Plant and Soil **158**: 287–297, 1994.

PLSO 3215

Is the isotopically exchangeable phosphate of a loamy soil the plant-available P?

C. MOREL and C. PLENCHETTE
Institut National de la Recherche Agronomique, Département d'Agronomie, 17 rue Sully, BV 1540, F-21034 Dijon, France

Received 26 May 1993. Accepted in revised form 2 October 1993

Key words: arbuscular mycorrhizae, available P, barley, *Glomus intraradices*, soil solution, ^{32}P uptake, P uptake, soybean

Abstract

This study compared the validity of using the isotopically exchangeable phosphorus (P) as an accurate measurement of plant available P by comparing the specific activity of P, i.e. the $^{32}P/^{31}P$ ratio, in soil solution (Ss) against the specific activity of P in plants (Sp) growing in a loamy soil after applying a ^{32}P-labelled fertilizer ($NaH_2PO_42H_2O$) at different rates (F) and specific activities (Sf). Non-mycorrhizal and mycorrhizal (*Glomus intraradices*) plants of two species (soybean and barley) were grown in greenhouse experiments. Ss values were determined on 1:10 soil suspension after periods of incubation ranged from 1 min to 35 d. At a given rate of P application, the Sp values of both non-mycorrhizal and mycorrhizal soybean and barley did not show significant difference although the plant P uptake varied 18 fold for all the (crop species × mycorrhizal infection) treatments over soil solution P values ranging from 0.02 to 5.46 mg P L^{-1} (0.6–176 μM). Ss values decreased with time and reached a steady state after 35 d of equilibration period. Both Sp/Sf and Ss/Sf increased with applied P and there is a 1:1 correspondence between Sp/Sf and Ss/Sf values. The identity between the isotopic composition of both P in soil solution and in plant indicates that the isotopically exchangeable P (E = F(Sf/Ss-1)) is the only source of phosphate in solid soil phase which replenishes P of the soil solution after P has been removed by the plant, i.e. the only source of P which participates in plant nutrition. The isotopically exchangeable P of a loamy soil is the P available to growing plants and mycorrhizal fungi increases the P uptake giving plants wider access to isotopically exchangeable P in soil, and not making previously non-exchangeable P available. An immediate application of the 1:1 correspondence between a soil parameter (Ss/Sf) and a plant parameter (Sp/Sf) concerns the agronomic evaluation of P fertilizers.

Abbreviations: R – amount of ^{32}P associated with the amount of ^{31}P (F) in fertilizer, Rp – amount of ^{32}P taken up by plant, Rs – amount of ^{32}P in soil solution, Qf – amount of ^{31}P taken up by plant from the fertilizer (Qf = FRp/R), Qs – amount of ^{31}P taken up by plant from the soil, Qt – total amount of ^{31}P taken up by plant (Qt = Qs + Qf), Wf – amount of ^{31}P in soil solution derived from the fertilizer (Wf = FRs/R), Ws – amount of ^{31}P in soil solution originated from the soil, Wt – total amount of ^{31}P in soil solution (Wt = Ws + Wf), Sf – specific activity of P in the applied fertilizer (Sf = R/F), Ss – specific activity of P in the soil solution (Ss = Rs/Wt), Sp – specific activity of P taken up by the plant (Sp = Rp/Qt). A value – FQs/Qf, L value – F(Sf/Sp-1), E value – F(Sf/Ss-1).

Introduction

The ability of soil to supply phosphorus (P) to plants, and the modification of this ability by agricultural practices, is usually assessed by correlating plant growth parameters such as yield or P uptake with extractable or isotopically exchangeable soil P over a great range of soil-fertilizer-crop combinations (Kamprath and Watson, 1980). Three radiotracer methodologies have been used to quantify plant-available P.

The first technique is based on the application of ^{32}P labelled fertilizer to a small volume of soil. It is assumed that the availability of fertilizer P is the same as the availability of P from the soil P pool, i.e. that the coefficient of P uptake from fertilizer (Qf/F) is the same as the coefficient of P uptake from soil available P (Qs/A). The amount of available P in soil (A value) can then be calculated with:

$$Qf/F = Qs/A \quad (1)$$

where Qs is the P amount taken up by plant from the soil, Qf, the amount of fertilizer P taken up by plant and, F, the amount of fertilizer P supplied. As the total amount of P in the plants (Qt) is the sum of Qs and Qf, the original formulation of A value is obtained (Fried and Dean, 1952):

$$A = F(1 - y)/y \text{ where } y = Qf/Qt \quad (2)$$

The calculation of A value is not based on the application of isotopic dilution principle.

The second isotopic methodology requires the application of the isotopic dilution principle. The analysis of the soil phosphate by isotopic dilution consisted of (i) introduction into soil solution of a ^{32}P labelled solution which the specific activity

$$Sf = R/F \quad (3)$$

i.e. ratio of the amount of ^{32}P (R) to the F amount, is known, and then (ii) the dilution of the ^{32}P-labelled PO_4-ions by the unknown amount of PO_4-ions in the solution and the solid soil phases. Larsen (1952) determined the amount of available P in the soil (L value) by assuming the specific activity of P taken up by plant (Sp) is the same as the specific activity of P in the total (L + F) pool, i.e.

$$Sp = R/(L + F) \quad (4)$$

The L value is obtained by combining Eq. [3] and [4]:

$$L = F(Sf/Sp - 1) \quad (5)$$

The calculation of the L value required to measure the amount of ^{32}P taken up by the plants (Rp) and the total amount of P in the plants (Qt) to obtain Sp value. The third radiotracer methodology was proposed by Russell et al. (1954) and determined the amount of the isotopically exchangeable P (E value) by assuming that the specific activity of P in soil solution (Ss) is the same as the specific activity of P in the total (E + F) pool, i.e.

$$Ss = R/(E + F) \quad (6)$$

The E value is given by combining Eq. [3] and [6]:

$$E = F(Sf/Ss - 1) \quad (7)$$

The calculation of E value required to measure the ^{32}P amount in soil solution and the total amount of P in solution (Wt) to obtain Ss value.

