FERTILIZER RESEARCH AND DEVELOPMENT SERIES

Number 58

Assessment of the availability of phosphorus with the measurement of phosphate-stress in seedlings of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Scots pine (*Pinus sylvestris* L.) using ^{32}P phosphorus

A. R. Sheriton and R. L. Aldhous

F. R. D. S. 58/74

December 1974

INTRODUCTION

Whilst it is recognised that the availability of phosphate in soils is a major factor limiting the productivity of many natural habitats or ecosystems, it is difficult to get a really reliable measure of the availability of the nutrient in a wide range of soils. Various laboratory procedures (chemical, anion exchange resin, or isotope dilution) can be used with varying degrees of success, but no one method appears entirely adequate.

The problem of finding an adequate test largely results from the fact that these tests are designed as "spot-tests". An inherent failure of these methods is that they do not take account of the complex and dynamic nature of the soil phosphorus-plant relationship.

Obviously, therefore, where time and manpower permit, the best approach to the assay of soil phosphorus availability is that which employs a plant response. In a recent article, Bowen (1971) has outlined a plant response method which could have considerable potential. The method determines the degree of phosphate stress or deficiency in seedlings grown for a short period in soils. In his research, Bowen has demonstrated large differences in phosphate stress in Pinus radiata, Wimmera rye-grass, wheat and, subsequently, sub-terranean clover, when grown for three to ten days on a phosphate-deficient soil and the same soil to which phosphate was added. The importance of this technique is that it may allow the detection of deficiency in the plants long before the symptoms become apparent externally.

We have followed up these findings using sycamore and birch seedlings and confirm that the method can produce results which may be useful as a sensitive bioassay of available soil phosphate. The following is a brief interim account of the work we have carried out and the conclusions which we have arrived at, so far.

Experiment 1

Forty-two sycamore seeds, seven per pot, were sown (after storage in moist sand at $2^{\circ}C$ for eight weeks) on 3rd February 1972 in phosphate-free silver

sand. Six levels of phosphate were added in a standard nutrient solution (Hewitt, 1952); these were 0, 2, 5, 10, 20 and 50 ppm. Two litres of these solutions were used per pot and were replenished weekly.

The seedlings germinated in March and were grown until 23rd May, by which time clear morphological differences between the treatments were becoming evident.

Differences are summarised:

1. No phosphate - cotyledons entirely brown and shrivelled. Two pairs of true leaves developed.

2. 2-5 ppm phosphate - cotyledons yellow-green. Three pairs of true leaves developed.

3. 10-50 ppm phosphate - cotyledons green with yellow tips. Four pairs of true leaves developed.

The seedlings were harvested and the root systems were washed thoroughly in water. The seedlings were then placed in 5×10^{-4} M calcium sulphate solution for thirty minutes. Calcium ions stimulate phosphate uptake (Miller, et al, 1972). They were then transferred to a solution of 5×10^{-4} M $CaSO_4$ and 5×10^{-6} M of potassium dihydrogen phosphate containing approximately 100 microcuries P^{32}-PO_4/litre at pH 6.5 for fifteen minutes at $18^{\circ}C \pm 1^{\circ}C$. Most of the unabsorbed phosphate was removed by a five minute wash in running water. 200-400 mg samples of the root system were cut, usually from terminal ends of lateral roots, and these samples placed in 15 ml distilled water in a counting vial. The $P^{32}O_4$ content of the root was counted using Cerenkov radiation in a Packard Liquid Scintillation Spectrometer. After a first counting, the root sample was removed and weighed accurately and the vial containing water recounted under identical conditions. This second counting allowed a correction to be made for any non-metabolically absorbed $P^{32}O_4$ diffusing from the root surface to the solution during the first counting. Quench corrections were applied where necessary using the sample channels ratio method (Stubbs and Jackson, 1967).

