MONTANA STATE UNIVERSITY

Phosphorus uptake from insoluble soil sources by five forage legumes, winter wheat, and buckwheat
by Robert Mark Jacques

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Abstract:
The absorption and utilization of phosphorus from insoluble tricalcium phosphate compounds commonly found in Montana soils by alfalfa (Medicago sativa L.), sainfoin (Onobrychis viciifolia Scop.) , yellow sweetclover (Melilotus officinalis L.), red clover (Trifolium pratense L.), subterranean clover (Trifolium subterranean L.), winter wheat (Triticum aestivum L.), and buckwheat (Fagopyrum esculentum Moench) were studied.

Legume seedlings in general had higher root surface area indexes (RSAI = wt of H2O removed from root surface/unit of root dry wt) then winter wheat and buckwheat seedlings. Ranking of species for root cation exchange capacity (CEC) depended on whether it was expressed on a dry wt basis or on a RSAI basis. Root CEC per unit of dry wt was significantly correlated with RSAI for all species. Since the density of cation exchange sites along a root surface normally varies little within a species, these correlations indicate that increases in root CEC per unit of dry wt within a species were likely a function of increased RSAI.

Sainfoin, yellow sweetclover, alfalfa, and buckwheat all responded well to insoluble calcium-phosphate P sources. Yellow sweetclover absorbed more P and had a higher % P in its tissue than the other forage legumes. Sainfoin absorbed less P from fluorapatite than both yellow sweetclover and alfalfa, but more efficiently translocated P absorbed from fluorapatite from roots to tops. Winter wheat roots absorbed considerable P from fluorapatite, but were very inefficient in translocating this P to tops. Yellow sweetclover, alfalfa, and sainfoin produced maximum dry matter while feeding on either pure fluorapatite or rock phosphate in small, restrictive containers, but only sainfoin responded to rock phosphate when the root density was much less.

RSAI generally increased as available P became more limiting for the forage legumes, but not for winter wheat and buckwheat. When no P was present, the density of cation exchange sites along the root surface decreased dramatically for yellow sweetclover, alfalfa, red clover, and subterranean clover, but not for sainfoin. An inverse relationship between root exocellular acid phosphatase activity and dry matter production occurred for most species. Forty-five sainfoin seedlings exhibited a wide range of rhizosphere pH values when subjected to P stress. Rhizosphere pH changes in response to P stress and Ca accumulation by plants from insoluble calcium-phosphate compounds appeared to be the most promising indicators of a plant's P feeding power from rock phosphate.

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Chairperson, Graduate Committee

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ABSTRACT

The absorption and utilization of phosphorus from insoluble tricalcium phosphate compounds commonly found in Montana soils by alfalfa (*Medicago sativa* L.), sainfoin (*Onobrychis viciifolia* Scop.), yellow sweetclover (*Melilotus officinalis* L.), red clover (*Trifolium pratense* L.), subterranean clover (*Trifolium subterranean* L.), winter wheat (*Triticum aestivum* L.), and buckwheat (*Fagopyrum esculentum* Moench) were studied.

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INTRODUCTION

Although the total phosphorus content of many soils is relatively high, much of this P is commonly found in the form of insoluble phosphate minerals. Minerals containing insoluble P can be mined from the soil and degraded to available P fertilizer, but this process is expensive and requires a considerable use of fossil energy. Therefore, the development of plants capable of utilizing unavailable soil P is desirable.

Renewed interest in the ability of cultivated forage legumes to absorb P from insoluble soil sources resulted from observations that sainfoin (*Onobrychis viciifolia* Scop.), a forage legume grown in the Rocky Mountain region, seldom responded to P fertilization. Quite often, on the same soil, alfalfa (*Medicago sativa* L.) responds to P applications, but sainfoin does not. This lack of response to P applications by sainfoin indicated that it was absorbing P from existing soil sources.

This study investigates the absorption and utilization of P from insoluble tricalcium-phosphate compounds commonly found in Montana soils by alfalfa, sainfoin, yellow sweetclover (*Melilotus officinalis* L.), red clover (*Trifolium pratense* L.), subterranean clover (*Trifolium subterranean* L.), winter wheat (*Triticum aestivum* L.), and buckwheat (*Fagopyrum esculentum* Moench). My objectives were to rank these species according to their P feeding power from insoluble sources, determine what factors make some plants better feeders of insoluble P sources than others, and possibly develop a simple screening technique to distinguish good P feeders.

