted							
		None	N	Р	S	K	Mg	Ca	
NH4NO3	(a)	27.00	0	27.00	27.00	27.00	27.00	27.00	
	(b)	0	0	0	0	0	0	0	
$NaH_2PO_4.2H_2O$	(a)	8.78	8.78	0	8.78	8.78	8.78	8.78	
	(b)	8.78	0	0	8.78	8.78	8.78	8.78	
K <sub>2</sub> SO <sub>4</sub> , anhydr.	(a)	7.35	7.35	7.35	0	0	7.35	7.35	
	(b)	5.48	0	5.48	0	0	5.48	5.48	
$MgSO_4.7H_2O$	(a)	2.22	2.22	2.22	0	2,22	0	2,22	
	(b)	2.22	0	2.22	0	2.22	0	2.22	
CaCO3, anhydr.	(a)	1.80	1.80	1.80	1.80	1.80	1.80	0	
	(b)	1.80	0	1.80	1.80	1.80	1.80	0	
HC1, N.	(a)	$36 \mathrm{ml}$	36  ml	36 ml	36  ml	36 ml	36 ml	0	
	(b)	36 ml	0	36 ml	36 ml	36 ml	36 ml	0	
NaCl	(a)	0	0	3.29	0	0	0	0	
	(b)	0	0	3.29	0	0	0	0	
Na <sub>2</sub> SO <sub>4</sub> , anhydr.	(a)	0	0	0	0	5.99	1.28	0	
	(b)	0	0	0	0	4.47	1.28	0	
$MgCl_2.6H_2O$	(a)	0	0	0	1.83	0	0	0	
	(b)	0	0	0	1.83	0	0	0	
KCl	(a)	0	0	0	6.29	0	0	0	
	(b)	0	0	0	4.69	0	0	0	

# TABLE 2

Composition of stock solutions of minor nutrients, in mg per litre, for use with leguminous and non-leguminous test plants

Constituent			Element s	ubtracted	1	
	None	В	Cu	Mn	Мо	Zn
H <sub>3</sub> BO <sub>3</sub>	3.0	0	3.0	3.0	3.0	3.0
CuCl <sub>2</sub> .2H <sub>2</sub> O	1.0	1.0	0	1.0	1.0	1.0
$MnCl_2.H_2O$	20.0	20.0	20.0	0	20.0	20.0
$(NH_4)Mo_7O_{24}.4H_2O$	0.4	0.4	0.4	0.4	0	0.4
ZnCl <sub>2</sub>	1.5	1.5	1.5	1.5	1.5	0

tracted, the complete major nutrient solution is used, according to indicator plant, with an appropriate solution from Table 2.

Need for nutrient solutions varies with observed growth rates, and volumes of solutions applied to pots therefore vary from 100 ml per day in summer to 100 ml per week in winter; additional water to make good transpiration loss is also needed. Control of moisture stress is essential to prevent cracking of

the soil through dehydration, with attendant large drainage channels and erratic growth. By trial and error it was found that for most soils 75 per cent of field capacity provided the best moisture condition; weight of water needed for this condition should be determined experimentally while test plants are germinating; thereafter, all pots should be adjusted with water to the required weight at least once per week.

#### Yield measurements and transpiration rates

Aerial parts of test plants are harvested at about 3 cm above the level of soil; weight of oven-dry material for 6 similarly treated pots constitutes yield per treatment per cut. On the morning of the day before plants are cut, all pots in a trial should be adjusted with water to 75 per cent of field capacity and re-weighed seven hours later. Weight differences, corrected by subtracting the mean weight loss from 6 pots containing bare soil, and expressed as grams per hour for 6 similarly treated pots, measure transpiration rates per treatment per cut.

#### Results and discussion

Because temperature and humidity do not vary with treatment or type of measurement, transpiration rate is closely correlated with yield. The degree of correlation for 5 trials with ryegrass and 5 with lucerne is given in Table 3 for various cuts; correlation coefficients were obtained from 11 pairs of treatment totals each containing 6 observations. Results from about 100 cuts in 30 trials have also shown that deficiencies measured from transpiration rates were in close agreement with those measured from yield data; the test could therefore be carried out without serious loss of information by measuring transpiration rates only.