The following results were obtained:

Table 1

ppm phosphate in culture solution	No. of seedlings[+]	No. root samples counted	C.p.m. per mg seedling root
0	4	20	366.4 ± 44.2
2	4	20	264.7 ± 25.7
5	2	10	241.8 ± 12.9
10	6	30	147.7 ± 7.3
20	2	10	118.3 ± 22.5
50	2	10	58.3 ± 0.39

[+] variation due to poor germination

These results were very encouraging and show that the $P^{32}O_4$ taken up during the fifteen minute immersion period is negatively related to the phosphate level added to the culture solution The overall difference between the amounts of $P^{32}O_4$ uptake was significant at the 0.1 per cent probability level, using either a reciprocal or a logarithmic transformation of the data. Bowen (1971) has already demonstrated differences between soils with and without added phosphate, but this experiment demonstrates a progressive response over a wide range of phosphate levels.

Experiment 2

The assay was repeated on 128 sycamore and 160 birch seedlings, which had been grown on thirteen different soils from North Wales and Lake District woodlands, in connection with another project (Helliwell, 1973) (see table in that report for soil properties). Half the replicates had phosphate added at the rate of 3 gm $NaH_2PO_4.2H_2O$ per pot. The seedlings were removed from the soils in the second week of September, the root systems washed thoroughly, and immersed in $CaSO_4$ and P^{32}-phosphate solutions as before.

Five root samples were taken from each seedling for P^{32} counting. The results are summarised in Table 2.

The counts per minute/mg root were, within any one soil treatment, fairly consistent. The general level of P^{32}-phosphate uptake was greater with birch than with sycamore. This result was surprising as birch is generally considered to be able to grow better than sycamore on poor soils. However an explanation may be that the roots of the birch seedlings were finer and therefore offered a larger surface area/volume for absorption of the P^{32}-phosphate.

The degree of correlation of the $P^{32}O_4$-uptake response with the soil phosphate variables, isotopically exchangeable phosphate, total P and P extractable in 2.5 per cent acetic acid were not as high as had been expected; nor was the correlation with height or weight of seedlings. It is possible that the low correlations are associated with the development of mycorrhiza. The nettle (Urtica dioica L.) which was used in the original testing of the isotopic method, is non-mycorrhizal (Pigott, 1973, pers. comm.), whereas the sycamore and birch seedlings appeared to have mycorrhizal fungi on their roots (Frankland, 1972, pers. comm.). Variability in the establishment of mycorrhizae on these seedlings could have masked the relationship. The relatively low concentrations could also be a result of the selection of soils. In this experiment, the selected soils fell within a pH range of 3.9 to 5.5. This range of pH is much narrower than in the soils used to test the method to measure isotopically exchangeable phosphate (Harrison, 1971). Soil pH is important in controlling the size of the labile pool and phosphate uptake by plants. Both tend to be positively correlated with pH. The omission of soils of high pH could therefore reduce markedly the probability of detecting a correlation between the measured availability of phosphate and phosphate stress in the plants.

The available soil mass may also be an important factor. In the previous work (Harrison, 1971), the nettles were grown on various soils the mass of which was restricted to 200 g per plant. In this case the mass was not so restricted. The amount of soil available for rooting may affect the relative importance of the intensity factor (given by mild extraction techniques) and the quantity factor (given by isotopic dilution techniques).

These points are to be investigated in further experiments.

The correlations between the $P^{32}O_4$-uptake responses of birch and sycamore seedlings were positive but not highly significant indicating some difference in response to the soils. It is probable that the level of phosphate stress in the seedlings grown on these different soils is influenced to some degree by factors other than that of phosphate availability and that these factors influence the two species differently. This is indicated by the results shown in Table 3. Only some 28 to 48 per cent of the variation in $P^{32}O_4$-uptake response is accounted for in terms of the phosphate variables (namely phosphorus extractable in 2.5 per cent acetic acid, the isotopically exchangeable phosphate and the reduction in isotopically exchangeable phosphate during plant growth), whereas between 76.5 and 98 per cent of the variation in $P^{32}O_4$-uptake response is accounted for by all soil variables. This suggests other soil factors are influencing soil phosphate uptake by the seedlings.