My results are reported in the following three chapters. The first chapter describes a relatively unknown method of estimating the surface area of an intact root system. The relationship between root surface area and root cation exchange capacity is also discussed. Chapter II deals with growth responses, P uptake rates, and root to top translocation efficiencies of P using fluorapatite as the sole source of P. The third chapter describes several plant characteristics which may enhance P uptake from rock phosphate. Possible screening techniques which might be used to distinguish good P feeders are also considered.

I. ROOT SURFACE AREA AND ITS RELATIONSHIP TO ROOT CATION EXCHANGE CAPACITY

INTRODUCTION

Root development is usually evaluated as a change in dry wt, moist wt, or volume. These measurements are often inadequate for plant nutrition studies. Root surface area is often a more important measurement, but is seldom used because it is difficult to measure accurately. Raper and Barber (10) stressed the importance of root surface area in determining the efficiency of seedling roots as nutrient absorptive surfaces. With relatively immobile nutrients such as P, increased root surface area could greatly enhance the efficiency of utilization (3). Root cation exchange capacity (CEC) measurements expressed per unit of dry wt are often inaccurate unless adjusted for differences in surface area per unit of dry wt (11).

Four methods used which estimate root surface area are: i) the titration method (12) in which root cation exchange sites are saturated with H^+ ions and subsequently titrated with a weak base, which is similar to the acid-washing technique (1) used to measure root CEC; ii) the gravimetric method (4) in which a beaker of $Ca(NO_3)_2$ solution is weighed, roots then dipped into it and lifted above the beaker to drain, and the remaining solution weighed to determine the amount of $Ca(NO_3)_2$ which was absorbed by the root surface; iii) calculations of root surface area from length and diameter measurements at representative segments of a root (1, 2, 5, 11); and iv) the differential centrifugation method (8) in which partial H_2O shells are removed from root surfaces and weighed.

Linford and Rhoades (9) reported a similar application, using the spinning tub of an automatic washing machine as a low speed centrifuge to remove excess surface H_2O from washed roots before determining moist weights. The differential centrifugation technique assumes that one can consistently remove a uniform fraction of the surface H_2O layer adhering to a root. Jeffrey (8) listed three basic assumptions that must be met for the technique to be accurate:

i. The surface tension and viscosity of surface H_2O films must be similar on roots to be compared;

ii. The centrifugal force generated at a given rpm will equilibrate with surface tension forces, leaving a uniform H_2O film adhering to the root surface; and

iii. H_2O can be removed from small capillary pores between root hairs.

Thus, if H_2O-saturated roots are centrifuged at a low force, followed by a higher force, the weight of H_2O spun off during the second centrifugation will be proportional to root surface area.

Root surfaces possess a net negative charge which attracts cations, but the charge density (charge per unit surface area) varies among plant species (6). Crooke et al. (5) found that the charge density of a root was highest at the root tip, possibly due to higher pectin content. They found that root sections from three segments above the root tip differed in CEC expressed on a dry wt basis but not on a surface area basis, which illustrates possible misinterpretations that can occur if the dry wt basis is used without caution. An accurate, reproducible means of estimating root surface area is needed to express root CEC on a surface area basis. The objectives of this experiment were:

1. To evaluate the differential centrifugation method of estimating root surface area; and
2. To evaluate the relationship between root CEC per unit of dry wt and root surface area index (RSAI = wt of H_2O removed from root surface/unit of root dry wt).

MATERIALS AND METHODS

Five forage legumes, alfalfa, sainfoin, yellow sweetclover, red clover and subterranean clover, and two check species, winter wheat and buckwheat, were pre-germinated on moist blotter paper for one day and transplanted to individual plastic test tubes (16 cm x 2.4 cm) filled with washed silica gravel. Seedlings were grown in a controlled environment chamber at 21$\pm$1C with a photoperiod of 16 hr at a photosynthetic photon flux density of 280 uEinsteins m^{-2} sec^{-1} provided by incandescent and fluorescent lights. Roots were harvested separately 35 days after germination of the forage legumes and 25 days after germination of winter wheat and buckwheat.

