# ADVANCING THE UNDERSTANDING AND INTERPRETATION OF PLANT AND SOIL TESTS FOR PHOSPHORUS IN MANITOBA

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

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## ABSTRACT

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Selected factors proposed to be limiting the effectiveness of the assessment of plant and soil P status by current testing methods were studied, to provide information for use in improving the processes and interpretations.

Growth and P status of field-grown spring wheat was monitored under a wide range of conditions. Plant P deficiency restricted growth early in the season, beginning one to two weeks after crop emergence, even where seed-placed P fertilizer was applied. However, within two to five weeks from emergence, shoot relative growth rates in the P-deficient treatments were at least as high as those in the high-P treatments. Plant analysis was most successful for predicting yield-limiting P deficiency if conducted during the first several weeks of growth. Determination of tissue total P concentration in the leaf or whole shoot, and of extractable inorganic leaf P concentration, could provide a good basis for assessing plant P status; comprehensive interpretive criteria were developed for each test. Leaf P concentrations generally declined until the stem extension stage, but shoot P concentrations declined throughout the growing season. For each test, concentrations tended to converge with time among sites and treatments.

Examination of spatial variability of extractable P in four field soils revealed a high degree of variability over very short distances (1-2 cm), even where fertilizer had not recently been

applied. Persistence of small localized zones high in P availability may enhance residual fertilizer P uptake by plants.

Extractability of fertilizer residual P compounds by the Olsen $NaHCO_3$ soil testing procedure was studied. Although the most highly soluble compounds dissolved rapidly, added octacalcium phosphate did not dissolve with soil present. Soil solutes, including $Mg^{2+}$, $Fe^{2+}$, and others, greatly retarded the dissolution. Octacalcium phosphate is an important P fertilizer reaction product in Manitoba soils and is moderately available to plants; its failure to dissolve may limit the effectiveness of the Olsen test in fully reflecting the residual availability of recently applied P fertilizer to crops. The simple solubility of a compound in a pure soil test extractant may not be a good indicator of the degree to which it will be recovered by the test in the presence of soil.

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My wife Terry has been a constant source of support and understanding in my decision to enter the doctoral program, during the studies and project work, and during the protracted period of thesis preparation. I am greatly indebted to her and to our children (Daniel, Adam, and Louisa) for their sacrifices to accommodate my completion of this work.

And my parents, for support in every way. I'm finished going to school now, Mom.

## FOREWARD

The presentation of this thesis is in manuscript format. None of the manuscripts have been published or submitted for publication in a refereed journal, as of January 2000. Portions of sections #3 - 6 have been presented at conferences with published proceedings. It is intended that sections #3, 4, and 6 will be submitted for publication as papers (#3 and 4 as companion papers), and section #5 as a note; specific journal in each case to be determined.

All the work presented herein is primarily the work of the author, in consultation with the major advising professor.

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| | |
|---|---|
| ATP | adenosine triphosphate |
| $CaCl_2$-P | inorganic P extractable with $CaCl_2$ solution |
| CNC | critical nutrient concentration |
| DAE | days after emergence |
| DCPD | dicalcium phosphate dihydrate |
| DMPT | dimagnesium phosphate trihydrate |
| DNA | deoxyribonucleic acid |
| dwb | dry weight basis |
| fwb | fresh weight basis |
| HAP | hydroxyapatite |
| MAP | monoammonium phosphate |
| $NaHCO_3$-P | inorganic P extractable with $NaHCO_3$ solution (Olsen procedure) |
| OCP | octacalcium phosphate |
| OM | organic matter |
| $P_i$ | inorganic P in orthophosphate form |
| $P_{id}$ | inorganic P as determined on the dried leaf samples; dry tissue weight basis |
| $P_o$ | organic P |
| $P_t$ | total P |
| RNA | ribonucleic acid |
| RY | relative yield |
| TMP | trimagnesium phosphate 22-hydrate |
| XRD | x-ray diffraction |

# 1. INTRODUCTION

Soil testing to predict fertilizer requirements of crops for the major plant nutrients, including phosphorus (P), has been in widespread use on the Canadian Prairies since the 1960's. Test correlation and calibration work, mostly from the 1950's and 60's, resulted in a recommended soil P test ($NaHCO_3$) for Manitoba and Saskatchewan and interpretive criteria which have changed little with time.