Many reports have shown that A, L and E values are highly correlated to plant P uptake, validating these three methods for evaluation of available soil P. But, although useful information was obtained, the methods often gave unsatisfactory predictions for other soil-plant-fertilizer combinations. The inconsistency can be explained by factors related both to plant species and to methodologies used.

A first source of variation of plant P uptake is the plant studied, since phosphorus requirements of plants differ widely with crop species. For instance, the external P requirement assessed by the P concentration in soil solution necessary to achieve 95% of the maximum yield (Fox, 1970), is about 10 fold higher for potatoes and several vegetable crops than for wheat (Memon and Fox, 1983). A second factor affecting the plant P uptake is presence of arbuscular mycorrhizal

infection, since the P uptake by a mycorrhizal plant can be up to 20 fold higher than by a non-mycorrhizal plant in sterilized soil. Level of P fertilization also changes plant P uptake. The synergistic and antagonistic interactions of these three factors on P uptake by plants add further complications since the phosphorus uptake by plants from soil varies with the rate and forms of P fertilizer and the variation depends on the level of plant P uptake from soil in the unfertilized P treatment (Morel and Fardeau, 1989; 1990).

The inconsistencies in the correlations between plant P uptake and either the A, E or L values are also caused by methodological problems. It has been shown that the basic hypothesis used to calculate A values, that the coefficients of P utilization from fertilizer and available P pool are identical, is not true (Fardeau and Guiraud, 1972; Morel and Fardeau, 1991). Furthermore, Ss in soil solution at steady-state is time-dependent indicating that both E and L values reflect changes in a multicompartmental system (Fardeau, 1993) whose size is defined by the time of isotopic exchange with P in soil solution. Therefore, these three isotopic procedures did not permit the determination of a single quantity of available soil P but were limited to the characterization of specific soil-plant relationships in P nutrition by means of the Ss and Sp data (Fardeau and Jappe, 1976). Very few reports concerned the comparison of Sp values among crop species and when it is possible to calculate Sp values from published data (Kalra and Soper, 1968; Kalra, 1971), the fertilizer was applied in small soil volume. The localization of fertilizer changes the Sp value for a given crop species (Nelson et al., 1947; Welch et al., 1949); there is no data on the comparison of Sp values between crop species.

Accordingly, for a given soil type (loamy soil) which is representative of soils in France and in temperate areas, the objective of this study were fivefold: (i) to compare the specific activity of P taken up (Sp) by 2 crop species with contrasting P requirements; (ii) to study the influence of arbuscular mycorrhizal (AM) infection on Sp values; (iii) to evaluate the variation in Sp over a wide range of P fertilization; (iv) to measure the time-dependent changes of the specific activity of P in soil solution (Ss) after applying ^{32}P labelled fertilizer; and (v) to compare Sp and Ss values.

Material and methods

Soil sample

A soil sample was collected from the upper 30 cm layer of a permanent pasture which had not received P fertilizer for at least 40 years, located near Châlons-sur-Marne (51), France. The soil is classified as an eluviated brown soil according to the French classification and as a loamy Luvisol with a depth > 1 m in the FAO soil taxonomy. The sample was air-dried, sieved at 2 mm and sterilized by gamma-irradiation (10 KGy). Its main physico-chemical characteristics were: pH in water 6.1; total C content 1.92%; P extractibility in 0.5 *M* $NaHCO_3$, pH = 8.5 (Olsen, 1954) was 7.9 mg P kg^{-1}.

Experimental determination of the L value

The L values were measured by a pot experiment which was a factorial combination of 3 factors: 10 P levels of fertilizer applied as ^{32}P-labelled (0.1 to 10 MBq/mgP) NaH_2PO_4; 2 plant species [barley, *Hordeum vulgare* L. var. Vodka and soybean, *Glycine max* (L.) Merr var. Maple Arrow]; and presence or absence of arbuscular mycorrhizal inoculation (*Glomus intraradices*) (AM). There were 4 replicates for each treatment.

Seeds of barley and soybean were germinated in petri dishes. One 6-day old barley or soybean plantlet was transplanted into each pot containing 0.5 kg of sterilized fertilized soil. Half of the pots were inoculated by adding 1 g of mycorrhizal inoculum to the transplanting hole. The inoculum used was pieces (about 0.5 cm long) of surface disinfected (ultrasonic treatment for 10 min in a 2% Chloramine T and 10% Mercryl solution) fresh leek (*Allium porrum* L. var. Olaf) roots infected by *Glomus intraradices*. The same weight of non-mycorrhizal and surface sterilized roots of leek was added to non-mycorrhizal treatments. The plants were grown until

maturity (about 80 d and 95 d for barley and soybean, respectively) in a glasshouse maintaining the water content of soil near the field capacity. A complete liquid basal fertilizer mixture (Long Ashton solution (Hewitt, 1966)), without P for barley, and without N and P for soybean which were inoculated with *Rhizobium* (2.5 mL/pot of 10^7 *Rhizobium japonicum*/mL), were applied every week. The pots were randomized weekly to minimize for possible variations within the glasshouse.

