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Bioassay of phosphorus deficiency in Queensland wheat soils by the *Azotobacter* plaque method

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Abstract

The *Azotobacter* plaque method was tested as a bioassay of phosphorus deficiency in Queensland soils used for wheat growing. The method was modified by using seven concentrations of supplied phosphate, inoculating replicate plaques with a pure culture of *Azotobacter chroococcum*, supplying other nutrients where required, and rating *Azotobacter* growth after three incubation periods. Tests were conducted on nine soils of varying phosphate status for which data on wheat response to rate of phosphate fertiliser application were available from a glasshouse experiment. From semi-quantitative curves of *Azotobacter* growth in response to increasing levels of supplied P, I assessed each soil for concentration of supplied P giving maximum growth of *Azotobacter*, concentration of P giving half-maximum growth, and growth rating at the lowest concentration of supplied P as a percentage of maximum growth.

Azotobacter in the plaques required greater concentrations of P for maximum growth than did wheat in pot culture. Concentration of supplied P resulting in half-maximum growth of natural *Azotobacter* in plaques after 5 days' incubation correlated best with parameters of wheat response to applied phosphate (r=0.86, $P<0.01$ with maximum yield increase and r=0.82, $P<0.01$ with linear response trend of wheat to applied P). *Azotobacter* response parameters to applied P were generally better correlated with water soluble phosphate in soil and P sorption measures than with acid- or bicarbonate-extractable P.

INTRODUCTION

In a previous study (Thompson 1987*a*), a soil plaque method based on growth of naturally occurring *Azotobacter* (Winogradsky 1928) was used in an attempt to bioassay 'long fallow disorder' in a black earth soil. The method proved sensitive to phosphorus and sulphur deficiencies but not to zinc deficiency. Since black earths (Stace *et al.* 1968) and other cracking clay soils or vertisols (Soil Survey Staff 1975) are extensively used in Queensland and northern New South Wales for wheat growing, the *Azotobacter* plaque method was tested as an aid to prediction of phosphorus fertiliser requirements on a range of vertisols for which response data of wheat to P fertiliser was available (Whitehouse and Hibberd 1969). Some modifications that were made to published *Azotobacter* plaque methods included the use of several rates of applied phosphorus to establish semi-quantitative response curves, the addition where necessary of 'complete nutrients' other than P, and tests conducted both with natural populations of *Azotobacter* and an inoculated culture of *Azotobacter chroococcum.*

MATERIALS AND METHODS

Soils

Nine soils from the wheat belt of Queensland selected to cover a range of phosphate levels and assessed for wheat response to phosphorus fertiliser in a glasshouse experiment (Whitehouse and Hibberd 1969) were assayed for phosphorus deficiency by the *Azotobacter* plaque method (Winogradsky 1928). A tenth soil tested in plaque experiments described by Thompson (1987*a*) was used in the development of the method. All samples were of

topsoil (0 to 0.1 m) that had been air-dried, crushed to <2 mm and stored in sealed glass jars for 3 months (soils 1 to 9 were subsamples of the soils used in the glasshouse experiment) or 14 months (soil 10). Some characteristics of these ten soils are given in Table 1.

Table 1. Some characteristics of soils used in the experiments

No.	Great soil group*	Soil association†	Locality	pH	Pa‡ (mg/kg)	Pb§ (mg/kg)	Colour	Texture
1	Black earth	Condamine	Daandine	7.3	27	21	dark grey	clay-loam
2	Black earth	Mywybilla	Norwin	6.9	73	20	dark grey	clay
3	Black earth	Mywybilla	Norwin	6.9	15	19	dark grey	clay
4	Black earth		Warwick	7.8	55	24	dark grey	clay
5	Black earth	Condamine	Haystack	7.3	15	22	dark grey	clay
6	Black earth	Condamine	Dalby	7.5	146	44	dark grey	clay
7	Brown clay		Biloela	6.5	74	51	brown	clay-loam
8	Black earth		Willowvale	8.3	11	9	dark grey	clay
9	Brown clay		Inglewood	8.2	54	40	dark brown	silty clay-loam
0	Black earth	Waco	Mt. Maria	8.5	380	70	dark grey brown	clay

* Great soil group (Stace *et al.* 1968).