Only a small fraction of fertilizer P is taken up by the crop in the year of application. Most remains in the soil ("residual P") and can contribute to the P available to subsequent crops. Consequently, with time crops draw their P increasingly from fertilizer residual sources in the soil and less from native soil P. A great deal has been learned in the last four decades about the reactions of fertilizer P with the soil (Sample et al. 1980), and the longer-term form and plant-availability of residual P. However, there is concern that the $NaHCO_3$ test may not reflect the plant-availability of residual P as effectively as the availability of native soil P.

Plant analysis to determine the nutrient status of plants has been a tool available to Prairie producers almost as long as soil testing. Although plant analysis can more directly indicate the nutrient status of plants, its use for annual field crops is more limited than soil testing because most nutrients must be applied before plant test results can be obtained. The more definitive diagnostic function of plant analysis for assessing current plant nutrient status can complement the predictive role of soil testing. However, for plant analysis on the Canadian prairies, little test developmental and calibration work has been carried out to determine the best approaches and

interpretive criteria. In the case of cereal plant tissue P tests, results have tended to fall within a relatively narrow range, making diagnosis often inconclusive.

The studies that follow were designed to improve plant and soil testing for P through increased understanding of 1) plant P in field-grown cereals, and of 2) factors influencing the nature and availability of soil and fertilizer residual P and their dissolution during soil test extraction. In particular, the growth and P status of field-grown wheat was monitored through the season by a number of measurements and under a wide range environmental and P availability conditions. Specific soil and residual P studies included the characterization of micro-scale spatial variability of extractable P in field soils, investigation of the dissolution of P fertilizer reaction products under soil test extraction conditions, and determination of soil Ca:Mg ratio effects on P availability and extractability.

# 2. INTRODUCTORY LITERATURE REVIEW

## 2.1 Plant P

### 2.1.1 Plant P Requirements

Phosphorus is classified, along with N and K, as a major plant nutrient, even though concentrations of P in plants are more similar to those of the secondary nutrients Ca, Mg, and S (Follet et al. 1981). The position of P as a primary fertilizer nutrient is well deserved, since P deficiency (and application of large amounts of P fertilizer to correct it) is widespread throughout the world.

Average annual crop removal of P as grain and hay is about 40,000 tonnes in Manitoba, and 220,000 for all three Prairie Provinces (1985-1989 data; Doyle and Cowell 1993). Laverty et al. (1980) estimated average annual removal of P by crops to be about 9 kg $ha^{-1}$ for both Manitoba and the Prairie Provinces, using 1974-1978 production levels.

Most plant tissues usually contain about 0.5 to 5 g $kg^{-1}$ of P, expressed on the dry weight basis (Bingham 1966). For many annual crops, total P concentrations in the leaves must be at least 2 to 4 g $kg^{-1}$ for optimum growth. The rate of dry matter accumulation tends to increase with time in proportion to the rate of P accumulation, resulting in declining plant P concentrations as plants mature (Bouma 1983, Racz et al. 1965, Walworth and Sumner 1988). This was clear for spring wheat in the studies of Boatwright and Haas (1961) and Elliott et al. (1997a); maximum plant P accumulation had occurred by the flag leaf to anthesis stage in all treatments.

### 2.1.2 Nature of P in the Plant

Phosphorus present as orthophosphate in the soil solution is taken up actively into plant roots. This is achieved by the plant despite inorganic P concentrations in the plant cells 1000 to 10,000-fold higher than in soil solutions (Bieleski 1973). Phosphate moves readily from the xylem to the phloem, so can be transported effectively throughout the plant. Although P can be rapidly incorporated into organic compounds, much of its transport within the plant is in the inorganic orthophosphate form (Mengel and Kirkby 1982). Organic phosphates transported include phosphorylcholine, sugar phosphates, and the nucleotide phosphates (e.g. ATP), which are important in transfer of energy (Pate 1976).