At harvest the dry weights of seed were recorded. Then each seed sample was calcined at 600 °C. Ashes were then dissolved in 10% HNO_3 and analysed for ^{31}P (Qt) by the colorimetric method of John (1970). Content of ^{32}P (Rp/R) was determined by Cerenkov counting in a liquid scintillation counter to calculate $L = F[(R/F)/(Rp/Qt) - 1]$. Roots were washed from soil to assess mycorrhizal infection by the gridlines intersect method (Giovanetti and Mosse, 1980) which yields the percentage of root length infected by mycorrhizal fungus.

Experimental determination of the E value

Because the amount of radioactivity in soil solution (Rs) (Morel and Fardeau, 1991) and the total amount of P in the soil solution (Wt) (Barrow and Shaw, 1975) are time-dependent, the specific activity of P in the soil solution, and thus E value will be time-dependent. The experimental procedure was performed to characterize the changes of all data as previously described (Morel and Fardeau, 1991): 20 g of soil were shaken for 18 h with 199 mL deionized water in a plastic bottle. Then, 1 mL of ^{32}P labelled fertilizer was added at $t = 0$ into the soil suspension and stirred with magnetic stirrer. Specific activities varied between 0.1 and 10 MBq/mgP, depending on the P fertilizer rates which were between 20 and 280 mgP kg^{-1} to obtain a range of P concentration in soil solution between 0.02 to 5.5 mgP L^{-1}. After 1, 10, 100, 1000 [about 17h], 10000 [about 7 d], 28605 [about 20 d] and 50270 min [about 35 d], a 15-mL aliquot was taken from suspension with a plastic syringe and filtered through a 0.01-μm pore-size membrane. Phosphorus was determined colorimetrically by the method of John (1970) to obtain Wt value, while Rs/R were measured by scintillation counting in order to calculate $E = F[(R/F)(Rs/Wt) - 1]$. Each analysis was performed in triplicate at all sampling times.

Mathematical and statistical analysis of data

Sp and Ss were expressed by the ratio of Sp/Sf and Ss/Sf in order to take into account the difference in the introduced activity (R) and the amount of P (F). The mean of Sp/Sf over all the (crop species × mycorrhizal inoculation) treatments and Ss/Sf ratios were fitted to the rate of applied P by nonlinear regression:

$$y = a - b.e^{-cx} \quad (8)$$

(c is the base of natural Log). Significant differences among plant species and mycorrhizal inoculation were determined by comparing the 95% confidence interval on the a, b and c constants of regression. The equation used to fit the kinetic data of Ss/Sf was:

$$Ss/Sf = d - g.Log(t) \quad (9)$$

with d and g constants. Ss/Sf and Sp/Sf values were fitted by a linear regression. All statistical analyses and figures were obtained with the NLIN, REG and GPLOT procedures of SAS-STAT and SAS-GRAPH software (SAS Institute Inc., 1987).

Results and discussion

Specific activity of plant P

In order to compare the specific activities of P uptake by mycorrhizal and non mycorrhizal barley and soybean ($Sp = Rp/Qt$) independently of the different additions of both ^{32}P activity (R) and P amount (F), Sp values were expressed by the dimensionless Sp/Sf ratio where Sf is R/F ratio, i.e. the specific activity of the added P (Table 1). For all crop species and mycorrhizal treatments, the average of Sp/Sf values increased with the rate of applied P and ranged from 0.208 to 0.755 for rates of application of 20 to 310 mgP kg^{-1}. The a, b and c regression

Table 1. Regression coefficients of the equation: $Sp/Sf = a - be^{-cF}$ for non-mycorrhizal and mycorrhizal barley and soybean

Crop treatments	Regression coefficients	Estimate	Asymptotic std. error	Asymptotic 95% confidence interval	
				Lower	Upper
Barley					
Non-mycorrhizal	a	0.742	0.034	0.663	0.798
	b	0.707	0.039	0.615	0.798
	c	0.014	0.002	0.010	0.018
	R^2	0.98			
Mycorrhizal	a	0.736	0.038	0.647	0.826
	b	0.705	0.046	0.596	0.813
	c	0.015	0.002	0.010	0.017
	R^2	0.97			
Soybean					
Non-mycorrhizal	a	0.731	0.030	0.661	0.801
	b	0.719	0.034	0.639	0.798
	c	0.014	0.001	0.010	0.017
	R^2	0.98			
Mycorrhizal	a	0.723	0.030	0.653	0.793
	b	0.699	0.035	0.615	0.782
	c	0.015	0.002	0.011	0.019
	R^2	0.98			

R^2 is the proportion of variation accounted for by the above equation.

constants of Eq. [8] between the Sp/Sf values and the rate of P application show no significant differences ($p > 0.05$) between non-mycorrhizal or mycorrhizal barley or soybean (Table 1). The set of Sp/Sf values vs. P rate for all (crop species × mycorrhizae) treatments was described by the following equation (Fig. 1):

$$Sp/Sf = 0.737 - 0.707e^{-0.014(\text{P rate})} \quad \text{with } R^2 = 0.97 . \qquad (10)$$

Although, there is no significant difference between the Sp/Sf values measured on 2 crop species, the amount of P taken up (Qt) by non-mycorrhizal and mycorrhizal soybean and barley widely differed over the range of applied P (Table 2):

(i) the P uptake by non-mycorrhizal barley was 2.6 times higher than P uptake by non-mycorrhizal soybean on nil P treatment.