†Soil association (Beckmann and Thompson 1960).

‡Pa=phosphate extracted with 0.02M H_2SO_4 (Kerr and von Stieglitz 1938).

§Pb=phosphate extracted with 0.5M $NaHCO_3$ (Colwell 1963).

Preparation of plaques

To prepare a plaque, the soil (20 g oven-dry equivalent) was mixed with 1 % w/w of carbon source (sucrose or glucose) by shaking in a polythene bag. The soil was spread in a clean Petri dish and solutions of the required rate of phosphorus as NaH_2PO_4 and other inorganic nutrients if needed, were added as evenly as possible by pipette to the soil. An additional, pre-determined volume of deionised water required to bring the soil to the 'sticky point' was added. The soil was moulded by hand to a putty-like consistency, working from the lowest to the highest concentration of added P; hands were washed and rinsed in alcohol between soils. The moulded soil was pressed into a 50 mm diameter Petri dish and the surface smoothed with a moistened stainless steel spatula to an 'iced', convex finish. All treatments were prepared in duplicate and plaques were smoothed in order from the lowest to the highest rate of phosphorus with thorough cleaning of the spatula and sterilisation by flaming in alcohol between soils. The plaques were placed in glass desiccators containing 0.5 M H_2SO_4 to absorb atmospheric ammonia and incubated at 28°C. *Azotobacter* growth on the plaques was rated after three incubation periods on colony size (Thompson, 1987*a*) with a rating system from 0=no visible growth to 5=mean colony diameter approximately 1.2 mm.

Experiment 1: Comparison of glucose and sucrose as carbon source for assessing *Azotobacter* response to a range of concentrations of applied P

The effect of either glucose or sucrose as carbon source on *Azotobacter* growth in response to six rates of phosphorus; that is, 0, 50, 100, 200, 400 and 800 μg P/g soil supplied as NaH_2PO_4 was tested in Waco soil (soil 10, Table 1). All treatments received a basal dressing of 22 μg S/g soil as Na_2SO_4 because this soil required sulphur for maximum

growth of *Azotobacter* (Thompson 1987*a*). A further treatment comprised a 'complete nutrient treatment' additional to 800 μg P/g soil and the basal sulphur. The 'complete nutrient' solution consisted of (g/L): $CaCl_2.2H_2O$ 0.162; KCl 0.084; $MgCl_2.6H_2O$ 0.368; $Fe_2(SO_4)_3$ 0.157; $CuSO_4.5H_2O$ 0.017; $ZnSO_4.7H_2O$ 0.039; $MnSO_4.4H_2O$ 0.036; $Na_2MoO_4.2H_2O$ 0.056; which when added at the rate of 10 mL per plaque supplied the following elements in μg/g soil: Ca 10, K 10, Mg 10, Fe 10, Cu 1, Zn 2, Mn 2 and Mo 5. Plaques were rated after 3, 4 and 7 days' incubation.

Experiment 2: Response of natural *Azotobacter* in a range of soils to a single rate of phosphorus and complete nutrients

This experiment was designed to determine for a number of potentially phosphorus deficient soils whether the natural populations of *Azotobacter* were sufficient to conduct the plaque tests, and whether additional inorganic nutrients were required to obtain maximum response in *Azotobacter* growth to applied phosphorus. Soils 1 to 9 (Table 1) were tested. All plaques were prepared with 1 % w/w sucrose and 1600 μg P/g soil as NaH_2PO_4. Each soil was treated in two ways; that is, no further nutrient addition or complete nutrients similar in composition to that applied in Experiment 1 but also containing Na_2SO_4 sufficient to supply 22 μg S/g soil. Growth of *Azotobacter* on the plaques was rated after 2, 3 and 5 days' incubation.

