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THE EFFECT OF ENVIRONMENT ON RESPONSE TO PHOSPHORUS OF WHITE CLOVER

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ABSTRACT

The response to phosphorus (P) of eight semi-natural populations and two cultivars of white clover (*Trifolium repens* L.) was investigated in seven experiments. Experimental conditions ranged from grazed mixed swards to solution culture. Differences among populations for attributes such as leaf size and proportion of stolon were reasonably consistent across experimental conditions. However, differences among populations in plant yield were consistent only under similar experimental conditions, while differences in response to P were even less consistent among experiments, indicating an overriding effect of experimental technique and environment on P response. There was an indication that the P response of populations collected from low-P sites changed more with experimental technique than that of populations collected from high-P sites. Similarly, the P response of populations that showed least response to P across all experiments was more affected by experimental technique than that of the more P-responsive populations. It was concluded that tolerance of soils low in P can be accurately identified only by screening in realistic field conditions.

KEY WORDS

Trifolium repens.

INTRODUCTION

During the last decade there has been considerable interest in selecting plants for tolerance to nutrient imbalances found in some soils. In New Zealand, low phosphorus (P) levels in hill country soils have limited white clover *(Trifolium repens L.)* distribution and production. Although this has to some extent been alleviated by superphosphate application, increasing costs (Stuart, 1984) have resulted in attempts to select white clovers that tolerate low-P soils and respond differently from each other to additions of P (Caradus *et al.*, 1980). There is considerable evidence that species (Bradshaw *et al.*, 1960; Pigott and Taylor, 1964; Andrew and Robins, 1969) and populations or genotypes (Snaydon and Bradshaw, 1962) adapted to low-P soils are less responsive to added P than those species or populations from high-P soils.

Historically, examinations for differences in response to P have been carried out in pot experiments where all environmental variables, other than P, are kept relatively constant (Smith, 1934). Pot and solution culture methods are popular ways of differentiating between genotypes for P response in some crop species (Coltman *et al.*, 1982; Gabelman and Gerloff, 1982). However, the reliability of screening for differences in P response in artificial environments is now being questioned.

The aim of the present study was to determine whether the results of glasshouse and growth cabinet P response studies were correlated with field P responses.

METHOD

A series of seven experiments was conducted to examine the effect of experimental technique on the P response of 10 white clover populations. A detailed description of methods is given by Caradus (1984); only a brief summary will be given here.

Description of populations

Eight semi-natural populations were collected from habitats which differed widely in soil P content and soil pH (Table 1). Two cultivars, Kent wild white and Grasslands Huia, were also included. There were 15 genotypes of each population. Stolon tips of each genotype were pre-rooted before planting. Shoot dry weight, leaf size, and proportion of stolon were measured.

Experimental

The seven experimental techniques used to measure P response of the 10 populations were:

- A grazed mixed sward with a soil of pH 4.8 and 3 ppm Olsen P, to which P was applied at 0 and 500 kg P/ha per year, and growth recorded after six and 18 months.
- A cut mixed sward with a soil of pH 5.3 and 1 ppm Olsen P, to which P was applied at 0 and 100 kg P/ha per year, and growth recorded after 22 months.
- A pure clover sward with a soil similar to that for (b), to which P was applied at 12 and 125 kg P/ha per year, and growth recorded after 10, 11 and 14 months.
- Soil culture in a glasshouse, using soil collected close to experiment (b), to which P was applied at the equivalent of 0 and 200 kg P/ha, and shoots harvested

Table 1. Description of sites of semi-natural populations of white clover.

Number	Origin	Grid reference	Soil-pH[2]	Extractable[3] soil P
1	Halfmoon common	SU 291168	4.7	0
2	Rothamsted[1]	(Plot 2d)	5.2	0
3	Rothamsted[1]	(Plot 2a)	7.1	2
4	Finedon	SP 915706	8.1	2
5	Furzluy	SU 289169	4.7	20
6	Hillfield	ST 633047	7.2	111
7	Rothamsted[1]	(Plot 7a)	6.7	117
8	Rothamsted[1]	(Plot 7d)	4.8	144

[1] Collected from the Park Grass Experiment.
[2] Sampled to 10 cm depth; measured in 1:1 soil:water mixture.
[3] Extracted in 0.5 M sodium bicarbonate.

after 16 and 35 weeks.