(ii) the P uptake by non-mycorrhizal soybean or barley increased 16 and 5 fold, respectively, over the range of applied P.

The observed difference in plant P uptake reflects the difference in the P requirement of the two crop species since the external P requirement of cereals is lower than the external P requirement of many vegetable crops (Memon and Fox, 1983).

Mycorrhizal infection assessed by the % infected root length (Table 3) indicated a high level of infection for each crop species in the nil P treatment. The rate of infection decreased with increasing applied P for barley and only for the highest rate of application for soybean. These observations reflect the low and high mycorrhizal dependency of barley and soybean, respectively (Plenchette et al., 1983). In the nil P treatment the rates of AM infection of barley and soybean were similar, but AM infection increased P uptakes 4.5 and 1.4 fold for soybean and barley, respectively (Table 2). Although both P requirement and mycorrhizal dependency of crop species were very different, and the wide range of P application produced large differences in plant P uptake, Sp/Sf values were not significantly different among the (crop species × AM inoculation) treatments for a given rate of P addition. Similar results have been reported and reviewed by Bolan (1991) for experiments where

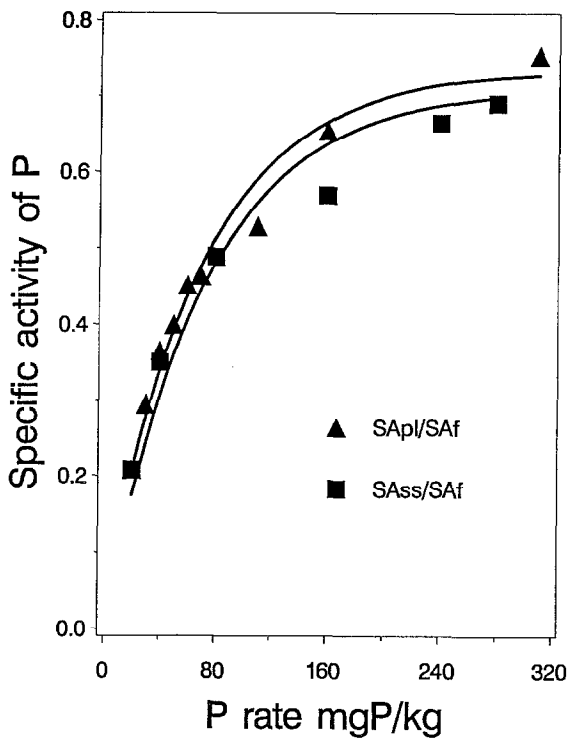

Fig. 1. The specific activity of P taken up by non-mycorrhizal and mycorrhizal barley and soybean plants ($Sp/Sf = 0.737 - 0.707e^{-0.014F}$, $R^2 = 0.97$) and the specific activity of P in soil solution ($Ss/Sf = 0.713 - 0.713e^{-0.014F}$, $R^2 = 0.95$) in relation to the rate of P application (F).

P was applied at a single rate and the plants tested were as different as onion (Hayman and Mosse, 1972; Powell, 1975; Sanders and Tinker, 1971), clover (Bolan et al., 1984; Powell, 1975) and oil palm (Blal et al., 1989).

Among the different methodologies used to assess the availability of P to plants, the L value is generally considered as a good predictor of the plant P uptake. The average of the 36 results about L values from the data set in Table 2 and Eq. [5] gives $L = 81.5\,mgP\,kg^{-1}$ (coefficient of variation (CV) = 15.8%). In this experiment a single L value is associated with a wide range of plant P uptake with the (crop species × AM inoculation) treatments. This example is likely to cover the extremes of plant effects on P uptake ever encountered in field situation, but it explains the inconsistencies in the characterization of the soil-plant relationships in plant nutrition by correlating the Qt vs. L value. The results show that the calculation of an L value reduces the amount of information obtained by the isotopic dilution method, since behind one L value there are 9 Sp/Sf values which are not dependent upon plant factors.