A gradient of P stress was established by differing levels of applied water-soluble and water-insoluble P. Each species was grown using 3 different P treatments in a randomized complete block design with 6 replications.

1) Three hundred forty mg of screened Idaho rock phosphate, provided by the J. R. Simplot Co., Pocatello, Idaho, were thoroughly mixed with washed silica gravel and the mixture added to each plastic test tube. Seedlings were watered every other day with a modified -P Hoagland's nutrient solution (7) containing, in mg/liter, N, 84(73 as NO_3 and 11 as NH_4); K, 68; Ca, 60; Mg, 12; S, 16; Cl, 0.8; B, 0.5; Mn, 0.5; Zn, 0.05; Cu, 0.02; Mo, 0.01; and Fe, 1.0 added as Fe.EDTA. The nutrient solution was adjusted to pH 6.0 using 1.0 N H_2SO_4 before use. The

gravel medium was flushed periodically with distilled water to avoid salt accumulation.

2) Seeds were planted in individual plastic test tubes filled with pure washed silica gravel and watered with the same nutrient solution as in treatment 1. No source of P was present.

3) Seeds were planted in individual plastic test tubes filled with pure washed silica gravel. Seedlings were watered with a complete nutrient solution including 8 mg/liter of water-soluble P in addition to the nutrients listed in treatment 1.

Root Surface Area

RSAI was determined as follows:

1. Wash roots gently with distilled H_2O to remove any adhering sand particles.
2. Equilibrate roots in distilled H_2O for 10 min.
3. Place roots in wire mesh basket in a 50 ml centrifuge tube (Figure I-1).
4. Balance tubes to constant wt by adding distilled H_2O and secure cap.
5. Centrifuge at 111 x g (center of tube) for 7 min.
6. Weigh wire mesh basket with roots inside on an analytical balance accurate to 0.1 mg.
7. Replace wire mesh basket in tube, secure cap, and centrifuge at 344 x g for 7 min.