Phosphate is a component of several types of organic compounds in plant tissues: 1) ester-P, including the energy transfer compounds, sugar phosphates, and phytic acid (storage P in seeds), 2) DNA and RNA (genetic coding, protein synthesis), 3) phospholipids (membranes), 4) phosphoproteins, and 5) coenzymes (Glass et al. 1980). Bieleski (1973) suggested 1.0:2.2:1.5 as a typical ratio in tissues of P in the first three groups above. The broad ranges of form and function of P-containing compounds involve them in almost all metabolic and growth processes.

Concentrations of inorganic P in plants vary much more widely with plant P nutritional status than do those of any other P fraction (Chapin and Bieleski 1982). For example, inorganic P accounted for 16% of the total P in fresh leaves of P-deficient bean plants vs. 65% in P-sufficient plants (Barr and Ulrich 1963). Bieleski (1968) observed a 40-fold reduction in concentration of inorganic P in *Spirodela* after transferring the plants to P-free medium, but only an overall four-fold reduction in organic P. Cells maintain cytoplasmic (metabolically active) inorganic P levels within narrow limits, while vacuolar P can fluctuate widely and serve as a plant P reserve accumulating at times of high P availability (Bieleski 1968, Mimura et al. 1990).

### 2.1.3 Assessment of Plant P Status

Visual identification of macro-scale plant deficiency symptoms has been the approach most widely used for diagnosis of nutrient deficiencies in cereals. Symptoms of P deficiency in wheat include generally reduced growth and yield, especially of the shoot; reduced tillering (fewer heads); delayed plant development; and smaller leaves, which are held more erect and may die back from the tip (Elliott et al. 1997a, Hewitt 1984, Hoffer and Krantz 1949). These symptoms are not definitive or pronounced enough to be very diagnostic, unless used in combination with a growth response trial in the field, i.e. by direct comparison of growth in adjacent P-fertilized and non-treated areas within a field. The purple pigment anthocyanin can form in the leaves and lower stems of P-deficient plants as a result of sugar accumulation. However, anthocyanin accumulation varies among cultivars, can result from stresses other than P deficiency, and is not pronounced in small grains (Follet et al., 1981)

Other diagnostic methods for P deficiency have been developed but not used on a commercial scale. Bouma (1983) described a growth response test conducted in the growth chamber on field-grown plants or leaves. McLachlan et al. (1987) developed biochemical tests based on increase in activity of phosphatase enzymes in plants under P deficiency.

Conventional plant analytical tests to determine nutrient status are widely available. Determinations are made of either the total elemental concentrations in tissue after drying (*total* or *plant analysis*) or of specific soluble fractions of the nutrients in a sap or extract easily obtainable from the fresh tissue (*tissue testing*; Tisdale et al. 1985). Interpretations are based on relationships established, usually empirically, between nutrient concentrations and growth responses to added nutrients (Smith 1962). Many variables affect those relationships and have been reviewed elsewhere: species and cultivar, plant part sampled and its physiological age, growing conditions and growth rate, nutrient interactions, and nutrient fraction determined (Bates

1971, Bouma 1983, Sheppard and Racz 1980, Smith 1962). Perhaps in part due to the lack of work done locally to establish interpretive criteria (hence limited accuracy and reliability of the interpretations), only occasional use of plant testing for nutrients is made in cereal crop production on the Canadian prairies. When plant analysis is carried out, total elemental analysis is used, usually for diagnosis of growth problems.

Tisdale et al. (1985) stated that the most critical stage for plant testing is at bloom stage of the crop, or bloom to early fruiting. Much of the plant analysis carried out is at heading or later. However, P deficiency symptoms are evident at much earlier stages (Elliott et al. 1997a, Hoffer and Krantz 1949). Phosphorus stress during the first weeks of growth reduces grain yields (Boatwright and Viets 1966, Classens 1990), and the effect cannot be reversed by later improvement in P nutrition (Batten and Wardlaw 1987).

## 2.2 P in Soil

### 2.2.1 Native P

Soil P originates from primary phosphate-containing minerals, mostly of the apatite group. Weathering releases the P by dissolution of the apatite during soil development, its further reaction to be controlled mostly by interaction with Ca in neutral to alkaline environments, or with Fe and Al under acidic conditions (Syers and Curtin 1989). The earth's crust contains about 0.12% P; soils contain 0.02 to 0.50%, averaging 0.06% (Lindsay 1979). For western Canadian chernozemic surface soils, 25 to 55% of the total P is in organic combinations, 10 to 30% of which is present as inositol phosphate and most of the remainder in unknown chemical forms (Stewart et al. 1980).