Experiment 3: Response of natural and introduced *Azotobacter* to multiple rates of phosphorus in a range of soils

This experiment was designed to bioassay the phosphorus status of soils 1 to 9 (Table 1) by assessing the response of *Azotobacter* to seven rates of supplied phosphorus; that is, 0, 50, 100, 200, 400, 800 and 1600 μg P/g soil as NaH_2PO_4. Based on the results of Experiments 1 and 2, all soils were supplied with 1 % w/w sucrose and soils 4, 6 and 8 were supplemented with the complete nutrients used in Experiment 2. Because two of the soils had too small a natural population of *Azotobacter* a second set of plaques of all soils was inoculated with a pure culture of *Azotobacter chroococcum*, strain WR-68 (Thompson 1977), which had been isolated from the rhizosphere of wheat growing in a Mywybilla black earth (Beckmann and Thompson 1960). To prepare suitable inoculum, growth of WR-68 from a 2-day-old slope culture of maintenance medium 22 (Thompson and Skerman 1979) was suspended in 20 mL sterile deionised water, shaken with glass beads to disaggregate the cells, then washed twice in 20 mL deionised water by centrifugation, decantation and resuspension of the cells. To inoculate plaques, the stainless steel spatula was dipped in the washed *Azotobacter* cell suspension instead of deionised water before smoothing the plaque surface. A separately prepared cell suspension was used for each soil to avoid transfer of nutrients between soils. Growth of *Azotobacter* on the plaques was rated after 2, 3 and 5 days' incubation.

Semi-quantitative response curves of *Azotobacter* growth to increasing level of supplied phosphorus were constructed from results for both uninoculated and inoculated plaques of each soil. From these curves I determined for each soil at the three rating times: concentration of supplied phosphorus giving maximum growth of *Azotobacter*; concentration of phosphorus giving half-maximum growth; and growth rating at the lowest concentration of supplied phosphorus as a percentage of the maximum growth rating. Correlation coefficients were calculated between these parameters of *Azotobacter* response and soil phosphate status as determined by chemical analysis and parameters of wheat response to four levels of applied phosphate; that is, 0, 15, 30 and 60 μg P/g soil in a glasshouse experiment (Whitehouse and Hibberd 1969).

RESULTS

Experiment 1: Comparison of glucose and sucrose as carbon source for assessing *Azotobacter* response to a range of concentrations of applied P

Azotobacter colonies on plaques grew somewhat faster and larger with sucrose than with glucose as a carbon source. Complete nutrients also stimulated early growth above that attained with P and basal S. However, maximum growth attained at 7 days with all P rates above 200 μg P/ g soil was similar irrespective of the nature of the carbon source or the addition of complete nutrients. As sucrose appeared a somewhat superior carbon source for *Azotobacter* growth in plaques to glucose as used previously (Thompson 1987*a*), sucrose was used in all subsequent experiments.

Experiment 2: Response of natural *Azotobacter* in a range of soils to a single rate of phosphorus and complete nutrients

The response to complete nutrients in growth of *Azotobacter* in the various soils after 2, 3 and 5 days' incubation is given in Figure 1. Although, soils 3, 5 and 9 contained few *Azotobacter*, their colony size could be rated. The other soils all contained large numbers of *Azotobacter*. Of these, soils 4, 6 and 8 required complete nutrients for maximum growth of *Azotobacter*.

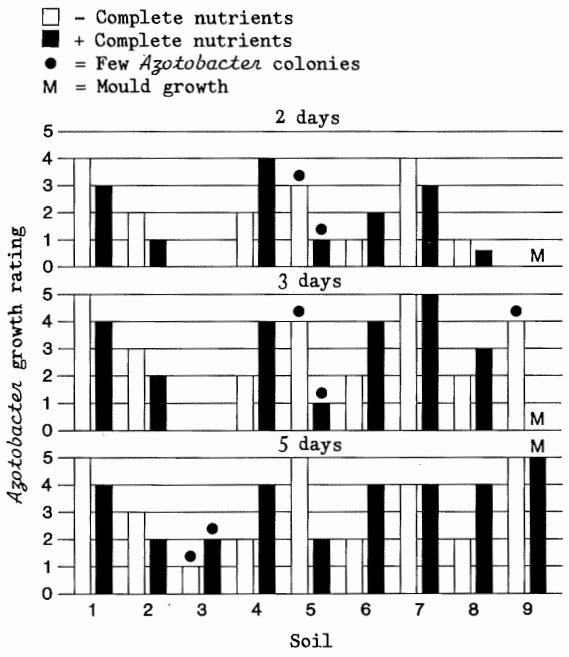

Figure 1. Responses of naturally occurring *Azotobacter* to addition of 'complete nutrients' including sulphur, in plaques of nine soils (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w) and phosphorus (1600 μg P/g soil).