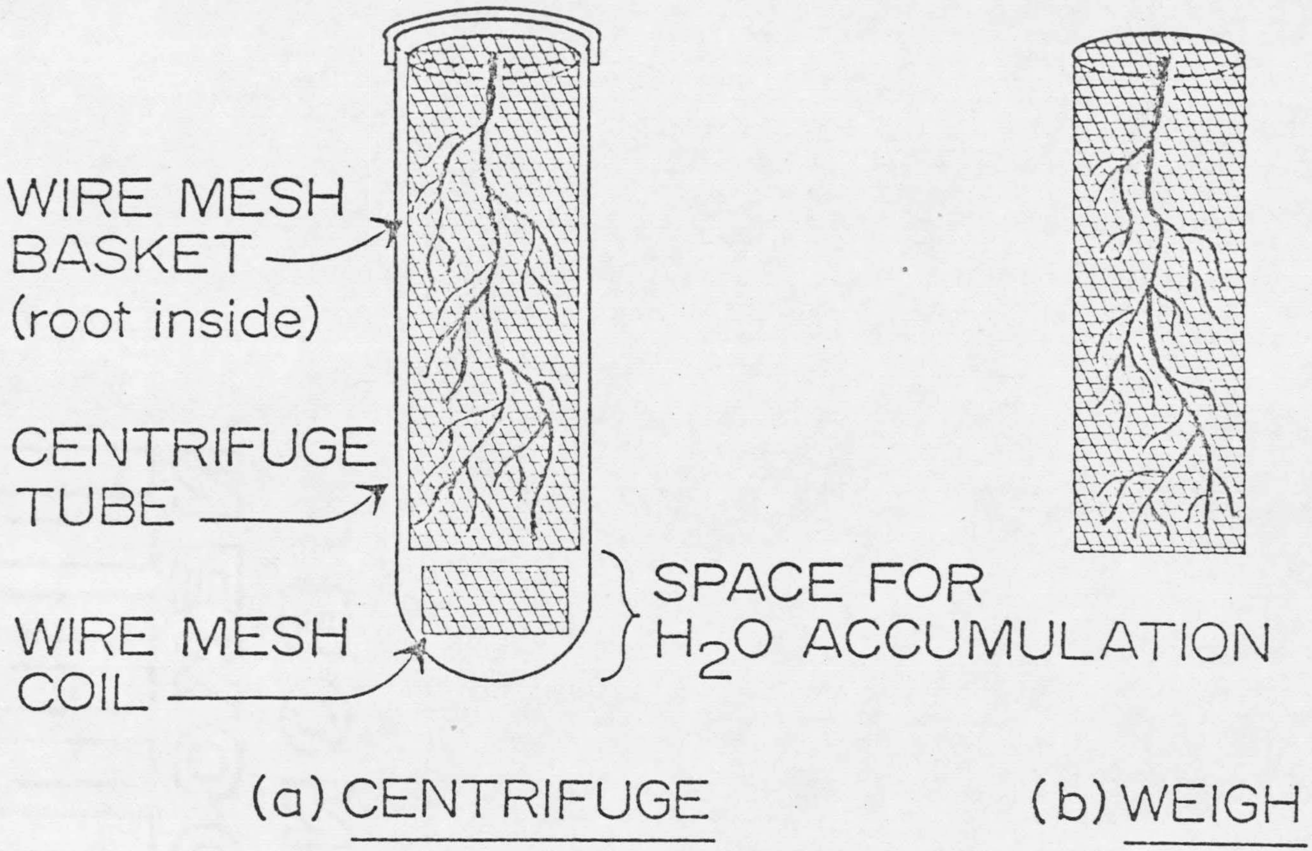

Figure I-1. Diagram of differential centrifugation technique: (a) roots inside wire mesh basket are centrifuged in a 50 ml capped centrifuge tube, and (b) only wire mesh basket with roots inside are weighed after centrifugation.

8. Weigh wire mesh basket with roots inside again.

   Difference in two wts = mg H_2O spun off root which is proportional to total root surface area.

9. Oven dry roots at 70C and weigh to obtain dry wt.

10. Calculated RSAI. RSAI = mg H_2O spun off roots/mg root dry wt.

<u>Root CEC</u>

The acid-washing technique of determining root CEC (1) was modified for small sample sizes (100-500 mg moist wt) and used as follows:

1. Wash roots gently with distilled H_2O to remove any adhering sand particles.
2. Allow excess H_2O to drain off roots.
3. Acid-wash roots in 100 ml refrigerated 0.1 N HCl for 5 min and rinse with distilled H_2O.
4. Repeat acid-wash 3 times with a thorough rinsing after the last acid-wash.
5. Allow roots to drain of excess H_2O.
6. Place roots in 50 ml of neutral, 1.0 N KCl.
7. Titrate to pH 7 over a 5 min period with .001 N KOH.
8. Rinse roots with distilled H_2O.
9. Oven dry roots at 70C and weigh to obtain dry wt.
10. Calculate root CEC.

$$\frac{\text{ml titrant (KOH) x .001 N}}{\text{g root dry wt}} \times 100 = \text{CEC (meq/100 g dry wt)}.$$

$$\frac{\text{ml titrant (KOH)}}{\text{RSAI}} = \text{CEC (meq/RSAI)}.$$

Root CEC and RSAI were measured on all samples. The order of measurement is not critical since neither measurement is influenced by small changes in metabolism that might occur during the experiment.

RESULTS AND DISCUSSION

Root Surface Area

Although fibrous-root systems are generally thought to have a larger root surface area/dry wt ratio than tap-root systems, the opposite may be true for seedlings. In this study, the five forage legumes and winter wheat were found to have higher RSAI than buckwheat (Table I-1). Most legume seedlings have a fine tap-root system during the first few weeks of growth. Winter wheat exhibits a fine fibrous-root system as a seedling, while buckwheat has a coarse fibrous system.

Abed and Hassan (1) reported that the average diameters of pea and clover roots were larger than corn and barley roots on plants sampled 35 days after germination. Surface areas calculated from length and diameter measurements at representative root segments appeared smaller per unit of fresh or dry wt for legumes than for grasses; however, differences between means were not tested for significance. My data show that legumes in general had higher RSAI than winter wheat and buckwheat during the seedling stage before a dominant tap-root was established (Table I-1). The discrepancy between their results and mine may be due to sampling technique. Root surface area is difficult to calculate since a large proportion of surface area is likely associated with very small lateral roots and root hairs, which are often ignored in calculating surface areas because of the difficulty involved in measuring them. I believe the differential centrifugation method is more reliable

TABLE I-1. ROOT SURFACE AREA INDEX (RSAI) AND ROOT CEC EXPRESSED PER UNIT OF DRY WT AND PER UNIT OF RSAI FOR FIVE FORAGE LEGUMES, WINTER WHEAT, AND BUCKWHEAT.