Soil Ca-phosphate minerals include, in order of increasing solubility in soil: fluorapatite, hydroxyapatite and other apatites, tricalcium phosphate, octacalcium phosphate, monetite, and brushite (Lindsay and Vlek 1977). The last three listed compounds are not sufficiently stable to exist in soil environments where P fertilizers have not been used.

The only fraction of soil P that is immediately available to plants is the inorganic phosphate in soil solution, which is absorbed by plants as the $H_2PO4^-$ and $HPO4^{2-}$ ions. Since the amount of soil P in this fraction is less than 1% of the amount of P required by crops in even a fertile field soil, rapid replenishment of the solution P is required for plant growth (Russell 1973). The greater control over the availability of soil P to plants, especially in the short term, is often attributed to release of P from sorbed forms, rather than to dissolution of crystalline forms (Murrmann and Peech 1969, Sadler and Stewart 1974, Syers and Curtin 1989). Sorption mechanisms and the quantitative relationships between sorbed and solution phosphate have been studied in detail (Barrow 1980, Sample et al. 1980). However, the indirect information usually obtained about the mechanisms at the molecular level still leaves question as to the roles of adsorption and surface precipitation (Sposito 1986).

### 2.2.2 Fertilizer Residual P

Despite use of highly soluble fertilizer compounds, uptake of fertilizer P in the year of application is generally less than 25%, often much less (Russell 1973, Sadler and Stewart 1974). However, numerous studies including some in Manitoba (Read et al. 1977, Ridley and Tayakepisuthe 1974, Spratt et al. 1980) have shown the fertilizer P not used in the first year ("residual P") can contribute substantially to the P supply for several following crops. Barrow (1980), Roberts and Stewart (1987), Sadler and Stewart (1974), and Spratt and Read (1980) reviewed the availability of residual P to crops.

Addition of soluble phosphates to Manitoba soils has generally caused precipitation of dicalcium phosphate dihydrate (DCPD), which usually slowly changed to octacalcium phosphate (OCP) over the following months (Racz and Soper 1967, Strong and Racz 1970); in addition, Mg phosphates were detected in the high-Mg soils only. Rather large amounts of Ca-phosphate can precipitate close to the fertilizer granule site, especially in calcareous soils; the required $Ca^{2+}$ originates from the soil exchange complex (displaced by the cation of the applied phosphate salt) and from dissolution of $CaCO_3$ due to reaction with $H^+$ ions released as the basic Ca-phosphate precipitates (Cho 1991). The persistence and importance of OCP as a residual P fertilizer compound of considerable plant availability has been shown indirectly (soil phosphate potentials) in Manitoba (Ridley and Tayakepisuthe 1974) and elsewhere (Olsen et al. 1983, Sadler and Stewart 1977). A wide range of other P compounds can also form upon reaction of P fertilizers with soil, and other species present can influence transformations of reaction products (thoroughly reviewed by Sample et al. 1980).

For Manitoba, the amount of P applied annually as fertilizer is similar to the annual removal of P as grain and hay (Doyle and Cowell 1993). Since little applied P is taken up in the year of application, it would follow that the P supply to crops is increasingly from fertilizer residual P sources over the soil native P source. Russell (1973) emphasized the importance of the very limited movement of P from fertilizer granule sites in the soil as a factor influencing the immediate and residual availability of P from fertilizers; unless the soil is thoroughly mixed, the granule sites remain as zones of high P availability.

### 2.2.3 Assessment of Soil P Status

Olsen and Khasawneh (1980) and Kamprath and Watson (1980) reviewed the wide range of methods used to assess soil P status, particularly the availability of the soil P to plants. Most

procedures used in research recognize two soil factors influencing soil P availability to plants. The *intensity factor* reflects the immediate electrochemical potential gradient which must be overcome for P uptake by a root. This factor is influenced by the composition of the soil solution only. It is usually estimated by calculation of a phosphate activity or potential using a P concentration measured in the soil solution or in a weak aqueous extract. The extensive factor (usually referred to as the *quantity factor*) reflects the size of the pool of solid phase P that can actively replenish the P in solution as it is removed by plant uptake (i.e. the *labile* P). Labile P is measured by isotopic or resin exchange, extraction with other stronger extractants, or determination of P adsorption parameters.