Experiment 3: Response of natural and introduced *Azotobacter* to multiple rates of phosphorus in a range of soils

The growth of *Azotobacter* at 2, 3 and 5 days in both uninoculated and inoculated plaques of the nine soils is given in Figures 2, 3 and 4. Naturally occurring *Azotobacter* were present in all soils but colonies were not evident in soils 3 and 9 until after 5 days'

incubation. Ratings of *Azotobacter* growth on uninoculated and inoculated plaques were similar in some soils but somewhat different in others. All soils responded to increasing rate of phosphorus addition with some showing reduction in growth at the highest rates of phosphorus.

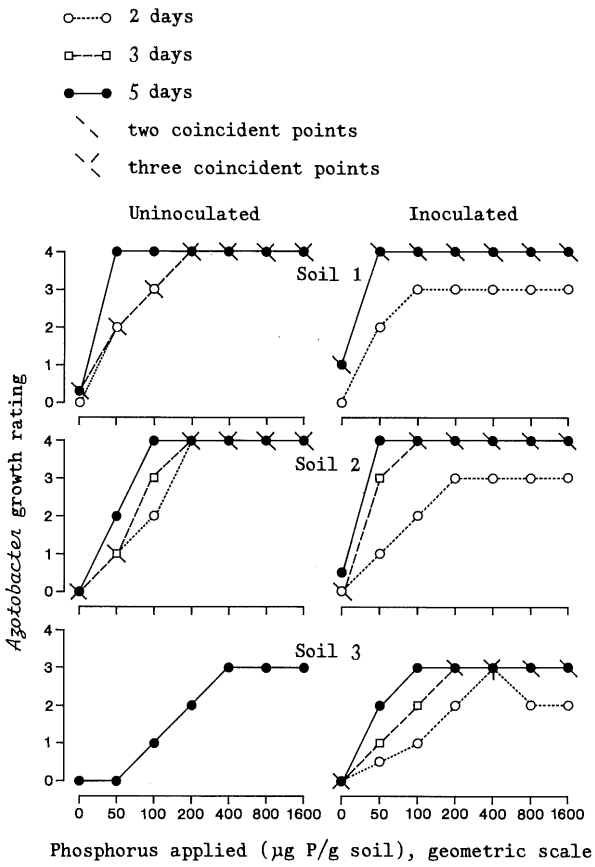

Figure 2. Response of *Azotobacter* to rate of phosphorus addition in uninoculated and inoculated plaques of soils 1 to 3 (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w).

Maximum growth of *Azotobacter* in the various soils was attained with rates of P from 50 to 800 µg P/g soil in the uninoculated plaques and from 50 to 400 µg P/g soil in the inoculated plaques. Correlation coefficients between concentration of applied P for maximum and half maximum response of *Azotobacter* and some chemical measures of soil phosphate (M. J. Whitehouse and D. Hibberd, unpub. data 1970) are given in Table 2. The concentration of applied P giving maximum response of either natural or inoculated *Azotobacter* at 2 or 3 days (Table 2, code numbers A1–2, A7–8) were generally well correlated positively with chemical measures of P sorption (Table 2, code numbers C5–7). The concentration of applied P giving half maximum response of natural *Azotobacter* (A4–6) also was well correlated positively with chemical measures of P sorption (C5–7) and additionally was well correlated negatively with concentrations of phosphate in a water leachate (C3) and a water extraction (C4) of soil. Similar trends but with generally lower correlation coefficients were evident between concentration of applied P giving half maximum response of inoculated *Azotobacter* (A10–12) and water leachates and extracts