		Root CEC	
Plant[1]	RSAI	meq/100 g dry wt	meq/RSAI
Subterranean clover	4.14 a[2]	42.7 b	7.7 bc
Sainfoin	3.93 a	58.7 a	10.1 ab
Red clover	3.34 ab	33.1 c	8.3 ab
Yellow sweetclover	3.19 ab	31.8 c	8.8 ab
Alfalfa	2.45 bc	29.3 c	11.5 a
Winter wheat	2.57 b	13.4 d	4.5 c
Buckwheat	1.54 c	26.9 c	8.4 ab

[1]Plants were grown without nutrient stress.

[2]Values within a column followed by a common letter are not significantly different at the 5% level.

because the entire surface area of an intact root system is measured.

Although Jeffrey (8) reported large differences in total surface area of roots with evident morphological differences, he did not establish the validity of the differential centrifugation method used to measure root surface area. It is important that H_2O is removed only from the shell surrounding the root surface during the centrifugation and not from inside the epidermis. Roots that were centrifuged at increasing forces were examined for possible plasmolysis. With the higher centrifugal force used (344 x g), no plasmolysis or excessive drying of small roots occurred. The forces used removed only part of the H_2O shell surrounding a root without damaging it.

This method depends upon exact duplication of the technique for all samples being compared. All centrifugation and weighing steps must be timed and reproduced accurately. Centrifuge speeds must be repeated accurately since small changes in force applied will have large effects on the amount of H_2O removed from the surface of a root. An accurate tachometer should be used to duplicate speeds. Baskets with roots inside should be weighed as quickly as possible after removal from the centrifuge tubes since equilibration with the atmosphere occurs rapidly. Several procedural problems that may cause errors are:

1. Centrifuge tubes not balanced to constant wt.
2. Centrifuge speeds not accurately repeated. A strobe tachometer is helpful in reproducing speeds.

3. Centrifugation steps not accurately timed.

4. Inconsistency during weighing. For example, taking 30 sec to weigh one basket and only 10 sec to weigh another.

5. Comparing surface areas of roots that have large differences in size.

RSAI among roots within a plant species can be expected to vary because of differences in root morphology within a species. Comparisons of surface areas determined by this method are most valid within a species since chemical differences among roots of different species will affect such properties as surface tension and viscosity of surface H_2O films surrounding a root. Although no totally acceptable method of measuring root surface area has been developed, I believe that this method of measuring RSAI provides a meaningful indicator of root morphology and warrants further study.

Root CEC

The amount of pectic material in cell walls at the root surface accounts for a large percentage of root CEC (5). Therefore, root CEC should be related to the density of carboxyl groups (potential exchange sites) arising from pectic material at the root surface. If the density of exchange sites is related to cell wall structure, then charge density per unit of root surface area would not be expected to vary much within a species unless cell wall structure is somehow appreciably changed. However, root CEC expressed per unit of dry wt often varies within a root system.

Root surface area/dry wt ratios change considerably throughout a root system. As the ratio increases, the number of cation exchange sites per unit of dry wt also increases if charge density per unit of surface area remains constant (Figure I-2). In other words, root CEC expressed per unit of dry wt will increase without a consequent increase in charge density per unit of surface area. If root CEC is to be used as an indicator of charge density, it should be expressed on a surface area basis. Root CEC expressed per unit of RSAI should truly indicate the density of potential exchange sites along a root surface.

The magnitude of values for root CEC depended on whether being expressed per unit of dry wt or per unit of RSAI (Table I-1). For example, root CEC per unit of dry wt for sainfoin was greater than that of alfalfa, but when expressed per unit of RSAI the reverse was true (Figure I-3).

In order to obtain roots within a species having a range of RSAI values, plants were grown under a gradient of P stress established by differing levels of applied soluble and insoluble P. Root CEC per unit of dry wt was significantly correlated with RSAI for all plants (Table I-2). Correlation coefficients were higher for the forage legumes than for winter wheat and buckwheat. Regression coefficients varied among species and were highest with red clover and lowest with subterranean clover.

The significant correlations between RSAI and root CEC per unit