The specific activity of P in soil solution

The P concentration in soil solution ranged between 0.02 to 5.46 mg P L^{-1} which is representative of P concentrations in cultivated agricultural soils. Both Rs/R and Wt values decreased when the period of incubation increased (Table 4) but the rate of decrease with time was higher for Rs/R than for Wt values. These data agree with the general characterization of the reactions between soil and phosphate where it is assumed that an initial rapid sorption reaction is followed by further slow reactions with phosphate in solid phase (Barrow and Shaw, 1975; Fardeau, 1981). Consequently, Ss/Sf values [$Ss/Sf = (R/Wt)/(R/F)$] also decreased with time. The decrease with time was fitted to the following nonlinear regression: $Ss/Sf = d\text{-}g.Log(t)$ [9], where d and g are the constants of regression (Table 5). After a 35 d-period of incubation, the Ss/Sf ratio appears to remain constant. In order to test if a steady state is reached over the period of cropping, the change in Ss/Sf [$\Delta(Ss/Sf)$] between 1 month (43200min) and 3 months (129600min) was calculated using Eq. [9] and is expressed as percentage of the Ss/Sf value at 1 month. $\Delta(Ss/Sf)$ values were between 21 and 1.9% (Table 5) which was less than the standard deviation of Ss/Sf. Therefore, the Ss/Sf values measured after a 35 d-period of incubation were considered representative of the steady-state. This result agrees with previous one obtained using a calcareous soil (17% $CaCO_3$), in which Ss reaches a constant value over the period of plant growth after 22 d of isotopic exchange (Morel and Fardeau, 1991). The relation between Ss/Sf values at steady-state and the rate of applied P was the following (Fig. 1):

$$Ss/Sf = 0.713 - 0.713e^{-0.014(P\ rate)}, \quad R^2 = 0.95. \qquad (11)$$

The average of the 6 E values [$E = F(Sf/Ss - 1)$] calculated from the Ss/Sf ratio measured after

Table 2. Phosphorus amount (Qt), proportion of radioactivity (Rp/R) uptakes and Sp/Sf ratio ranged from 0 to 310 mg P kg^{-1} rates of fertilizer application (F) and measured on non-mycorrhizal (NM) and mycorrhizal (M) soybean and barley plants

F (mg Pkg^{-1})	Qt (mg P/0.5 kg)		Rp/R (%)		Sp/SAf[a]	
	NM	M	NM	M	NM	M
Soybean						
0	2.6	11.6				
20	4.7	15.3	8.2	31.9	0.171	0.209
30	5.5	16.6	9.8	31.6	0.266	0.286
40	7.4	18.4	12.3	33.7	0.324	0.366
50	8.6	18.9	13.2	31.7	0.383	0.420
60	10.1	21.8	14.4	32.2	0.432	0.444
70	12.7	24.6	16.6	32.4	0.461	0.460
110	17.5	27.7	16.1	27.1	0.504	0.538
160	22.9	30.2	18.6	23.9	0.648	0.633
310	41.8	47.1	20.0	22.9	0.741	0.754
Barley						
0	6.8	9.7				
20	10.2	11.3	22.8	26.1	0.222	0.232
30	11.3	13.0	23.6	27.1	0.314	0.313
40	11.4	13.0	21.7	25.3	0.380	0.391
50	12.0	14.7	18.2	24.3	0.381	0.414
60	13.4	17.2	20.1	27.7	0.448	0.484
70	14.5	17.0	18.4	23.0	0.443	0.472
110	18.8	21.8	19.0	20.8	0.555	0.524
160	22.8	26.1	18.5	22.3	0.650	0.684
310	36.3	36.7	17.9	18.0	0.763	0.762

[a] Sp/Sf = (Rp/Qt)/(R/F).

Table 3. Percentage of infected root length inoculated with *Glomus intraradices* for both soybean and barley species

P rate (mg P/kg^{-1})	Infected root length (%)	
	Soybean	Barley
0	99 a[a]	97 a
30	100 a	78 b
50	100 a	76 bc
70	100 a	77 bc
110	95 a	66 c
310	55 b	71 bc

[a] Mean range by Duncan test: values in each column following by the same letter are not significantly different at the 0.05 level.

35 d of incubation over the 6 rates of P application (Table 4) is: E = 100.2 mg P/kg (CV = 24.5%). The higher CV value for the E value than for the L value is explained by the lower levels in Rs/R and Wt than in Rp/R and Qt for the lowest levels of P application which caused a greater standard error in the Ss/Sf ratio than in Sp/Sf ratio. The same remarks given for the L value are also valid for the E value since many different Ss/Sp values are used in calculation of single E value.

Comparison between Sp/Sf and Ss/Sf values

The calculation of Ss/Sf values by Eq. [11] for the P rates (F) used in the plant experiment gave the following (Ss/Sf–F) data pairs: (0.174–20), (0.245–30), (0.306–40), (0.356–50), (0.405–60), (0.446–70), (0.560–110), (0.637–160) and (0.704–310). The Sp/Sf values were fitted to the above Ss/Sf values by a linear regression:

$$Ss/Sf = -0.05 + 1.04Sp/Sf, \quad R^2 = 0.98 . \quad (12)$$

The regression coefficient (1.04, standard error = 0.06) between Sp/Sf and Ss/Sf values was not significantly different from 1 and the intercept value (−0.05, standard error = 0.029) not significantly different from 0. These results showed that the isotopic composition of P ab-

Table 4. The time dependent changes of experimental data used to calculate Ss/Sf in relation to the rate of P application (F)