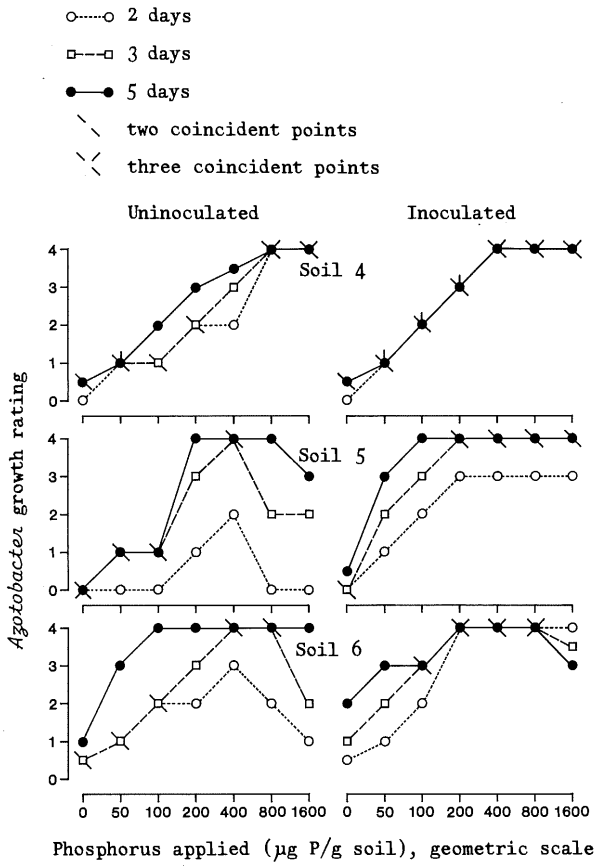

Figure 3. Response of *Azotobacter* to rate of phosphorus addition in uninoculated and inoculated plaques of soils 4 to 6 (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w) and soils 4 and 6 received 'complete nutrients'.

Table 2. Correlation matrix between some measures of *Azotobacter* response to applied phosphorus and some chemical measures of soil phosphate status

		Measure of *Azotobacter* response to phosphorus											
	Inoculation:	Uninoculated						Inoculated					
Chemical measure of soil phosphate	P concentration for:	Maximum response			Half maximum response			Maximum response			Half maximum response		
	Rating time (days):	2	3	5	2	3	5	2	3	5	2	3	5
Code No. Variable	Code No.:	A 1	A 2	A 3	A 4	A 5	A 6	A 7	A 8	A 9	A 10	A 11	A 12
C 1 0.005 M H_2SO_4 extractant		0.00	−0.02	−0.26	−0.41	−0.26	−0.67	−0.17	−0.14	−0.04	−0.29	−0.20	−0.46
C 2 0.5 M $NaHCO_3$ extractant		−0.18	−0.18	−0.20	−0.37	−0.34	−0.44	−0.43	−0.29	−0.30	−0.50	−0.07	−0.44
C 3 Water leachate (at field capacity)		−0.61	−0.56	−0.70*	−0.91**	−0.79*	−0.81**	−0.64	−0.65	−0.53	−0.62	−0.63	−0.83**
C 4 Water extract (1:10)		−0.46	−0.44	−0.59	−0.68	−0.61	−0.77*	−0.47	−0.38	−0.32	−0.44	−0.44	−0.67*
C 5 Sorbed P at 0.1 µg P/mL		0.74	0.71	0.47	0.86*	0.88**	0.75*	0.67*	0.66*	0.53	0.64	0.46	0.44
C 6 P buffer capacity at 0.1 µg P/mL		0.81*	0.81*	0.47	0.80	0.88**	0.57	0.66*	0.67*	0.59	0.47	0.42	0.35
C 7 Linear P sorption trend		0.81*	0.82*	0.47	0.81*	0.88**	0.58	0.66*	0.67*	0.58	0.45	0.41	0.35

* Statistically significant at $P<0.05$.

** Statistically significant at $P<0.01$.

and P sorption measures (C3–4). Generally, measures of *Azotobacter* response to applied phosphate were poorly correlated with concentrations of phosphorus in soil extracts with 0.005 M H_2SO_4 (C1) or 0.5 M $NaHCO_3$ (C2). One exception was a significant negative correlation between the concentration of applied P giving half-maximum response of naturally occurring *Azotobacter* (A6) and the P concentration in 0.005 M H_2SO_4 extracts (C1). The third measure of *Azotobacter* response; that is, growth rating at the lowest concentration of supplied phosphorus as a percentage of the maximum growth rating, was not significantly correlated with any chemical measure of soil phosphate.