#### TABLE 3

rate, using test plants differing in nutritional status, but with surrounding temperature and humidity constant							
Trial and test plant	Cut 1	Cut 2	Cut 3				
Galatea, ryegrass	0.968	0.987	0.997				
Hinemaiaia "	0.973	0.985	0.993				
Kaharoa "	0.996	0.988	0.997				
Poronui "	0.862	0.933	0.986				
Tarawera "	0.985	0.995	0.975				
Oruanui D, lucerne	0.983	0.960	0.964				
"E"	0.982	0.955	0.952				
"F,	0.927	0.980	0.943				
Otamatea D 🛛 ,,	_	0.943	0.960				
"E"	0.989	0.951	0.960				

Coefficients (r) for correlation of yield with transpiration

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## TABLE 4

Degree of nutrient deficiency diagnosed from reduction in yield (Y) or transpiration rate (T) both expressed as percentages of a control (\* Not significantly different from 0)

Soil type	Nutrient	Cu	Cut 1		Cut 2		Cut 3	
test plant.		Y	т	Y	Т	Y	T	
Poronui sand,	Nitrogen	82	81	92	91	94	93	
ryegrass	Phosphorus	91	100	100	100	100	100	
	Sulphur	49	62	79	100	93	100	
	Potassium	24	26	34	44	70	100	
	Magnesium	16	89	82	100	94	100	
	Calcium	7*	0	11*	0	21	25	
	Boron	0	0	0	0	0	13*	
	Manganese	6*	7*	5*	0	0	9*	
	Nil (water)	90	100	100	96	100	98	
Tarawere gravel,	Nitrogen	76	75	91	99	100	100	
ryegrass	Phosphorus	72	80	84	78	86	81	
	Sulphur	41	23	74	92	87	87	
•	Potassium	7*	12	18	21	28	22	
	Magnesium	0	13	0	8*	0	0	
	Calcium	0	0	0	0	4*	0	
	Boron	42	32	27	25	14	0	
	Manganese	14	12	0	6*	14	0	
	Nil(water)	75	72	91	98	100	100	
Oruanui sand,	Phosphorus	92	96	100	100	100	100	
lucerne	Sulphur	5*	0	20	26	18	39	
	Potassium	0	0	20	0	32	35	
	Magnesium	7*	0	9*	8*	0	31	
	Boron	11*	0	8*	6*	10*	9*	
	Copper	0	0	14	0	19	26	
	Manganese	0	0	0	0	0	12	
	Molybdenum	25	10*	34	11*	28	22	
	M.E.	13	0	12*	5**	23	21	
	Nil (water)	75	89	100	100	100	100	
Otamatea	Phosphorus	98	—	100	100	100	100	
stony sand,	Sulphur	24	-	25	24	35	25	
lucerne	Potassium	34		53	40	66	30	
	Magnesium	0		0	27	30	43	
	Boron	0		15	8*	12*	8*	
	Copper	0		0	6*	0	0	
	Manganese	0		0	0	0	0	
	Molybdenum	39	_	34	19	36	21	
	M.E.	46	-	42	33	43	21	
	Nil (water)	84		87	100	100	100	

Immediate and incipient deficiencies. Marked deficiencies, evident when test plants are cut for the first time, need correction in the field in any event; others, which develop in subsequent cuts are important under field conditions only after the more immediate deficiencies have been corrected, in other words, after reserves in the soil have been depleted through intensified cultivation. Visual symptoms of deficiencies of nitrogen, phosphorus, sulphur, potassium, magnesium, and molybdenum are shown in Figure 1.

Results from 30 different sites agree with results from field trials. Detailed results for four sites are given in Table 4, in which degree of deficiency is the difference between control and subtracted treatment expressed as percentage of the control; 'Nil' means that only water was applied, and 'M.E.' that no minor nutrient elements (including iron) were applied; all non-zero results except those marked with an asterisk were significantly different from the control at the 10 per cent level or better.

Using ryegrass as a test plant, marked deficiencies of nitrogen, phosphorus, and sulphur, have been diagnosed for two sites, moderate potassium and magnesium deficiencies at one site, and slight potassium, boron, and manganese deficiencies at the other. Using lucerne as a test plant, marked deficiencies of phosphorus are shown for two sites, slight boron and molybdenum deficiencies for one site, and moderate sulphur, potassium, and molybdenum deficiencies for the other.

The results in Figure 1 and Table 4 clearly show that the test provides a general picture of nutrient deficiencies, major and minor, immediate and incipient, for particular soils, and also a quantitative estimate of the degree or severity of a deficiency. In the tests, plant growth per pot is dependent on various nutrients, supplied in solution or obtained from the soil; also on several physical conditions, including temperature, light intensity, and soil structure and aeration. Supply of water and nutrients is standardised, but physical conditions vary from cut to cut and from trial to trial, and their relation to corresponding conditions in the field is not easy to establish. Within cuts, however, physical conditions do not vary with treatment, and, by expressing yield per treatment as a percentage of the corresponding control yield, a direct comparison of deficiencies is possible between cuts and between trials, and therefore between different soil sites.

The four soil sites in Table 4 form part of a survey of nutrient deficiencies in the root zone of grass/clover pastures derived from pumice near Lake Taupo, New Zealand. From results of field trials and chemical tests, it has been concluded <sup>5</sup> that fertiliser requirements of these soils call for a potassic, molybdated, serpentine, superphosphate, occasionally supplemented with sulphur; in other words for a balanced mixture of phosphorus, sulphur, potassium, magnesium, and calcium, fortified with a trace element. Results from subtractive tests and field trials are therefore in substantial agreement.

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