In almost all cases, the $P^{32}O_4$-phosphate taken up by the roots was markedly reduced in the seedling replicates receiving phosphate. This result conforms to what had been expected. Birch showed this reduction in all cases and sycamore in eleven out of thirteen cases. Responses to added phosphate by the two species were however not correlated. The fact that a significant correlation was obtained (Table 4) between the growth of sycamore seedlings and $P^{32}O_4$-uptake in soils with added phosphate but not in soils without added phosphate is, at first sight, somewhat surprising. However, by reference to Table 2, it can be seen that the variation in $P^{32}O_4$-uptake in soils withadded phosphate is greater than it is without added phosphate, i.e. some soils respond well to the addition of phosphate, whereas others give no increase in growth; or, in two cases, even a slight decrease. The addition of phosphate has helped to show more clearly those soils which retain phosphate in a form which is not available to sycamore seedlings. Therefore, although the average amount of $P^{32}O_4$ taken up is smaller in seedlings grown in soils with added phosphate, the amount which is taken up is better correlated with the height and dry weight of the seedlings.

Nearly all the correlations between $P^{32}O_4$-uptake and plant variables (Table 4) were negative, though most were not statistically significant. These results would suggest that phosphorus stress (i.e. deficiency) is greater the poorer the growth of the plants, and this relationship agrees with what was expected.

These points are to be investigated in further experiments.

The correlations between the $P^{32}O_4$-uptake responses of birch and sycamore seedlings were positive but not highly significant indicating some difference in response to the soils. It is probable that the level of phosphate stress in the seedlings grown on these different soils is influenced to some degree by factors other than that of phosphate availability and that these factors influence the two species differently. This is indicated by the results shown in Table 3. Only some 28 to 48 per cent of the variation in $P^{32}O_4$-uptake response is accounted for in terms of the phosphate variables (namely phosphorus extractable in 2.5 per cent acetic acid, the isotopically exchangeable phosphate and the reduction in isotopically exchangeable phosphate during plant growth), whereas between 76.5 and 98 per cent of the variation in $P^{32}O_4$-uptake response is accounted for by all soil variables. This suggests other soil factors are influencing soil phosphate uptake by the seedlings.

In almost all cases, the $P^{32}O_4$-phosphate taken up by the roots was markedly reduced in the seedling replicates receiving phosphate. This result conforms to what had been expected. Birch showed this reduction in all cases and sycamore in eleven out of thirteen cases. Responses to added phosphate by the two species were however not correlated. The fact that a significant correlation was obtained (Table 4) between the growth of sycamore seedlings and $P^{32}O_4$-uptake in soils with added phosphate but not in soils without added phosphate is, at first sight, somewhat surprising. However, by reference to Table 2, it can be seen that the variation in $P^{32}O_4$-uptake in soils with added phosphate is greater than it is without added phosphate, i.e. some soils respond well to the addition of phosphate, whereas others give no increase in growth; or, in two cases, even a slight decrease. The addition of phosphate has helped to show more clearly those soils which retain phosphate in a form which is not available to sycamore seedlings. Therefore, although the average amount of $P^{32}O_4$ taken up is smaller in seedlings grown in soils with added phosphate, the amount which is taken up is better correlated with the height and dry weight of the seedlings.

Nearly all the correlations between $P^{32}O_4$-uptake and plant variables (Table 4) were negative, though most were not statistically significant. These results would suggest that phosphorus stress (i.e. deficiency) is greater the poorer the growth of the plants, and this relationship agrees with what was expected.

The relationships between $P^{32}O_4$-uptake responses and the nutrient contents of the seedlings have yet to be investigated.