The greatest correlations between measures of *Azotobacter* response and wheat yield response to applied P were between the rate of applied P giving half maximum response of natural *Azotobacter* at 5 days and on the maximum yield increase of wheat to applied P (r=0.855, P<0.01), relative yield of wheat; that is, yield without P as a percentage of maximum yield with P fertiliser (r=−0.70, P<.05) and the linear response trend, b, (r=0.817, P<.01) from a fitted equation of form $y=a+bx+cx^2$ where y=wheat yield and x=rate of applied phosphorus. Most other correlations between measures of *Azotobacter* response and wheat yield response to applied P were not statistically significant.

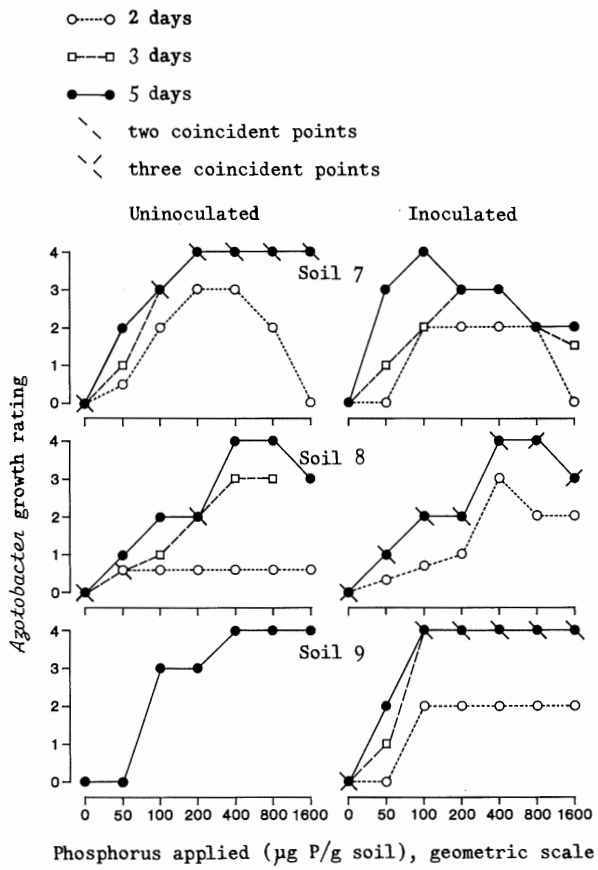

Figure 4. Response of *Azotobacter* to rate of phosphorus addition in uninoculated and inoculated plaques of soils 7 to 9 (descriptions given in Table 1) after 2, 3 and 5 days' incubation. All plaques were supplied with sucrose (1% w/w) and soil 8 received 'complete nutrients'.

DISCUSSION

The vertisols studied here were suitable for application of the *Azotobacter* plaque method for bioassay of phosphorus deficiency, because their chemical and physical properties are naturally favourable for growth of *Azotobacter*. Thus, there is no need for major amendments that could modify phosphorus availability, such as raising the pH of acid soils (Young 1933) or enriching sandy soils with kaolinite (Sackett and Stewart 1931). Although some of the soils contained few *Azotobacter*, any possible problem was overcome by inoculating a second set of plaques with a pure culture of *Azotobacter chroococcum*. In previous studies with soils from temperate areas the method has tested growth of *Azotobacter* at a single high rate of added phosphate in comparison with a control without added phosphate (Winogradsky and Ziemecka 1928; Sackett and Stewart 1931; Ziemecka 1932; Young 1933; Halversen and Hoge 1942). The degree of phosphorus deficiency was then assessed from the relative growth of *Azotobacter* colonies on the two treatments. However, Greene (1933) indicated that *Azotobacter* growth and nitrogen-fixation in culture media follow the law of decreasing increment or Mitscherlich function to increasing rate of phosphorus. He claimed that the *Azotobacter* plaque method related best to plant response in the field for extremes of deficiency and sufficiency but less well for intermediate levels. In the present study, a range of phosphorus rates was used in the plaques to obtain better discrimination between soils of varying degrees of phosphorus deficiency.