F (mgP kg^{-1})		Time (min)						
		1	10	100	1000	10000	28605	50270
20	Rs/R%	43.6	24.6	9.2	2.0	0.40	0.26	0.23
	Wt mg P kg^{-1}	13.0	7.4	3.6	1.1	0.3	0.2	0.2
	Ss/Sf[a]	0.674	0.664	0.529	0.372	0.328	0.262	0.208
40	Rs/R%	53.6	36.0	17.7	4.3	0.74	0.46	0.37
	Wt mg P kg^{-1}	31.0	21.7	11.1	3.4	0.7	0.5	0.4
	Ss/Sf	0.692	0.674	0.635	0.497	0.461	0.352	0.350
80	Rs/R%	65.4	49.0	28.7	8.73	1.68	0.88	0.78
	Wt mg P kg^{-1}	64.3	55.4	33.2	11.0	2.6	1.4	1.3
	Ss/Sf	0.817	0.708	0.691	0.633	0.524	0.506	0.489
160	Rs/R%	76.1	63.9	46.5	23.8	7.6	4.7	3.3
	Wt mg P kg^{-1}	159	127	95.7	45.8	18.8	12.1	9.3
	Ss/Sf	0.763	0.805	0.779	0.832	0.647	0.628	0.570
240	Rs/R%	83.6	73.7	58.4	35.9	16.1	10.7	8.9
	Wt mg P kg^{-1}	254	216	174	97	55.2	37.8	32.1
	Ss/Sf	0.791	0.816	0.804	0.884	0.699	0.682	0.664
280	Rs/R%	86.7	77.8	64.1	43.0	22.8	15.6	13.6
	Wt mg P kg^{-1}	302	270	231	–	90.2	63.5	54.6
	Ss/Sf	0.804	0.808	0.778	–	0.708	0.687	0.691

[a] Ss/Sf = (Rs/Wt)/(R/F).

Table 5. The d and e constants of nonlinear regression: Ss/Sf = d-g.Log(time). R^2 was the proportion of variation accounted for by the above equation. Δ(Ss/Sf) is the difference in Ss/Sf between 1 and 3 month of incubation calculated by the above equation and expressed in % of the Ss/Sf value after 1 month of incubation.

P rate (mgP kg^{-1})	d	g	R^2	Δ(Ss/Sf) (%)
20	0.716	0.045	0.96	21.0
40	0.737	0.034	0.94	10.0
80	0.808	0.029	0.98	6.4
160	0.799	0.017	0.81	3.0
240	0.828	0.014	0.83	2.3
280	0.821	0.012	0.95	1.9

sorbed by the plants was identical to the isotopic composition of P in soil solution, in accordance with previous data (Fardeau and Jappe, 1976).

The identity between Sp/Sf values and Ss/Sf values at steady state meant that the soil P involved in plant nutrition has the same isotopic composition as P in soil solution. As the contribution of P in soil solution by mass flow process represents only a few percent of the plant P uptake (Barber, 1984), 99 to 90% of the P taken up by plant comes from the P in the solid phase of soil which equilibrates with P in soil solution, and has the same specific activity as P in soil solution after 35 d of exchange or beyond. For instance, after applying 20 mgP kg^{-1}, the dry matter yields of non-mycorrhizal soybean was 2.21 g/0.5 kg and soil solution P, 0.02 mg P L^{-1} (Table 4). Assuming the plant transpires about 0.3 L of water during the synthesis of each 1 g of dry matter, the P supply by mass-flow process is 0.01 mg P which represent according to the data in Table 2, 0.2% (0.01*100/4.7) of the P uptake. More than 99% of the P uptake must have been replenished from the solid soil phase by diffusion

processes, and the replenished P is derived from the isotopically exchangeable P because of the identity of isotopic composition between P in the plants and that in the soil solution. In this example, P in soil solution was replenished 470 (4.7/0.01) times to obtain the P amounts taken up by non-mycorrhizal soybean. The results obtained in the present experiment also indicates that the role of mycorrhizal fungi in the uptake of P by plants is to give a much wider access to isotopically exchangeable P in soil either by explorating a larger soil volume and by increasing movement of P into mycorrhizal hyphae (Bolan, 1991), and not rendering previously non-isotopically exchangeable soil P plant-available.

The usual approach to the characterization of soil-plant relationships in plant nutrition was to correlate Qt vs. E or L values over a great number of soil-plant combinations. The present data showed that with one single L or E value many Qt values are associated which differ widely according to the plant factors involved in P nutrition such as the crop species or the AM inoculation which is often an uncontrolled factor. But it appears possible to establish a 1:1 correspondence between a plant parameter, the Sp/Sf ratio, and a soil parameter, the Ss/Sf ratio after or beyond 35 d of exchange. This 1:1 relationships between soil and plant parameters justifies the measurement of the Ss/Sf ratio (Russell's E value) rather than the Sp/Sf ratio (Larsen's L value) because it is a more simple, rapid and less time-consuming methodology. This 1:1 correspondence between isotopic composition of P in plant and isotopically exchangeable P is also an evidence that available soil P to plants is the isotopically exchangeable P.

Agronomic application of the proposed methodology

Examination of the Sp/Sf ratio [(Rp/Qt)/(R/F)] shows that it is the amount of P from applied P taken up by plant [F(Rp/R)] divided by the total amount taken by plant (Qt), i.e. the proportion of P derived from fertilizer taken up by plants (Sp/Sf = Qf/Qt). This parameter generally expressed as a percentage has been intensively used since the first ^{32}P radiotracer studies (Dean et al., 1947). In the present study, the Sp/Sf values increase from 0.17 to 0.76 when the rate of applied P increases from 20 to 310 mgP kg^{-1} (Table 2). In the same way, the Ss/Sf ratio (FRs/RWt) is the amount of P from fertilizer in soil solution (Wf) divided by the total of P in soil solution (Wt), i.e. the proportion of P in soil solution derived from fertilizer (Ss/Sf = Wf/Wt). Because the Sp/Sf and Ss/Sf values are not significantly different at a given rate of application, Ss/Sf value can be considered as a predictive value for Sp/Sf. Consequently, the proposed methodology has an immediate application in the field of the agronomic evaluation of P fertilizers.