- Sand culture in a glasshouse, using four P levels (13, 52, 208 and 832 μM P), with plants harvested after 32 days.
- Long term solution culture in a glasshouse, using two P levels (0.3 and 300 μM P), with plants harvested after 11 weeks.
- Short term solution culture in a growth cabinet, using two P levels (0.3 and 300 μM P), with plants harvested after 24 days.

Statistical analysis

P response, measured as log-yield at high-P minus log-yield at low-P, of populations when grown in a grazed grass sward were compared with all other experimental systems using linear correlation analysis.

The change in P response of populations with change in experimental systems (environment) was calculated using a Finlay and Wilkinson (1963) analysis. All regressions between mean P response for each environment and P response of individual populations were significant ($P < 0.05$) for all populations, and ranged from 0.66 to 0.98.

RESULTS AND DISCUSSION

Comparison of experimental techniques

P response of populations was inconsistent across experiments (Table 2). The P response of populations in a grazed grass sward was not significantly and positively correlated with their P response in either a cut grass sward, a pure sward, soil, sand, or solution culture. As the experimental system became less natural there was a tendency for correlation coefficients to become negative. The response to P, in the second year, of populations grown in a grass sward was also not significantly correlated with that obtained in the first year. Jessop (1974) obtained only a moderate correlation ($r = +0.55$ $P < 0.05$) between response to P of 17 wheat cultivars grown at the same site in two years.

Shoot yield of populations was also not consistent across experiments (Table 3). In other words, the expected

Table 2. The correlation between a grazed grass sward (Year 1) and other experimental systems for response to phosphorus (log high-P yield minus log low-P yield) of 10 white clover populations. * significant at P< 0.05.

Experimental system	r
Field	
grazed grass sward (year 2)	+0.41
cut grass sward	+0.17
pure sward (year 1)	+0.27
pure sward (year 2)	+0.23
Glasshouse	
soil culture (harvest 1)	+0.30
soil culture (harvest 2)	−0.03
sand culture (P_1)[1]	−0.64*
sand culture (P_2)[1]	−0.32
sand culture (P_3)[1]	+0.45
solution culture	−0.29
Growth cabinet	
solution culture	−0.46

[1] Low-P levels were P_1 - 13, P_2 - 52, and P_3 - 208 μM P.

Table 3. The correlation between a grazed grass sward and six other experimental systems for shoot yield leaflet size and proportion of stolon, of 10 white clover populations. * significant at P<0.05.

Experimental system	Shoot yield	Leaflet size	Propn stolon
Field			
cut grass sward	+0.76*	n.m.	n.m.
pure clover sward	+0.24	+0.84*	+0.07
Glasshouse			
soil culture	+0.26	+0.67*	+0.80*
sand culture	−0.10	+0.72*	+0.57
solution culture	−0.42	n.m.	+0.61
Growth cabinet			
solution culture	−0.22	+0.69*	n.m.

n.m. = not measured.

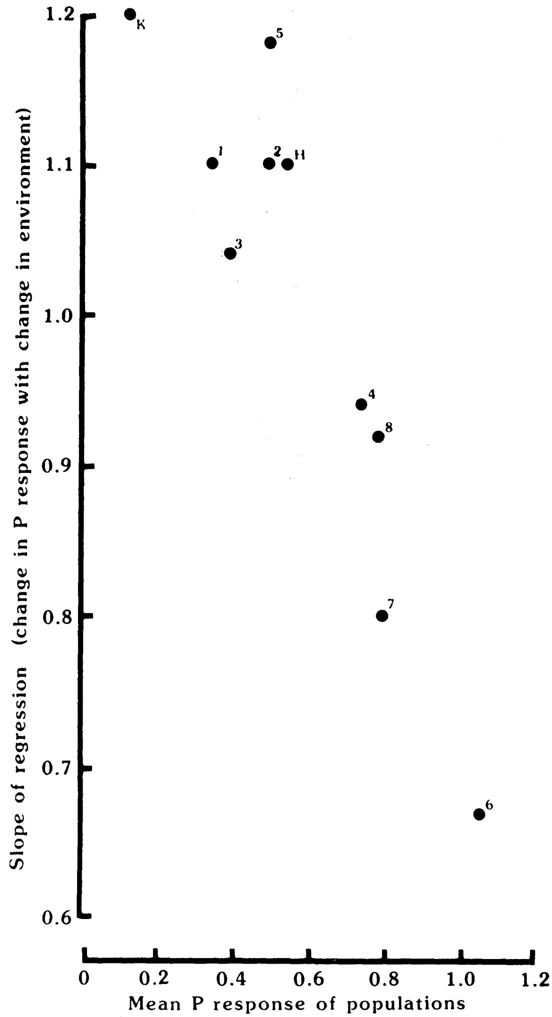

Figure 1. Relationship between the mean P response (log-yield at high-P minus log-yield at high low-P) of white clover populations (descriptions given in Table 1, H = Huia, K = Kent wild white) with the slope of the regression of population P response on mean experiment P response. (r = -0.90, P< 0.001).

yield and P response of populations grown in the field could not be reliably estimated from measurements made in the glasshouse or growth cabinet. Morphological characters of populations such as proportion of stolon and especially leaf size, were much more consistent across experiments (Table 3).