Azotobacter in the plaques required greater concentrations of added phosphorus for maximum growth than did six-week-old wheat plants in pot culture (60 μg P/g soil) (Whitehouse and Hibberd 1969). The apparently greater phosphorus requirements of *Azotobacter* in plaques than of crops has been noted before; for example, Young (1933) and Wieringa (1939). This possibly reflects the high demand by nitrogen-fixing *Azotobacter* for phosphorus; for example, Becking (1961) found 65 and 130 μg P/mL culture medium were required for maximum nitrogen fixation by a temperate and a tropical strain of *A. chroococcum* respectively. Probably, the even higher concentrations of added phosphorus needed for maximum growth of *Azotobacter* on plaques is also due to the poor mobility of phosphate in soil and the inability of an *Azotobacter* colony to move to undepleted soil once it has depleted the available phosphorus in the soil it contacts. In contrast, plant roots can grow to undepleted zones of soil and this process is greatly aided if the roots are colonised with mycorrhizal fungi as demonstrated for Queensland vertisols by Thompson (1987*b*).

Although the absolute requirements for phosphorus of *Azotobacter* in soil plaques and wheat in pots of soil may differ, correlations between parameters of the respective response curves to applied phosphorus could make the plaque method useful for predicting the fertiliser requirements of wheat. The greatest correlation coefficients between parameters of wheat response to phosphorus and *Azotobacter* response were obtained with the concentration of applied P giving half maximum response of natural *Azotobacter* after 5 days' incubation. The correlation coefficients were not as great as those obtained by Whitehouse and Hibberd (1969) between parameters of plant response and H_2SO_4 extractable phosphorus (r=0.89 to 0.97).

Because measures of *Azotobacter* response were better correlated with either a water leachate or a water extract than with acid or bicarbonate extracts of soil phosphate, *Azotobacter* seems more sensitive to intensity of phosphate in the soil solution than to the capacity of the soil to supply phosphate from its labile reserves. Possibly this reflects the relatively short time (2–5 days) of the *Azotobacter* test, whereas with the longer times involved in plant growth, replacement of phosphate removed from solution becomes a more important factor. Measures of *Azotobacter* response to applied phosphorus were also correlated with chemical measures of phosphorus sorption by the soils. These correlations

probably reflect the extent to which the various soils' sorption properties reduce the concentration of phosphate remaining in soil solution from the phosphorus applications and hence still available for *Azotobacter* growth.

Although the results indicate some interesting relations between measures of soil phosphate, *Azotobacter* response and wheat response to applied phosphate, they indicate that the *Azotobacter* plaque method offers no advantage over chemical methods for predicting phosphate availabity to wheat. However, the results may partly depend on the paricular set of test soils. Although Whitehouse and Hibberd (1969) found for this set of soils that acid-extractable phosphate was the best predictor of wheat response to phosphorus fertiliser, Whitehouse (1970) later found bicarbonate-extractable phosphate was better for a larger set of vertisols. Likewise, density of naturally occurring *Azotobacter* populations in another set of vertisols (J. P. Thompson, unpub. data 1971) was better correlated with bicarbonate-extractable phosphorus than other chemical measures of soil phosphate.

The system of rating *Azotobacter* growth and derivation of parameters to semi-quantitative response curves was adequate for the present purposes. However, more definitive results might be obtained if the method was quantitative to allow a better mathematical treatment of the *Azotobacter* response curves. Nitrogen fixed by *Azotobacter* is directly related to cell growth, and measuring nitrogen fixed in the soil plaques in response to rates of applied P would make the method quantitative. This might be achieved with Kjeldahl analysis for total nitrogen content. However, despite the active nitrogen fixation resulting from the addition of sucrose, total nitrogen content might be too insensitive a measure at low rates of applied P because of the large background of combined nitrogen in the soil organic matter. A preferable method might be to assay the nitrogenase in the *Azotobacter* cells on the soil plaques or within a mass of incubated soil by the acetylene reduction method as already applied to a Queensland black earth by Okafor and Macrae (1973). If the assay were to measure the activity of *Azotobacter* cells throughout the mass of soil instead of only those colonies on the plaque surface, the problem of poor phosphorus movement to the *Azotobacter* colonies might be reduced. The test might then better relate to plant response to phosphorus. Plants have mycorrhizal networks of roots and fungal hyphae that permeate the soil mass to overcome the limitation of poor mobility of phosphorus in soil.