However, all the results available so far suggest that the method may be a very useful assay for studying the plant-soil phosphate relationship The method appears to have considerable potential, for it could be applied:

i) to the classification of soils in relation to growth potential and phosphate nutrition of a particular plant species.

ii) to the assay of phosphate stress of plants growing in their natural environment, and

iii) to studies in the variation in the sources of available soil phosphate to individual species and/or factors affecting phosphate uptake by plants.

Further investigation of this assay is therefore justified.

Acknowledgements

We are indebted to Mr. J. N. R. Jeffers for statistical analyses of the data and particularly the application of analysis of variance, where differing numbers of seedlings occurred in different treatments.

We are also most grateful to Miss Tracey Stoddard for assistance.

References

Bowen, G. D. (1970). Early detection of phosphate deficiency in plants. Soil Science and Plant Analysis 1(5): 293-298.

Harrison, A. F. (1971). An isotope (P^{32}) method for determining readily available phosphate in Lake District woodland soils. Merlewood R. & D. Paper No. 30.

Helliwell, D. R. (1973). The growth of sycamore seedlings in different woodland soils. Merlewood R. & D. Paper No. 48.

Hewitt, E. J. (1952). Sand and water culture methods used in the study of plant nutrition. Tech. Commr. No. 22. Commonwealth Agric. Bureaux p. 189.

Miller, R. J., Peverly, J. H. and Koeppe, D. E. (1972). Calcium stimulated ^{32}P accumulation in corn roots. Agron. J. 64: 262-266.

Stubbs, R. D. and Jackson, A. (1967). Channels ratio colour quenching correction in Cerenkov counting. Int. J. appl. Radiation Isotopes 18: 857.

Table 2

Uptake of $P^{32}O_4$ by sycamore and birch seedlings grown in thirteen woodland soils

Soils	Grid ref.	Sycamore[a]		Birch[c]	
		- phosphate[b]	+ phosphate	- phosphate[d]	+ phosphate
1. Lyulph's Tower, Ullswater, Cumb.	NY 407203	42.57 ± 3.65	55.4 ± 6.9	214.86 ± 13.0	49.48 ± 4.35
2. Loughrigg, Ambleside, Westmld.	NY 342059	63.8 ± 4.22	41.81 ± 3.01	258.8 ± 10.11	143.88 ± 10.5
3. Coed Berthlwyd, Dolmelynllyn, Ganllwyd. Merioneth.	SH 726237	61.4 ± 5.15	26.43 ± 1.83	182.2 ± 30.29	71.01 ± 25.17
4. Birks Brow, Winster, Westmld.	SD409916	72.2 ± 6.17	38.23 ± 8.64	134.3 ± 11.39	42.31 ± 5.72
5. Low Fell, Shap, Westmld.	NY 562110	29.29 ± 3.04	9.26 ± 2.16	63.51 ± 6.9	50.69 ± 5.73
6. Vaynol spruce plantn. Felynheli, Caerns.	SH 532698	56.75 ± 5.84	14.34 ± 3.67	117.63 ± 5.42	43.47*
7. Vaynol pine plantn. Felynheli, Caerns.	SH 527690	40.35 ± 4.42	26.8 ± 3.28	115.07 ± 18.61	42.51 ± 7.57
8. Bron Eifion, Criccieth, Caerns.	SH 492397	29.4 ± 7.59	17.59 ± 2.93	58.41 ± 3.46	20.18 ± 2.89
9. Low Fell Wood, Winster, Westmld.	SD 419909	35.87 ± 3.85	26.46 ± 3.79	99.61 ± 8.82	35.9 ± 4.93
10. Coed Gorswen, Ro Wen, Conwy, Caerns.	SH 755760	51.6 ± 4.19	30.87 ± 4.51	131.01 ± 14.37	60.93 ± 4.6
11. Wintering Park, Newby Bridge, N. Lancs.	SD 367869	28.6 ± 3.34	32.4 ± 2.92	131.06 ± 12.43	81.64 ± 8.51
12. Spring Wood, Town End, Hawkshead, N. Lancs.	SD 363982	71.47 ± 7.14	48.12 ± 4.27	126.96 ± 15.0	87.09 ± 7.43
13. The Howe, Troutbeck, Windermere, Westmld.	NY 417024	40.6 ± 5.43	36.56 ± 3.72	119.1 ± 9.73	59.35 ± 4.68