The change in P response of populations across experiments was calculated by regressing the P response of the population on the mean P response for each experiment. The latter was therefore an indicator of the P response potential of the environment in which each experiment was conducted. This showed that populations whose P response changed most across environments were those collected from low-P soils (r = -0.74* for correlation of soil-P content at population origin with slope of regression). The correlation of mean population P response across environments with the slope of the regression was highly significant (R = +0.90***). This suggested that the P response of populations that were less responsive to P was more affected by environment (experimental system) than that of populations that were generally more responsive to P (Fig. 1).

Although intraspecific differences for P response have been shown (Snaydon and Bradshaw, 1962; Caradus, 1984) the present study suggests that screening for plants adapted to low-P soils in artificial environments is not likely to be successful. Fox (1978) and Fox *et al.* (1978) compared the P response of maize hybrids in the same soil in the field, in sunken drums in trenches, and in pots of soil and solution culture in the glasshouses. They concluded that yield and P response in the field could not be reliably predicted from sunken drums or pot tests.

Artificial environments have often been used to identify nutritional differences assuming

- there is a single limiting factor and that it can be identified (e.g., P level)
- this factor does not interact with other factors, and
- differences in nutrient uptake are the important adaptive mechanism.

Such assumptions may not be justified.

Environmental effects on P response

Many factors, both biotic and abiotic, affect the P response in populations. They include non-specific effects such as site or year (Jessop and Palmer, 1976), as well as specific factors such as moisture and temperature (Finn and Mack, 1964), photoperiod (Millikan, 1957), defoliation management (Hoglund and Brock, 1983), the presence of absence of mycorrhiza (Toth *et al.*, 1984) and other nutrient imbalances (Paulsen and Rotimi, 1968). Also of importance are companion species, plant density, and the presence of absence of certain pathogens or pests.

Inconsistent effects of some environmental variables on P-response differences suggest that interaction between environmental factors and P response can be very complex. Screening for differences in P response must therefore be carried out in environments very similar to those for which the final selections are to be used.

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SYMPOSIUM DISCUSSION

Dr R.D. Burdon, Forest Research Institute

You showed very poor correlations between phosphorus responses to the respective experimental conditions. What were the apparent repeatabilities of the differential phosphorus responses within the experimental situations?

Caradus

They were better — I did not do that comparison in all the experiments but in the glasshouse it was a lot better. I could not make comparisons in the field other than the year to year comparison which was not particularly good. In the experiment of Jessop, although there was a significant correlation in year to year data, it was still a very low correlation, only explaining about 25% of the variation. It is a very difficult problem and the interactions appear to be extremely complex.

Dr G.W. Burton, USDA, Georgia

Does your experience question the validity of the Olsen soil test in determining what clover can get from the soil?

Caradus

I was only using the soil test results as guides. On the table describing populations, some of the values are zero and obviously plants cannot grow on zero phosphorus. So I think they should just be taken as ballpark figures — the zeros as low figures.

Dr J.A. Lancashire, Grasslands Division, DSIR

Have you got a feeling for the direction of selection?

Caradus

Originally we did quite a lot of glasshouse screening work, with not a great deal of success. Now we have gone to the empirical screening system — putting the material into grazed hill country grass swards with several levels of phosphorus applied. It makes the experiment larger, more complicated and more difficult to manage, but I think the gains will be a lot greater, than from the earlier work.

Dr R.D. Burdon, Forest Research Institute

This would seem to reflect an evolutionary principle — if one particular feature of phosphorus uptake was consistently limiting, then it might give very good correlation across a range of conditions. But if you did have such a consistently limiting feature, then the whole weight of natural selection would be coming to bear on it and it would be taking up the slack pretty quickly. So the situation would cover its own tracks.

Caradus

There has been a lot more success in this area in selecting for micronutrient mineral differences and differences in tolerances to toxicity where there are possibly just a few genes involved. But where macronutrients are involved selection for differences has been much less successful.