The usual approach in the agronomic evaluation of P fertilizer is (Terman and Engelstad, 1976): (i) to establish the response curve, i.e. the relation between the yield or the plant uptake and increasing rates of P application for different forms of P fertilizers; (ii) to compare the regression constants of the equation fitted to the response curves; and (iii) to express the results in relation to a standard form of P fertilizer which is water-soluble in order to calculate the Relative Agronomic Effectiveness (RAE) of the tested P fertilizer. Although the factors involved in the RAE of the various P fertilizer forms relating to soil properties have been studied more than a century, it is apparent in the literature on the agronomical evaluation of P fertilizers that it is still an actual agronomic problem since the sets of RAE values of a given P form are often contradictory (Bolland and Gilkes, 1987). Many methodological factors can explain this discrepancy, but it basically indicates this approach doesn't take into account the complexity of the process and the interactions involved between the rate of P application and its effects on agronomic response. Both soil and plant parameters are involved in the determination of RAE, with plant parameters varying with crop species and cultivar and with soil parameters varying with soil type. Because of synergestic and antagonistic interactions of soil-fertilizer combination with the plant factors involved in the plant P uptake (Morel and Fardeau, 1989, 1990), the RAE value is closely dependent on the plant-soil-fertilizer combination. This explains why it is impossible to extend a given information to another plant-soil-fertilizer situation. Accurate information on ag-

ronomical evaluation of P fertilizer is however essential to give adequate P fertilizer recommendations.

The Sp/Sf values are not dependent upon plant factors and furthermore agreement between Sp/Sf and Ss/Sf values is close to 1:1. This means that Ss/Sf value predicts the participation of phosphorus derived from fertilizer in plant nutrition and can therefore be considered an agronomic evaluation of P fertilizers. This is the fundamental agronomical interest of this approach. It is independent of plant factors, unlike the usual agronomic evaluation, and depends only on the combination of soil and fertilizers. Furthermore, the measurement of Ss/Sf does not require crop experiments facilitates the gathering of information about numerous soil-fertilizer combinations for a better understanding of the relationships between soil properties and agronomical effects of various P fertilizer forms in plant nutrition.

Conclusion

After (i) diluting a ^{32}P labelled solution of water-soluble P fertilizer in a loamy sterilized soil and (ii) cropping both non-mycorrhizal or mycorrhizal soybean and barley, the isotopic composition of plant P was not significantly different between (crop species × mycorrhizal inoculation) treatments although the plant P uptake differed greatly. The specific activity of P taken up by plant was also identical to the specific activity of P in soil solution after about 1 month of incubation, confirming previous results obtained in calcareous soil (17% $CaCO_3$) and ryegrass as a test plant. Two main conclusions can be derived from the above results:

1. soil available P in loamy soils is the isotopically exchangeable phosphate in soil.
2. the agronomical significance of this result is that it is possible to predict the participation of P fertilizer in plant nutrition only by determining the P in soil solution derived from fertilizer which can consequently, be considered as an agronomical evaluation of P fertilizer.

The proposed methodology requires measurement of Ss/Sf value which must be representative of Sp/Sf. In the present study, where a loamy soil was tested, and in a previous one, where a calcareous soil was tested (Morel and Fardeau, 1991), Ss/Sf did not significantly change with time after about 1 month of equilibration in 1:10 soil:solution suspension. But, as the rate of reaction between applied P and soil particles was increased when temperature of reaction increased (Barrow and Shaw, 1975), it might be interesting to verify if an increase in temperature could faster the reaction on Ss/Sf values in order to develop a more rapid procedure. Another possibility could be to measure Ss after a relatively short period in soil incubated at similar moist conditions as plant experiments because it has been shown that pseudo-equilibrium in Ss is more quickly reached when the soil:solution ratio increases. Further work is also required to determine the validity of this result across different soil types, especially in acidic soils with high P sorption capacity, and for other crop species like, particularly those like rape which are well known for their ability to mobilize P from rock phosphate (Hoffland, 1992).

Acknowledgements

We sincerely acknowledge Dr Jean-Claude Fardeau from Commissariat à l'Energie Atomique and Dr John Stewart and Dr Holm Tiessen from the University of Saskatchewan, Soil Science Department, for their critics and suggestions on this manuscript. We also express our gratitude to Dr R L Bieleski for his review and his helpful comments.

References

Barber S A 1984 Soil Nutrient Bioavailability. A mechanistic approach. Wiley Interscience and Sons, New-York. 398 p.

Barrow N J and Shaw T C 1975 The slow reactions between soil and anions: 2. Effect of time and temperature on the decrease in phosphate concentration in the soil solution. Soil Sci. 119, 167–177.

Blal B, Morel C, Gianinazzi-Pearson V, Fardeau J C and Gianinnazi S 1990 Influence of vesicular-arbuscular mycorrhizae on phosphate fertilizer efficiency in two tropical acid

soils planted with micropropagated oil palm (*Elais guineensis* Jacq.). Biol. Fertil. Soils 43, 43–48.