The primary goal of soil analysis, as widely conducted in soil testing for production agriculture, is to provide information on the soil nutrient status which can be used to make efficient fertilizer use recommendations (Follet et al. 1981). The inorganic phosphate extracted by a single procedure is usually determined. The extractant must remove a consistent portion of the labile P, as well as account for P intensity (Leitch et al. 1980). Several procedures have been developed, varying with respect to the chemical composition of the extractant, and hence the primary mechanisms of P removal (Kamprath and Watson 1980).

For areas of dominantly neutral to alkaline soils, including calcareous soils, the Olsen $NaHCO_3$ test (Olsen et al. 1954) is most widely used and recommended. For a wide range of soil types, Sibbesen (1983) concluded that $NaHCO_3$ methods were inferior to anion-exchange resin methods but more suitable than other procedures. Olsen et al. (1954) cite the major mechanisms of P extraction by $NaHCO_3$ to be enhancement of dissolution of Ca-phosphates and replacement of surface phosphate ions. Despite the general success in use of the test, there is evidence that it may not adequately reflect the full availability to plants of residues of added fertilizer P until very a long reaction period has passed (Bolland and Baker 1987, Esilaba et al. 1992).

## 2.3 Research Needs

Plant analysis can provide useful, often-conclusive, information about crop nutrient status to complement soil testing in a fertility management program. However, plant analysis is not widely used, especially for cereal crops. The methods normally used (especially sampling time) would not appear to be optimum for detection of yield-limiting P deficiency. Also little local (western Canadian) work has been conducted to establish best methods and interpretive criteria. Nutrient levels in tissues required for optimum growth vary with crops, growth stages, conditions, etc. The need for development and evaluation of the tests and interpretive criteria under local field conditions is often emphasized (Bates 1971, Kamprath and Watson 1980, Bouma 1983, Savoy and Robinson 1990). Also, establishment of tissue criteria for P based on soluble phosphate in the plant is needed to facilitate development of field tissue quick-tests (Saarela and Sippola 1990).

Field correlation and calibration was carried out during the development of the traditional soil tests, but most of this was at a time when native soil P (rather than fertilizer residues) was still supplying most of the P to plants. There is a need to re-visit the interpretation of soil tests for P in light of the increasing importance of fertilizer residual P. In particular, the chemical form and spatial distribution of residual P may have implications for its plant availability and effects on soil P test levels.

# 3. TISSUE PHOSPHORUS TESTS FOR WHEAT AND THEIR RELATIONSHIPS WITH GRAIN YIELD

## 3.1 Abstract

Plant analysis is not widely used for assessment of nutritional status of small grains on the Canadian prairies. This may be in part due to limited success with the practices used for reliably diagnosing P deficiency. Three plant P tests were evaluated and calibrated, using results from eight site-years of field P-response studies with spring wheat in Manitoba. Concentrations of total P ($P_t$) in the whole-shoot, and of $P_t$ and extractable inorganic P ($P_i$) in the leaf, were monitored for about seven weeks after crop emergence. Results were then related to grain yields as affected by P treatments.

All P test concentrations declined early in the growing season. By approximately the stem extension stage, leaf $P_t$ and $P_i$ concentrations tended to stabilize, but shoot $P_t$ continued to decline. Tissue P concentrations showed pronounced differences among sites and fertilizer treatments during early growth stages, but converged as the season progressed for each test. Inorganic P concentrations showed much greater proportional variation than $P_t$. This wide spread in $P_i$ test values with P supply would reduce the analytical accuracy needed for reliable diagnosis (desirable under the "quick-test" conditions to which the $P_i$ test could be adapted).

The grain yield response to P was closely correlated ($r^2>0.8$) with plant P test level from about mid-tillering to flag leaf stage, for each of the three tests, across all sites and P treatments. The correlation for shoot $P_t$ declined much more sharply with time after the early boot stage,