Values are cpm/min/mg fresh wt root. These should not be compared with the results in Table 1, as a lower level of $P^{32}O_4$ was added to the $CaSO_4/PO_4$ solution.

a and c difference between without and with added phosphate significant at 0.1% level

b and d difference between soils without added phosphate significant at 0.1% level

* This value was estimated by a "missing value" technique, as the data for this group were not obtained because of a plant handling error

Table 3

Correlation coefficients between $P^{32}O_4$-uptake and soil variables

Soil variables	Sycamore	Birch	Both species combined	Sycamore P added	Birch P added	Both species combined
1. Loss-on-ignition	- .27	- .66*	- .69**	- .60*	- .36	- .48
2. % Total P	.01	- .26	- .22	- .03	- .30	- .25
3. % Total N	- .51	- .58	- .62*	- .50	- .23	- .35
4. Extr. K	- .05	- .32	- .30	- .15	- .37	- .36
5. Extr. Ca	- .29	- .37	- .39	- .69*	- .24	- .42
6. Extr. P (2.5% acetic)	- .47	- .52	- .57*	- .48	- .42	- .49
7. Isotopically Exch. PO_4 (before plant growth)	- .39	- .28	- .338	- .47	.19	.003
8. Reduction in isotopically exch. PO_4 during plant growth	- .512	- .24	- .330	- .58*	.15	- .065
9. pH	- .03	.06	.04	- .32	- .13	.21
Proportion of variation accounted for by all variables	92.27	72.49	84.51	86.33	92.91	98.37
Proportion of variation accounted for by variables 6, 7, 8	43.87	28.24	35.83	46.32	48.46	44.85

Table 4

Correlations between $P^{32}O_4$-uptake and plant variables

	Sycamore -P	Birch -P	Both Sp.	Sycamore +P	Birch +P	Both sp.
Mean height						
Sycamore -P	-.02	-.24	-.21	-.17	-.28	-.25
Birch -P	-.43	-.55*	-.58*	-.32	-.50	-.48
Sycamore +P	-.31	-.33	-.36	-.60*	-.33	-.47
Birch +P	-.43	-.47	-.51	-.36	-.59*	-.57*
Mean dry wt.						
Sycamore -P	-.07	-.31	-.02	-.36	-.28	-.25
Birch -P	-.37	-.49	-.52	-.21	-.44	-.44
Sycamore +P	-.50	-.35	-.42	-.62*	-.19	-.30
Birch +P	-.24	-.15	-.59	-.35	-.33	-.33
Total Production (no. seedlings times mean seedling wt.)						
Sycamore -P	-.23	-.19	-.22	-.54	-.12	-.27
Birch -P	-.39	-.46	-.49	-.14	-.37	-.35
Sycamore +P	-.22	-.16	-.20	-.35	-.12	-.22
Birch +P	-.49	-.53	-.58*	-.35	-.54	-.55*
Root/shoot ratio Sycamore	-.13	.21	.15	.32	.25	-.06
Change in R/S with added P	.02	.13	.12	.25	-.16	.23

Table 5

Intercorrelations between $P^{32}O_4$-uptake by seedlings

	Sycamore -P	Birch -P	Both combined	Sycamore +P	Birch +P	Both combined
Sycamore -P	1	.45	.63*	.45	.40	.48
Birch -P		1	.98**	.67**	.71**	.79**
Both combined			1	.68**	.71**	.80**
Sycamore +P				1	.44	.68**
Birch +P					1	.96**
Both combined						1