Bolan N S, Robson A D, Barrow N J and Aylmore L A G 1984 Specific activity of phosphorus in mycorrhizal and non-mycorrhizal plants in relation to the availability of phosphorus to plants Soil Biol. Biochem. 16, 299–304.

Bolan N S 1991 A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134, 189–207.

Bolland M D A and Gilkes R J 1987 How effective are calciphos and phospal? Fertil. Res. 12, 229–239.

Dean L A, Nelson W L, Mackenzie A J, Armiger W H and Hill W L 1947 Application of radioactive tracer technique to studies of phosphatic fertilizer utilization by crops. Soil Sci. Soc. Am. Proc. 12, 107–112.

Fardeau J C and Guiraud G 1972 Determination of available soil and fertilizers phosphorus by the isotopic dilution method. Phosphorus in Agric. 60, 19–26.

Fardeau J C and Jappe J 1976 Nouvelle méthode de détermination du phosphore assimilable du sol par les plantes: extrapolation des cinétiques de dilution isotopique. C.R. Séances Acad. Sci. série D, 282, 1137–1140.

Fardeau J C 1981 Cinétique de dilution isotopique et phosphore assimilable des sols. Thèse de Doctorat d'Etat, Université P. et M. Curie, Paris 6, 198p.

Fardeau J C 1993 Le phosphore biodisponible du sol: un système pluricompartimental à structure mamellaire. Agronomie 1, 1–13.

Fox R L and E J Kamprath 1970 Phosphate sorption isotherm for evaluating the phosphate requirements of soil. Soil Sci. Soc. Am. J. 70, 902–906.

Fried M and Dean L A 1952 A concept concerning the measurement of available soil nutrients. Soil Sci. 73, 263–271.

Giovanetti M and Mosse B 1980 An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.

Hayman D S and Mosse B 1972 Plant growth response to vesicular-arbuscular mycorrhiza. III. Increased uptake of labile P from soil. New Phytol. 71, 41–47.

Hewitt E J 1966 Sand and Water culture Methods used in the Study of Plant Nutrition. Tech. Comm. 22 (2nd revised Edition). Commonwealth Agricultural Bureaux, London.

Hoffland E 1992 Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. Plant and Soil 140, 279–289.

John M K 1970 Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. Soil Sci. 68, 171–177.

Kalra Y P and Soper R J 1968 Efficiency of rape, oats, soybeans and flax in absorbing soil and fertilizer phosphorus at seven stage of growth. Agron. J. 60, 209–212.

Kalra Y P 1971 Different behaviour of crop species in phosphate absorption. Plant and Soil 34, 535–539.

Kamprath E J and Watson M E 1980 Conventionnal soil and tissue tests for assessing the phosphorus status of soils. *In* the Role of Phosphorus in Agriculture. Eds. F E Khasawneh, E C Sample and E J Kamprath. pp 433–469. ASA Madison, WI.

Larsen S 1952 The use of ^{32}P in studies on the uptake of phosphorus by plants. Plant and Soil 4, 1–10.

Memon K S and Fox R L 1983 Utility of phosphate sorption curves in estimating the phosphorus requirements of cereal crops: wheat (*Triticum aestivum*). *In* Comptes Rendus du 3eme Congres sur les Composes Phosphates. pp 217–230. Imphos Casablanca, Morocco.

Morel C and Fardeau J C 1989 Native soil and fresh fertilizer phosphorus uptake as affected by rate of application and P fertilizers. Plant and Soil 115, 123–128.

Morel C and Fardeau J C 1990 Uptake of phosphate from soils and fertilizers as affected by P availability and solubility of phosphorus fertilizers. Plant and Soil 121, 217–224.

Morel C and Fardeau J C 1991 Phosphorus bioavailability of fertilizers: a predictive laboratory method for its evaluation. Fertil. Res. 28, 1–9.

Nelson W L, Krakntz B A, Colwell W E, Woltz W G, Hawkins A, Dean L A, Mackenzie A J and Rubins E J 1947 Application of radioactive tracer technique to studies of phosphatic fertilizer utilization by crops: field experiments. Soil Sci. Soc. Am. Proc. 12, 113–118.

Plenchette C, Fortin A and Furlan V 1983 Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. mycorrhizal dependency under field conditions. Plant and Soil 70, 199–209.

Powell C L 1975 Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Uptake of P by onion and clover infected with different Endogone spore types in ^{32}P labelled soils. New Phytol. 75, 563–566.

Russell R S, Rickson J B and Adams S N 1954 Isotope equilibrium between phosphate in soil and their significance in the assessment of fertility by tracer methods. J. Soil Sci. 67, 1–16.

SAS Institue Inc 1987 SAS/STAT Guide for personal computer, Version 6 Edition. Cray, NC: SAS Institute Inc. 1028 p.

Sanders F E and Tinker B P 1971 Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature 233, 278–279.

Soper R J and Kalra Y P 1969 Effect of mode of application and source of fertilizer on phosphorus utilization by buckwheat, rape, oats and flax. Can. J. Soil Sci. 49, 319–326.

Terman G L and Englestad O P 1976 Agronomic Evaluation of Fertilizers: Principles and Practices. Bull. Y-21 TVA, Muscle Shoals, Alabama. 45 p.

Welch C D, Hall N S and Nelson W L 1949 Utilization of fertilizer and soil phosphorus by soybeans. Soil Sci. Soc. Am. Proc. 13, 231–235.

Section editor: H Lambers