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References

Becking, J. H. (1961), Studies on nitrogen-fixing bacteria of the genus *Beijerinckia*. II. Mineral nutrition and resistance to high levels of certain elements in relation to soil type, *Plant and Soil* **14**, 297–322.

Beckmann, C. G. and Thompson, C. H. (1960), *Soils and land use in the Kurrawa area, Darling Downs, Queensland*, CSIRO Soils and Land Use Series No. 37, Melbourne, Australia.

Colwell, J. D. (1963), The estimation of the phosphorus fertiliser requirements of wheat in southern New South Wales by soil analysis, *Australian Journal of Experimental Agriculture and Animal Husbandry* **3**, 190–97.

Greene, R. A. (1933), The relation of phosphorus to biological nitrogen fixation and the conformity to the law of decreasing increment, *Soil Science* **36**, 383–86.

Halversen, M. V. and Hoge, W. G. (1942), The *Azotobacter* plaque test as applied to the determination of phosphate deficiency in Idaho soils, *Journal of the American Society of Agronomy* **34**, 503–12.

Kerr, H. W. and von Stieglitz, C. R. (1938), *The laboratory determination of soil fertility*, Bureau of Sugar Experiment Stations, Technical Communication No. 9, Queensland, Australia.

Okafor, N. and Macrae, I. (1973), The influence of moisture level, light, aeration and glucose upon acetylene reduction by a black earth soil, *Soil Biology and Biochemistry* **5**, 181–86.

Sackett, W. G. and Stewart, L. C. (1931), *A bacteriological method for determining mineral deficiencies by use of the soil plaque*, Bulletin Colorado Agricultural Experiment Station No. 375, 1–36.

Soil Survey Staff (1975), *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*, Handbook USDA No. 436, Government Printer, Washington DC.

Stace, H. C. T., Hubble, G. D., Brewer, R., Northcote, K. H., Sleeman, J. R., Mulcahy, M. J. and Hallsworth, E. G. (1968), *A Handbook of Australian Soils*, Rellim Technical Publications, Glenside, South Australia.

Thompson, J. P. (1977), *Catalogue of the Queensland Wheat Research Institute Collection of Azotobacteraceae Cultures*, No. 447. Brisbane: World Data Centre for Microrganisms, Computer Data Base.

Thompson, J. P. (1987*a*), Bioassay of long fallow disorder by the *Azotobacter* plaque method, *Queensland Journal of Agricultural and Animal Sciences* **44**, 123–32.

Thompson, J. P. (1987*b*), Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower, *Australian Journal of Agricultural Research* **38**, (in press).

Thompson, J. P. and Skerman, V. B. D. (1979), *Azotobacteraceae: The Taxonomy and Ecology of the Aerobic Nitrogen-fixing Bacteria*, Academic Press, London.

Whitehouse, M. J. and Hibberd, D. (1969), Studies of phosphorus availability, *Queensland Wheat Research Institute Annual Report 1968–1969*, 40–42.

Whitehouse, M. J. (1970), Soil phosphorus studies (a) Available soil P measurement, *Queensland Wheat Research Institute Annual Report 1969–1970*, 36–38.

Wieringa, K. T. (1939), Determination of the fertility of the soil by microbiological methods, *Antonie van Leeuwenhoek Journal of Microbiology and Serology* **6**, 53–70.

Winogradsky, S. (1928), Sur l'application agronomique d'une epreuve microbiologique, *Comptes Rendues de l'Academie de Sciences* **14**, 161–65.

Winogradsky, S. and Ziemecka, J. (1928), Etudes sur la microbiologie du sol. Troisieme memoire. Sur le pouvoir fixateur des terres, *Annals de l'Institut Pasteur* **42**, 36–62.

Young, A. W. (1933), The Winogradsky spontaneous culture method of determining certain soil deficiencies, *Iowa Agricultural Experiment Station Research Bulletin* **157**, 1–24.

Ziemecka, J. (1932), The *Azotobacter* test of soil fertility applied to the classical fields at Rothamsted, *Journal of Agricultural Science* **22**, 797–810.

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